THE EFFECT OF IODINE SUPPLEMENTATION ON BIOMARKERS OF IODINE STATUS, THYROID FUNCTION, RESTING METABOLIC RATE, AND BODY COMPOSITION IN WOMEN, 18-45 YEARS OF AGE

A DISSERTATION

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DEDICATION

To my mother, Sumathy,

For her sacrifices, resilience, and unconditional love.

To my brother, Pritish,

For staying by mom, each and every time I could not.

Most importantly, to my husband, Ramesh,

For his patience, because he ALWAYS understood.

And, in loving memory of my father, Kishore.

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ABSTRACT

PALLAVI PANTH

THE EFFECT OF IODINE SUPPLEMENTATION ON BIOMARKERS OF IODINE STATUS, THYROID FUNCTION, RESTING METABOLIC RATE, AND BODY COMPOSITION IN WOMEN, 18-45 YEARS OF AGE

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The purpose of this dissertation was to investigate the efficacy of iodine supplementation vs. placebo, in reproductive-age women, 18-45 years, in improving iodine status, thyroid function, resting metabolic rate, and body composition in a six-month, randomized-double-blinded-placebo-controlled trial. Non-pregnant (euthyroid, normal thyroid stimulating hormone (TSH), mean = 1.57mIU/mL) women were randomized into two groups: 12.5mg Iodoral® (IG, *n* = 65) or placebo (PG, *n* = 38). Assessments included iodine status determination (24-hr urine iodine (UI), %-iodine saturation (% IS), sodium-iodide-symporter-ratio (NIS), saliva and serum iodide concentrations), thyroid function (serum TSH, free-thyroxine (T_4), and free-tri-iodothyronine (T_3) concentrations), body composition analysis using Dual Energy X-ray Absorptiometry (DXA), resting metabolic rate (RMR) testing, and analysis of three-day dietary records, health, demographic, and physical activity questionnaires. Analysis of the data revealed dietary iodine intake to be significantly below standard recommended dietary allowance (RDA) of 150 µg iodine/d for IG and PG at baseline and six months. For the first time, associations were observed between dietary iodine intake and body composition, with decreased dietary iodine intake being associated with higher body fat content ($p<0.01$). Iodine status indicators, 24-hr UI and % IS were also significantly below normal, indicating iodine deficiency in the study population. Although 24-hr UI and % IS for IG and PG showed an increased trend from baseline

to six months, statistical significance was not observed for between and within group effects, indicating that a longer duration of supplementation may be needed to improve iodine status in deficient populations. Saliva iodide increased significantly in IG ($p = 0.041$), and PG ($p = 0.013$) at the end of six months; however, NIS ratio remained unchanged, indicating normal functioning of the NIS. Free-T_4 increased significantly at six months in IG and PG ($p < 0.001$), however other thyroid function parameters remained unchanged, indicating that the high dose iodine supplement may be better tolerated than expected. RMR significantly increased in IG and PG ($p < 0.001$) at six months, and was positively correlated ($p < 0.01$) with all body composition variables. Overall, participants demonstrated a generalized lack of awareness of iodine nutrition and the implications of iodine deficiency in reproductive-age women, indicating a significant public health concern that needs to be addressed.

ABBREVIATIONS

% IS	Percent iodine saturation
µg	Microgram
µg/d	Microgram/day
24-hr	24 hour
ACSM	American College of Sports Medicine
A-G ratio	Android-gynoid ratio
AI	Adequate intake
ALPCO	American Laboratory Products Company
ANOVA	Analysis of Variance
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CPL	Clinical Pathological Labs
D1	Type 1 iodothyronine deiodinase
D2	Type 2 iodothyronine deiodinase
D3	Type 3 iodothyronine deiodinase
DIT	Diiodothyronine
DRI	Dietary Reference Intakes
DV	Daily Value
DXA	Dual-energy-X-ray absorptiometry
EAR	Estimated average requirement
ELISA	Enzyme linked immuno-sorbent assay
FDA	Food and Drug Administration
FFP	Flechas Family Practice
FFQ	Food Frequency Questionnaire
FNB	Food and Nutrition Board
F-T_3	Free T_3
F-T_4	Free T_4
g	Grams

GE	General Electric
H_2O_2	Hydrogen peroxide
I	Iodine
I^-	Iodides
I/Cr	Iodine-to-creatinine ratio
I_2	Elemental iodine
IBM	International Business Machines Corporation
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IDD	Iodine deficiency disorders
IG	Iodine group
IGN	Iodine Global Network
In^3	Inches cubed
IO_3^-	Iodates
IOM	Institute of Medicine
IRB	Institutional Review Board
IU	International units
IU/mL	International units per milliliter
IWH	Institute for Women's Health
Kcals	Kilocalories
Kcals/d	Kilocalories/day
Kcals/kg/hr	Kilocalories per kilogram per hour
Kg/m^2	Kilogram per meter squared
KI	Potassium iodide
lbs	Pounds
MET	Metabolic Equivalent Score
mg	Milligram
mg/24-hr	Milli grams per 24 hours
mg/L	Milli grams per liter
MIT	Monoiodothyronine
mIU/L	Milli-international units/liter
ml/kg/min	Milliliter per kilogram per minute

NADPH	Nicotinamide adenine dinucleotide phosphate
NFNAP	National Food and Nutrient Analysis Program
ng/dL	Nano grams per deciliter
NHANES	National Health and Nutrition Examination Surveys
NIS	Sodium iodide symporter
PFAS	Perfluoroalkyl acids
PG	Placebo group
pg/mL	Pico grams per milliliter
R & D	Research and development
RDA	Recommended dietary allowance
REE	Resting energy expenditure
RMR	Resting metabolic rate
RNI	Recommended nutrient intake
ROS	Reactive oxygen species
rT_3	3, 3′, 5′- triiodothyronine or Reverse T_3
RXR	Retinoid X receptor
SEM	Standard error of the mean
SPSS	Statistical Package for Social Sciences
T_2	3, 5- di-iodo-L-thyronine
T_3	3, 3′, 5-triiodothyronine
T_4	3, 3′, 5, 5′-tetra-iodothyronine or Thyroxine
TBG	Thyroxine binding globulin
Tg	Thyroglobulin
TgAb	Thyroglobulin antibody
THR	Thyroid hormone receptor
TPO	Thyroid peroxidase
TPOAb	Thyroid peroxidase antibody
TRE	Thyroid hormone response element
TRH	Thyrotropin releasing hormone
TSH	Thyroid stimulating hormone
TWU	Texas Woman’s University

UCr	Urinary creatinine
UI	Urinary iodine
UNICEF	United Nations International Children's Emergency Fund
UNT	University of North Texas
USDA	U.S. Department of Agriculture
USPS	U.S. Postal Service
VAT	Visceral adipose tissue
WHO	World Health Organization

TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Iodine, an essential trace mineral, is required for the production of thyroid hormones. Recommended dietary intake (RDI) of iodine for adults as determined by the Institute for Medicine (IOM) is 150 µg/d (IOM, 2001). Urinary iodine (UI) concentrations above 150 µg/L indicate iodine sufficiency (WHO/UNICEF, 1994). Iodine intake in the U.S. diet has steadily decreased over the past few decades. The median UI concentration in the U.S. was 320 µg/L in 1974, and 164 µg/L in 2002 (Caldwell, Jones, & Hollowell, 2005). It decreased even further to 144 µg/L in 2010, suggesting a more than 50% reduction in dietary intake (Caldwell et al., 2013). According to the International Council for Control of Iodine Deficiency Disorders (ICCIDD), since renamed the Iodine Global Network (IGN), 17% of the U.S. population was iodine deficient, with a median urinary iodine concentration <150 µg/L, which meant that over 53 million individuals were at the risk of iodine deficiency in the U.S. (ICCIDD, 2013).

Iodine deficiency manifests in the thyroid gland as clinical or subclinical hypothyroidism, goiter, myxedema, cretinism and other non-specific conditions such as stunted growth, mental retardation, and decreased intelligence (Ahad & Ganie, 2010; Zimmermann, 2011). Women of childbearing age, 15-44 years, are at the greatest risk of iodine deficiency. Among pregnant and lactating women, 49% were consuming iodine below the Recommended Dietary Allowance (RDA) of 150µg/d (Pearce, Bazrafshan, He, Pino, & Braverman, 2004). The American Thyroid Association recommends that women of reproductive-age, and planning to become pregnant, consume between 220-290 µg/d of iodine, to compensate for fetal and maternal requirement and losses (Stagnaro-Green, Dogo-Isonaige, Pearce, Spencer, & Gaba, 2015). According to the

National Health and Nutrition Examination Survey (NHANES) 2009-2010, median UI was 134 μg/L for all U.S. females, and 124 μg/L for women of childbearing age (Caldwell et al., 2013). Thirty six percent of U.S. women had low UI levels (<100 μg/L) with 16% of women having levels below 50 μg/L, which would mean 2.2 million U.S. women had low or deficient iodine intakes (Blount, Pirkle, Osterloh, Valentin-Blasini, & Caldwell, 2006).

Iodine intake is a crucial determinant of iodine status, but it is difficult to assess. UI concentration is the method most commonly used to assess dietary iodine intake and is based on the assumption that >90% of iodine consumed from foods and supplements is absorbed. The amount of iodine excreted in the urine reflects recent iodine intake. Measures of thyroid function, including concentrations of serum thyroid stimulating hormone (TSH) and thyroglobulin (Tg), also reflect iodine status (Swanson & Pearce, 2013).

Iodine is an integral part of the thyroid hormones, triiodothyronine, T_3 (59% of the molecular weight of T_3) and thyroxine or tetra-iodothyronine, T_4 (65% of the molecular weight of T_4) (Erdman, Zeisel, & MacDonald, 2012). Dietary iodine is concentrated at the thyroid basal cell membrane by a sodium iodide symporter (NIS). Tg present in the gland is iodinated and the hormones, T_3 and T_4, are formed by the coupling of monoiodothyronine (MIT) and diiodothyronine (DIT) mediated by thyroid peroxidase enzyme (TPO). T_3 and T_4 are stored as part of the iodinated Tg in the thyroid follicles until they are needed, at which time, Tg residues pass into lysosomes to release T_3 and T_4 into the circulation. Iodine deficiency leads to increased stimulation of thyrotropin-releasing hormone (TRH) in the hypothalamus, leading to increased TSH secretion by the pituitary gland, increased iodine uptake and turnover, decreased iodine excretion, and enhanced production of the active hormone, T_3. Deficiencies of micronutrients

such as iron, vitamin A, zinc, copper, and selenium have also been shown to adversely affect iodine metabolism and thereby thyroid function (Hess, 2010).

Thyroid hormones play essential roles in regulating energy homeostasis by modifying basal metabolic rate and thermogenesis (Silva, 2003). A large number of people in the U.S. unknowingly have hypothyroidism and should be screened (Hollowell et al., 2002). Mild or sub-clinical hypothyroidism, defined as elevated TSH (4.0–10.0 mIU/L), with T_3 and T_4 levels within normal limits, is found in about 7.5% of females (Staub et al., 1992). Hypothyroidism causes weight gain and alterations in body composition with increased fat deposition. Adipose tissue contains receptors for TSH and the thyroid hormones T_4, and T_3. It is possible that obese patients have a central thyrostat reset, which makes them less sensitive to the circulating thyroid hormones (Michalaki et al., 2006). Low thyroid function, even within the clinically normal range of serum T_3, T_4, and TSH, could lead to obesity (Asvold, Bjøro, & Vatten, 2009). Low levels of free T_4 or high levels of TSH in euthyroid individuals are associated with high body mass index (BMI) (Knudsen et al., 2005; Makepeace et al., 2008). Thyroid volume is directly correlated with body weight, BMI, and lean body mass. Thyroid volume is directly regulated by amounts of TSH and iodine from the diet (Wesche & Wiersinga, 2001). In a group of weight stable but post-obese women, resting metabolic rate was significantly lower than a group of never obese women matched for age, fat mass and fat-free mass; and the post-obese group had significantly lower free T_3 concentrations (Astrup, Buemann, Toubro, Ranneries, & Raben, 1996). A positive correlation between BMI and serum TSH and a negative association between BMI and free T_4 levels was observed in the Danish investigation on iodine intake and thyroid disease (Dan Thyr Study) (Laurberg et al., 2006). A population-based study in Norway reported an association between BMI and serum TSH in non-smokers (Nyrnes, Jorde, & Sundsfjord, 2006).

Although iodine deficiency is the most common cause of thyroid dysfunction, it is rarely evaluated during regular annual checkups, or in conjunction with thyroid testing in clinical practice. It is possible that a simple correction of iodine deficiency in vulnerable populations such as reproductive-age women could result in correction of iodine status, thereby resulting in improved thyroid function, basal metabolism, and body composition.

Statement of the Purpose

The purpose of this study was to determine the impact of a 12.5 mg Iodoral® (iodine) supplement vs. placebo (glucose tablet) on iodine status, thyroid function, resting metabolic rate, and body composition in reproductive-age women, 18-45 years of age in a parallel, randomized, double-blind, placebo-controlled trial over six months.

Null Hypotheses

For the present study, the primary null hypothesis was that six months of iodine supplementation compared to placebo will not improve iodine status, thyroid function, RMR and body composition in reproductive-age women, 18-45 years of age. This study examined the following null hypotheses at significance levels of $p \leq 0.05$.

1. Iodine supplementation for six months will not improve iodine status by increasing concentrations of 24-hr UI excretion, percent iodine saturation, and sodium iodide symporter (NIS) ratio in a group of reproductive-age women, 18 – 45 versus a placebo.
2. Iodine supplementation for six months will not improve thyroid function by increasing serum concentrations of thyroid hormones, free thyroxine (T_4), free triiodothyronine (T_3), and decreasing thyroid stimulating hormone (TSH) in a group of reproductive-age women, 18 – 45 versus a placebo.

3. Iodine supplementation for six months will improve resting metabolic rate (RMR) in a group of reproductive-age women, 18 – 45 versus a placebo.
4. Iodine supplementation for six months will not improve body composition by decreasing BMI (kg/m^2), percent body fat, and increasing percent lean mass in a group of reproductive-age women, 18 – 45 versus a placebo.

Specific Aims

1. To examine the impact of iodine supplement versus placebo on iodine status measured by evaluating concentrations of 24-hr UI excretion, percent iodine saturation, and NIS ratio in a group of reproductive-age women, 18 – 45.
2. To examine the impact of iodine supplement versus placebo on thyroid function measured by evaluating serum concentrations of thyroid hormones, free thyroxine (T_4), free triiodothyronine (T_3), and thyroid stimulating hormone (TSH) in a group of reproductive-age women, 18 – 45.
3. To examine the impact of iodine supplement versus placebo on resting metabolic rate (RMR) in a group of reproductive-age women, 18 – 45.
4. To examine the impact of iodine supplement versus placebo on body composition, including BMI (kg/m^2), percent body fat and percent lean mass, measured by dual-energy X-ray absorptiometry (DXA) in a group of reproductive-age women, 18 – 45.

Expected Outcomes

Females between 18-45 years have a greater need for iodine in their diets and therefore a higher prevalence of iodine deficiency disorders (IDD) and thyroid dysfunction than the general population. Therefore, the results of this study will be directly applicable to the female population in the 18-45-year age range. Iodine supplementation is expected to replenish iodine stores in the

body resulting in an increase in 24-hr UI excretion as well as % IS, and normalization of NIS ratio, resulting in an overall improvement of iodine nutrition status. Iodine from the iodine supplement is expected to be directly taken up by the thyroid gland resulting in an increased production of the thyroid hormones T_3 and T_4, and decreased TSH secretion by the pituitary gland. Iodine supplementation is expected to increase RMR due to its direct effects on improving thyroid function, also resulting in a decrease in BMI and % body fat as well as an increase in % lean mass, thereby improving overall body composition.

CHAPTER II

REVIEW OF LITERATURE

Iodine and the Thyroid Gland

Iodine, an essential trace micronutrient, is an integral part of the thyroid hormones, 3, 3′, 5-triiodothyronine, T_3, and 3, 3′, 5, 5′-tetra-iodothyronine, T_4, produced by the thyroid gland (See Figure 2.1). The body of a healthy adult contains 15-20 mg of iodine, of which 70-80% is in the thyroid gland; extra-thyroidal utilization of iodine has also been documented, but the full role is unknown (Venturi, Donati, Venturi, & Venturi, 2000; Venturi, Donati, Venturi,Venturi, & Grossi et al., 2000). Iodine is found in various forms such as iodides (I^-) and iodates (IO_3^-), organic monoatomic iodine (I), and elemental iodine (I_2) (Zimmerman, 2009).

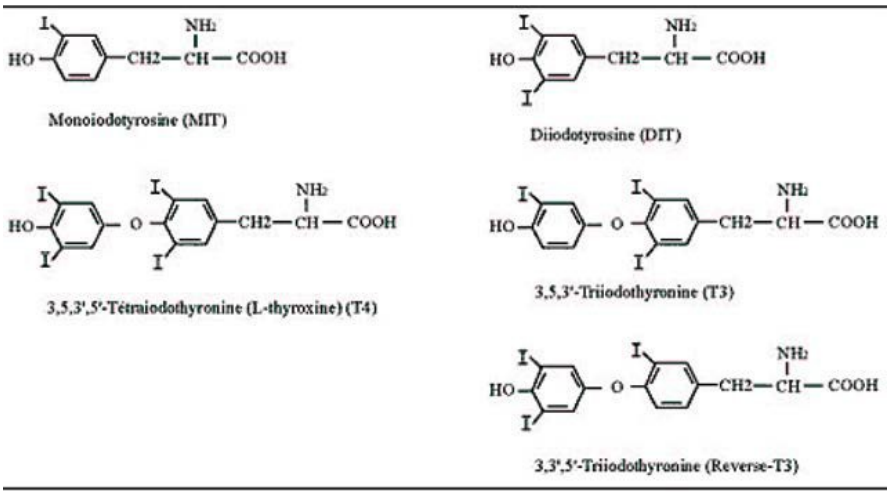

Figure 2.1. Structure of thyroid hormones (Thyroid Disease Manager, "Chapter 2 Thyroid hormone synthesis and secretion", 2015)

Synthesis and Regulation of Thyroid Hormones

Facilitated diffusion transports dietary iodine and I^- is absorbed via the NIS in the gastric mucosa, thyroid gland, mammary gland and other tissues that utilize iodine (See Figure 2.2). Thyroid

gland basal cell membrane concentrates iodide from the serum using NIS and oxidizes it at the apical membrane, attaching it to tyrosyl residues within thyroglobulin (Tg) to make MIT and DIT. Iodine that is released from MIT and DIT is recirculated (Dunn & Dunn, 2001).Thyroid peroxidase (TPO), hydrogen peroxide (H_2O_2), pendrin, and nicotinamide adenine dinucleotide phosphate (NADPH) are involved in this process (Rousset, 2015). Iodinated Tg is then acted upon by TPO and H_2O_2 to form the inactive or prohormone, T_4, and the active form T_3 (Dunn & Dunn, 2001). These hormones are stored in the thyroid follicular lumen as part of the iodinated Tg until they are needed, at which time, the Tg residues pass into lysosomes and are broken down to release T_3 and T_4 into the circulation. Active thyroid hormone, T_3, predominantly acts through its nuclear receptors, thyroid hormone receptor (THR). The functional THR complex consists of a heterodimer with the retinoid X receptor (RXR) that binds to a thyroid hormone response element (TRE) to modulate gene expression. Removal of outer ring iodine from T_4 produces T_3, thereby increasing thyroid hormone activity, this critical step being catalyzed by type 1 and 2 iodothyronine deiodinases (D1 and D2) (Brent, 2012; Hollenberg & Forrest, 2008). Type 3 deiodinases (D3) convert T_3 into 3, 5- di-iodo-L-thyronine (T_2) and T_4 into 3, 3′, 5′-triiodothyronine (reverse T_3 or rT_3) which inactivates thyroid hormone signaling (Cheng, Leonard, & Davis, 2010; Gereben et al., 2008).

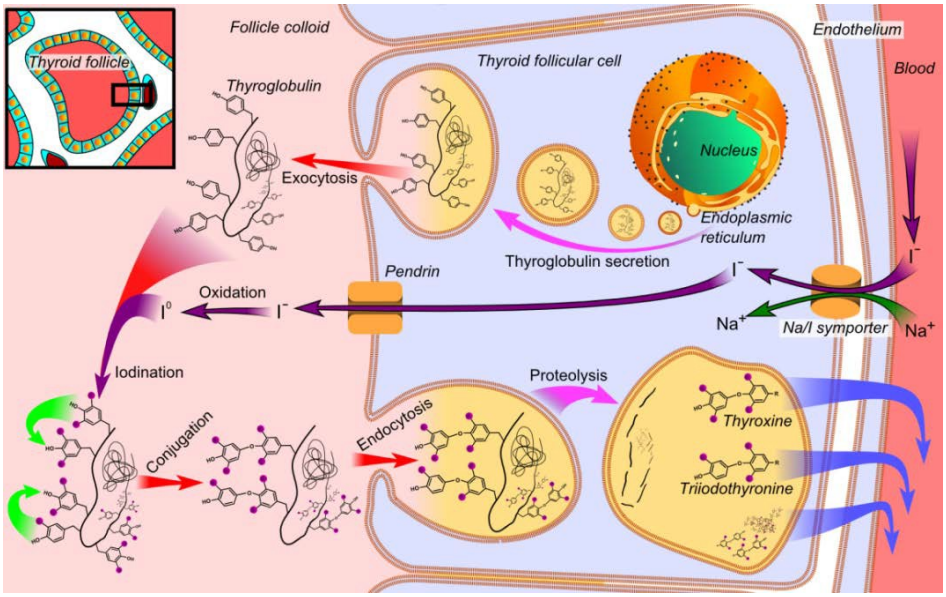

Figure 2.2. Thyroid hormone synthesis (Reproduced with permission from "Thyroid hormone synthesis.png - Wikimedia Commons," 2016)

The micronutrient, selenium, is an integral part of deiodinase enzymes, and its deficiency has been shown to have a significant impact on iodine and thyroid metabolism. Thyroid functioning is strictly regulated by the hypothalamic-pituitary-thyroid axis and circulating levels of TSH (See Figure 2.3). TSH mediates many aspects of iodine metabolism in the thyroid gland, including increased production of Tg and NIS, through stimulation of transcription factors, increased active hormone T_3 formation relative to the inactive form T_4, and appropriate distribution of iodine among tyrosyl residues (Taurog, 2000). Iodine supply regulated by dietary iodine intake is another factor that affects iodine metabolism. Iodine deficiency leads to increased stimulation of TRH in the hypothalamus, leading to increased TSH secretion by the pituitary gland, increased iodine uptake and turnover, decreased iodine excretion and enhanced production of T_3. Deficiencies of micronutrients such as iron, vitamin A, zinc, and copper have also been shown to affect iodine metabolism adversely and thereby thyroid function (Hess, 2010; Zimmermann, Burgi, & Hurerll, 2007).

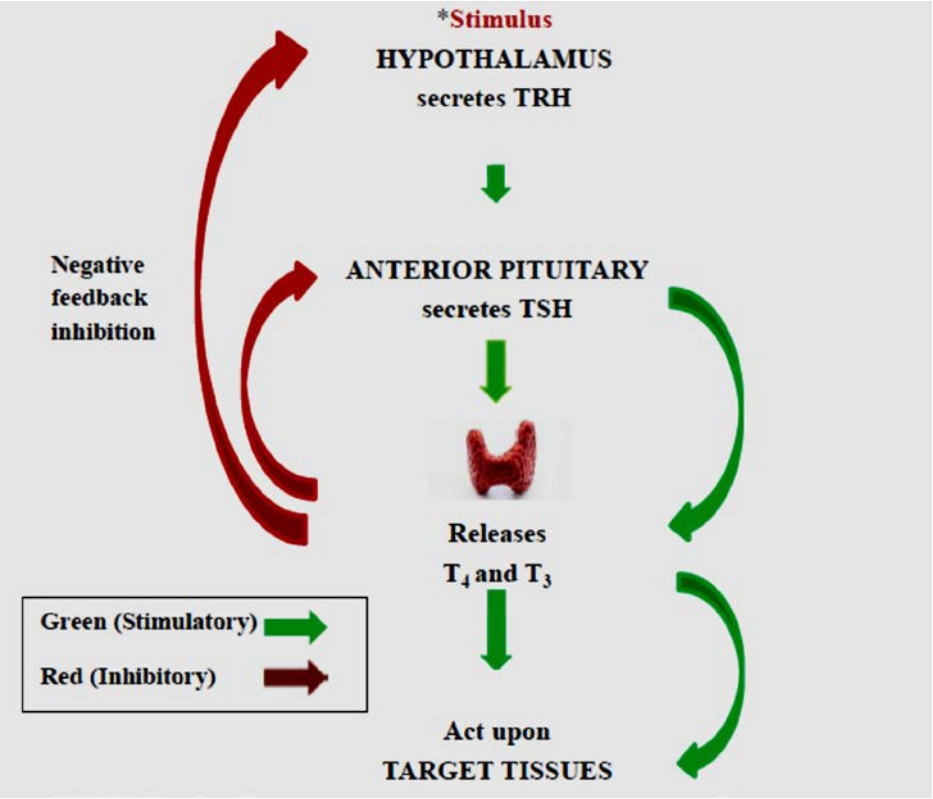

Figure 2.3. Regulation of thyroid hormones

The thyroid gland adapts to low dietary iodine intake by marked modification of its activity, triggered by increased secretion of TSH by the pituitary gland. If iodine intake falls below100 µg/d, TSH secretion is increased, resulting in increased plasma iodide clearance, reduced renal clearance, the breakdown of Tg and preferential synthesis and release of the active hormone T_3 into the blood (Abrams & Larsen, 1973). As dietary intake of iodine falls further, iodine content of the thyroid gland is depleted resulting in a spectrum of disorders described as Iodine Deficiency Disorders (IDD). Iodine deficiency manifests in the thyroid as clinical or subclinical hypothyroidism, goiter, myxedema, cretinism, and other non-specific conditions such as stunted growth, mental retardation, and decreased intelligence (Ahad & Ganie, 2010; Zimmermann, 2011). Apart from thyroid dysfunction, iodine deficiency also causes disorders of

the salivary gland, mammary gland, and stomach, as these organs are involved primarily in the extrathyroidal uptake of iodine (Venturi & Venturi, 1999; Verheesen & Schweitzer, 2008).

Dietary Iodine

Primary dietary sources of iodine in the U.S. are dairy products such as milk, bread, seaweed and seafood, and iodized salt in households that report using it (Murray, Egan, Kim, Beru, & Bolger, 2008). Dairy products contain iodine partly due to the use of iodine feed supplements and iodophors sanitizing agents in the dairy industry (Pennington & Young, 1990). Seafood, such as fish and seaweed contain high iodine content because marine plants and animals concentrate iodine from seawater. Iodine content of grain products is due to the use of iodate based dough conditioners which preserve the shelf life of bread. Fruits and vegetables contain iodine in varied amounts depending on the iodine content of the soil, fertilizer use, and irrigation practices (IOM, 2001). According to its label, iodized salt in the U.S. contains 45 μg iodine/g salt; however, the majority of salt intake in the U.S. comes from processed foods, and food manufacturers almost always use non-iodized salt in processed foods (Dasgupta, Liu, & Dyke, 2008).

Iodine intake in the U.S. was considered to be sufficient since iodization of salt and other food products began in the 1920s. However, iodine intake in the U.S. is declining and not as adequate as it is assumed to be (Global Scorecard of Iodine Nutrition, 2017; Iodine Global Network, 2016). It is estimated that among Americans, 7-8% of sodium intake is contributed by table salt and only 70% of consumers choose iodized salt (Becker et al., 2006). It is also not possible to accurately determine dietary exposure to iodine in the U.S. because national food composition tables do not currently include iodine amounts from foods and beverages. The U.S. Department of Agriculture's (USDA) Nutrient Database Website (USDA Food Composition

Database, 2017) lists the nutrient content of many foods, but this list does not currently include iodine.

The approximate iodine content of foods that provide over 20% of the daily value (DV) for iodine, and therefore are considered high sources of iodine are shown in Table 2.1 (Iodine, 2011; Office of Dietary Supplements-Iodine, 2011). The Food and Drug Administration (FDA) does not require food labels to list iodine unless a food item has been fortified with iodine.

Table 2.1

Selected Food Sources of Iodine Providing >20% DV of Iodine

Food	Approximate Micrograms (µg) per serving	Percent DV
Seaweed, whole or sheet, 1 g	16-2,984	11-1,989%
Cod, baked, 3 ounces	99	66%
Yogurt, plain, low-fat, 1 cup	75	50%
Iodized salt, 1.5 g (app. 1/4 teaspoon)	71	47%
Milk, reduced fat, 1 cup	56	37%
Fish sticks, 3 ounces	54	36%
Bread, white, enriched, 2 slices	45	30%
Fruit cocktail in heavy syrup, canned, 1/2 cup	42	28%
Shrimp, 3 ounces	35	23%
Ice cream, chocolate, 1/2 cup	30	20%

Legend: µg, micrograms; DV, daily value
Note. (Adapted from Iodine, 2011; Office of Dietary Supplements - Iodine, 2011)

The recommendations for dietary iodine intake are provided by following the Dietary Reference Intakes (DRIs) developed by the U.S. Food and Nutrition Board (FNB) at the Institute of Medicine (IOM) and the World Health Organization (WHO) and are shown in Table 2.2. Following these recommendations, the RDA for iodine for adults > 14 years was determined to be 150 µg/d. The RDA for iodine for pregnant and lactating women is higher at 220 -290µg/d to compensate for fetal and maternal requirement and losses (Benoist, 2004). Commonly consumed

foods and beverages have low iodine content, and only provide 3-80 µg iodine per serving, which is far below the RDA of 150 µg/d for the general population (Haldimann, Alt, Blanc, & Blondeau, 2005; Pearce et al., 2004).

Table 2.2

Recommendations for Iodine Intake (µg/d) by Age or Population Group

	IOM			WHO
Age or population group	EAR	AI or RDA	Age or population group	RNI
Infants 0-12 mo		110-130	Children 0-5 yrs	90
Children 1-8 yrs	65	90	Children 6-12 yrs	120
Children 9-13 yrs	73	120		
Adults ≥ 14yrs	95	150	Adults >12 yrs	150
Pregnancy	160	220	Pregnancy	250
Lactation	200	290	Lactation	250

Legend: AI, Adequate intake; EAR, Estimated average requirement; RNI, Recommended nutrient intake

Note. (Adapted from DRI, 2001; IOM, 2001)

Iodine intake can be measured by analyzing 24-hr recalls and food frequency questionnaires (FFQs) (Rasmussen et al., 2001). 24-hr recalls rely heavily on the participants memory and ability to recall food intake accurately, and may be beneficial in large population surveys such as in national nutrition surveys, however, due to the sizeable intra-individual variation in dietary intake, single 24-hr food recalls are unlikely to accurately determine nutrient intakes in smaller groups (De Keyzer et al., 2011; Raina, 2013). Weighted dietary records are considered the gold standard to measure nutrient intake as they provide a detailed description of foods consumed in a specified period, however, they are associated with significant respondent burden (Henríquez-Sánchez et al., 2009). A major limitation associated with these methods of assessment includes difficulty in accurately assessing the quantity of iodine derived from iodized salt (Rana & Raghuvanshi, 2013; Winger, König, & House, 2008). FFQs can be used to

determine usual intake and analyze dietary patterns. However, accuracy of FFQs for iodine requires validation against a reference and tables for iodine content of different foods which is unavailable in many regions. Using a validated iodine-specific FFQ focuses on assessing the consumption of iodine-rich foods such as seafood and the type of salt used, making it the preferred method in studies that assess iodine intake (Bath et al., 2016; Combet & Lean, 2014; Condo, Makrides, Skeaff, & Zhou, 2015; Rasmussen et al., 2014; Tan, Charlton, Tan, Ma, & Batterhamm, 2013).

Iodine intake in the U.S. diet has steadily decreased over the past few decades (Hollowell et al., 1998). Reduction of dietary iodine intake in the U.S. has been attributed to multiple factors: elimination of iodate-based dough conditioners in bread, reduced use of iodine-based disinfectants in the dairy industry, and increased consumption of processed foods which do not contain iodized salt are the most prominent reasons. Other factors also include environmental exposure to thiocyanate from cigarette smoke and perchlorate that decreases absorption of dietary iodine, recommendations for reduced salt intake, and an absence of mandatory salt iodization program in the U.S. (Dasgupta et al., 2008). Deficiency of iodine may be exacerbated by the consumption of substances known as goitrogens which interfere with iodine- thyroid metabolism. Cruciferous vegetables, cassava, lima beans, soy and millet flavonoids, perchlorate, and thiocyanates are known goitrogens and may have significant clinical effects with co-existing iodine deficiency (Braverman et al., 2006; Zimmermann, 2006; Zimmermann & Köhrle, 2002).

Iodine Status Determination

Iodine deficiency is one of the most prevalent nutrition deficiencies that often goes unchecked. Iodine status of a population can be assessed by biochemical and dietary assays, with biochemical assays being the most common method of assessment (Ristic-Medic et al., 2009).

Measurement of iodine concentration in urine (UI) and TSH, T_3, T_4, and Tg levels in blood are the most common biochemical assays used to evaluate iodine status (Pearce & Caldwell, 2016).

Dietary iodine intake is most commonly assessed by evaluating UI concentrations because it reflects recent iodine intake. It is based on the assumption that ≈90% of dietary iodine is excreted in urine. Higher excretion of UI therefore, indicates high recent dietary iodine intake (Soldin, 2002). UI concentrations can be determined in spot samples or 24-hr urine samples. UI determination in 24-hr urine samples is considered the gold standard because iodine excretion in this sample represents daily iodine intake and takes diurnal variation into account (Vejbjerg et al., 2009). A single 24-hr UI collection may also be insufficient to assess iodine status as UI excretion varies considerably from day to day due to changes in iodine intake, renal clearance, and urine volume; however, collection of multiple 24-hr urine samples is next to impossible in most clinical and population studies because of the associated respondent burden (Johner, Shi, & Remer, 2010; König, Andersson, Hotz, Aeberli, & Zimmermann, 2011; Rasmussen, Ovesen, & Christansen, 1999). For this reason, the collection of spot urine samples is the most common method of UI determination in population studies. Median UI concentrations are used to classify iodine status of populations (Ji et al., 2015). Table 2.3 shows the WHO/UNICEF/ICCIDD criteria for the classification of iodine deficiency based on median UI.

In spite of the relative ease of use in large-scale population studies, some of the disadvantages of using spot UI samples include high daily variation in UI excretion, and the inability to quantify individuals with low or excess iodine intakes (Andersen, Karmisholt, Pedersen, & Laurberg, 2008; Busnardo et al., 2006; Karmisholt, Laurberg, & Andersen, 2014). Iodine-to-creatinine ratio (I/Cr) is another method used to determine UI excretion, based on the assumption that urinary creatinine (UCr) excretion is constant. However, UCr excretion is not

constant from day to day in individuals, and can be affected by lean muscle and therefore age (Andersen, Hvingel, Kleinschmidt, Jørgensen, & Laurberg, 2005; Barr et al., 2005).

Table 2.3

Median Urinary Iodine Values and Iodine Nutrition in a Population

Median UI (μg/L)	Corresponding iodine intake (μg/d)	Iodine nutrition
<20	<30	Severe deficiency
20-49	30-74	Moderate deficiency
50-99	75-149	Mild deficiency
100-199	150-299	Optimal
200-299	300-449	More than adequate
≥299	>449	Possible excess

Legend: μg/L, micrograms per liter; μg/d, micrograms per day; UI, urinary iodine
Note. (Adapted from WHO, 2007)

Serum TSH concentrations are primarily used to diagnose thyroid dysfunction. TSH may not be a reliable indicator of population iodine status. Only severe iodine deficiency may cause TSH levels to rise above the reference range (Zimmermann & Andersson, 2012). Individuals or populations with mild iodine deficiency may not show any changes in TSH, and for this reason, TSH may not be a sensitive marker for iodine status (Skeaff, 2012; Zimmermann & Boelaert, 2015). Similarly, thyroid hormones, serum T_3, and serum T_4 have been reported to show no changes in populations with mild to moderate iodine deficiency, and therefore considered poor biomarkers to assess iodine status of populations (Rohner et al., 2014). Thyroglobulin concentrations, which can be measured using dried blood spot methods, on the other hand, are sensitive to iodine intake and may be indicative of iodine deficiency. Therefore, thyroglobulin could be considered a long-term biomarker of iodine status (Zimmermann et al., 2013).

Clinical assessment of thyroid volume can be conducted by palpation or ultrasonography. Ultrasonography is more sensitive in detecting subtle increases in thyroid volume; however, no

criteria exist to classify iodine status in adults using thyroid volume (Krejbjerg et al., 2014; Zimmermann, Jooste, & Pandav, 2008).

The Iodine Project

Based on the findings of Ghent, Eskin, Low, and Hill (1993), where women with fibrocystic disease of the breast showed beneficial effects with ingestion of 5mg iodine daily for one year, Guy Abraham, M.D, in 1997, decided to study iodine supplementation and started the Iodine Project. This project was based on the hypothesis that whole body sufficiency of iodine requires a daily intake of 12.5 mg of iodine. The body contains about 50 mg of iodine with 70-80% of total body iodine stored in the thyroid gland. According to the Iodine Project, whole body sufficiency exists when individuals excrete 90% of the iodine ingested (Abraham, 2004). Based on this hypothesis, a loading test was developed wherein individuals consume 50 mg of iodine and urine excreted in the next 24 hours is assessed for iodine content with the expectation that individuals would excrete at least 90% of the 50 mg dose. However, a majority of the individuals tested retained a substantial amount of iodine, with many of them requiring high doses of iodine intake for several months before they excreted over 90% of the loading dose of iodine (Miller, 2006). Thousands of patients in this project reported iodine intakes at more than 12.5 mg/d with their thyroid function remaining unchanged (Abraham & Brownstein, 2005; Abraham, Brownstein, & Flechas, 2005). This range of daily intake of iodine was labeled orthoiodinesupplementation, and subjects reported subjective benefits such as improved overall well-being, increased energy, and improvement in bowel movements. Overweight and obese subjects showed weight loss, decreased body fat and increased muscle mass (Abraham et al., 2005) This relatively newer method of assessing iodine status in addition to measuring UI excretion and iodine saturation, also measures the activity of the sodium iodide symporter (NIS)

to rule out functional defects in the NIS which may lead to decreased iodine uptake by the thyroid gland despite adequate dietary iodine intake (Abraham et al., 2005).

Significance of Iodine in Reproductive-age Women

Women of childbearing age, 15-45 years, are at the highest risk for iodine deficiency. Adequate intake during pregnancy and lactation is especially critical to the healthy brain development of the fetus (Hetzel et al., 2004). Iodine deficiency is the leading and most preventable cause of mental retardation in the world (Bleichtodt & Born, 1994). Iodine requirements increase >50% during pregnancy to support trans-placental transport of thyroid hormones to the fetus, alterations in maternal iodine metabolism, and higher maternal urinary loss due to increased renal clearance (Lazarus, 2011; Yarrington & Pearce, 2011). During a healthy pregnancy, the increase in plasma volume and plasma concentration of thyroxine-binding globulin (TBG) results in a several-fold increase in the T_4 pool (Glinoer, 2006a). Increased renal blood flow and glomerular filtration lead to an increased iodine clearance, resulting in decreased plasma iodide and an increased requirement for iodine intake (Burrow, 1993; Glinoer, 2006b). Women who are iodine-sufficient at conception have natural intra-thyroidal reserves to support the increased requirements during pregnancy (Liberman, Pino, Fang, Braverman, & Emerson, 1998). If iodine intake decreases and the rate of iodine loss exceeds intake, iodine deficiency occurs, therefore making it imperative to maintain appropriate iodine levels by supplementing iodine either from the diet or supplementation before and during pregnancy, and also lactation (Leung, Braverman, & Pearce, 2012). During pregnancy, while physiological adaptations provide the necessary increases in thyroid hormone production when iodine intake is sufficient, this is replaced by pathological alterations when iodine intake is insufficient, thus revealing the underlying deficits (Soldin & Soldin, 2009).

When maternal iodine deficiency occurs in the first trimester, it can lead to irreversible neurological complications and mental retardation in the offspring due to the critical period of organogenesis that occurs before many women know they are pregnant (Gahche, Bailey, Mirel, & Dwyer, 2013). Maternal hypothyroxinemia due to iodine deficiency may result not only in the birth of children with neurological cretinism but also in decreased mental and psychomotor development in the remainder of the population not exhibiting cretinism (Bleichtodt & Born, 1994).

A Boston study reported that 49% of pregnant and lactating women consumed iodine below the RDA of 150µg/d (Pearce et al., 2004). NHANES data demonstrates that 36% of women (2.2 million U.S. women) have low UI levels (<100 µg/L) because of low or deficient iodine intakes (Blount et al., 2006). From NHANES 2009- 2010, median UI for all females was 134 µg/L, and for women of childbearing age was 124 µg/L (Caldwell et al., 2013), indicating significantly low median UI concentrations in this vulnerable population. The U.S. median UI decreased from 320 µg/L in 1974 to 160 µg/L in 2002, suggesting a 50% reduction in dietary intake (Caldwell et al., 2005). According to the 2013 ICCIDD scorecard, 17% of the U.S. population is iodine deficient, with a median urinary iodine concentration 144 µg/L.

American and European Thyroid Associations recommend that women of reproductive-age, and those planning to become pregnant, consume at least 220 µg/d of iodine either from the diet or in the form of iodine supplements (Becker et al., 2006). Iodine supplements are considered safe for consumption with adverse metabolic effects primarily reported in patients with organification defects (e.g., Hashimoto's thyroiditis) in which severe hypothyroidism ensues (Braverman, Ingbar, Vagenakis, Adams, & Maloof, 1971). From NHANES 2006, although 77% of pregnant women reported taking one or more dietary supplements, only 22% were taking an

iodine-containing dietary supplement (De Leo, Pearce, & Braverman, 2017). Among reproductive-age women who reported using supplements with iodine, mean daily iodine intake was 107 μg/d, which was significantly below the recommended RDA of 220 μg/d during pregnancy (Gahche et al., 2013; Pessah-Pollack, Eschler, Pozharny, & Davies, 2014).

Awareness of the importance of adequate iodine nutrition, particularly during pregnancy and lactation, among the U.S. public is lacking (Leung, Braverman, & Pearce, 2007) indicating alarm for a significant public health concern that needs to be addressed.

Thyroid Dysfunction

Environmental iodine deficiency is the most common cause of hypothyroidism on a worldwide basis (Andersson, de Benoist, Delange, & Zupan, 2007). However, iodine status screening is not routinely undertaken in clinical practice when screening for thyroid disease. According to NHANES, the U.S. is considered to be an iodine-replete nation (Andersson, de Benoist, & Rogers, 2010). Although iodine intake may be decreasing, iodine insufficiency is not considered to be a cause of goiter or thyroid dysfunction in the U.S. (Fein & Cooper, 2009).

Suppressed serum TSH concentrations characterize hyperthyroidism, typically < 0.1 mIU/L, whereas hypothyroidism is defined as reduced T_4 and T_3, and elevated TSH secretion (>4.0 mIU/L). Prevalence of hypothyroidism in a reference U.S. population is 4.6% as determined from serum TSH (mean 1.4 mIU/L), and T_4 (112.3 nmol/L). Prevalence increases with age, from 4% of women and 3% of men at age 18-24 years to 21% of women and 16% of men over 74 years of age (Evered, Ormston, Smith, Hall, & Bird, 1973). Subclinical hypothyroidism is defined as elevated TSH (4.0–10.0 mIU/L), with T_3 and T_4 levels within reasonable limits. It is found in about 7.5% of females (Staub et al., 1992) with rates as high as 24% among women older than sixty years (Canaris, Manowitz, Mayor, & Ridgway, 2000).

Females with sub-clinical hypothyroidism have a 15% lifetime risk of developing overt hypothyroidism, therefore making it necessary to screen these individuals for the presence of thyroid dysfunction. A large number of people in the U.S. unknowingly have hypothyroidism and should be screened (Hollowell et al., 2002).

The presence of circulating antibodies against thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) is frequent in people with thyroiditis, especially Hashimoto's autoimmune disease. The association between iodine intake and presence of thyroid antibodies is complex and not fully understood (Kahaly, Dienes, Beyer, & Hommel, 1998). Populations, where goiter is common due to iodine deficiency, may have increased concentrations of antibodies in circulation (Pedersen et al., 2003). A sudden increase in iodine intake in an iodine deficient population may induce enhanced thyroid autoimmunity, but this may be a transient phenomenon, especially if they are already selenium deficient (Zimmermann, Moretti, Chaouki, & Torresani, 2003). Individuals with thyroid antibodies are at a higher risk of developing thyroid dysfunction when iodine intake is high and should monitor their iodine intakes (Li et al., 2008).

Given the association between iodine deficiency, thyroid dysfunction, and the high prevalence of thyroid disease in the female population, including iodine status assessments as a part of routine screening or evaluation of thyroid function may help identify individuals at risk for thyroid disease.

Iodine Excess

Excess iodine intake can be challenging to define. The recommended tolerable safe upper limit for iodine is 1100 µg/d (IOM, 2001). The Japanese have been reported to consume an average of 5.2-14.5 g/d of seaweed providing at least 45 mg/d of iodine (Abraham & Brownstein, 2005). Average iodine consumption, from all sources, in the Japanese population is 1200-5280

µg/d, whereas it is 209 µg/d in the U.S. (Teas, Pino, Critchley, & Braverman, 2004; Zava & Zava, 2011). Many individuals regularly consume high doses of iodine (10-200 mg/d) without apparent adverse effects. Conventional sources of high doses of iodine are medications such as amiodarone, which contains 75 mg iodine/200 mg capsule, kelp, and iodine-containing dyes used in radiologic procedures; generally these high intakes of iodine have been shown to be well tolerated (Luo et al., 2014). Excess salt consumption has never been documented to be responsible for excess iodine intake. Individuals without evidence of underlying thyroid disease almost always remain euthyroid even with excess iodine consumption (Braverman, 1993).

Wolff and Chaikoff (1948) reported that rats given large amounts of iodine intra-peritoneally showed an inhibition of thyroid hormone synthesis resulting from increased intra-thyroidal iodine stores; this phenomenon was defined as the Wolff-Chaikoff effect. They next demonstrated that this inhibitory effect of excess iodide was transient, lasting from 26–50 h, and that the thyroid escaped or adapted to prolonged iodide excess, resuming near-normal hormone synthesis which was labeled the escape from or adaptation to Wolff-Chaikoff effect (Wolff, Chaikoff, Goldberg, & Meier, 1949). This adaptation to the acute Wolff-Chaikoff effect was postulated to have been caused by an abrupt decrease in iodide transport into the thyroid, which reduced the intra-thyroidal iodide concentrations (Braverman & Ingbar, 1963). Failure to escape from the acute Wolff-Chaikoff effect may result in iodine-induced hypothyroidism, which may be transient or permanent in individuals with predisposing risk factors (Leung & Braverman, 2012).

Certain health conditions and risk factors predispose individuals to iodine-induced thyroid dysfunction. Individuals with underlying thyroid disease such as Grave's disease and Hashimoto's thyroiditis, euthyroid individuals with a history of subacute thyroiditis, post-partum thyroiditis, postpartum hemithyroidectomy, type-2 amiodarone-induced thyroiditis, history of

interferon α therapy, elderly individuals with sub-clinical hypothyroidism, patients with certain non-thyroidal diseases such as cystic fibrosis, patients taking amiodarone for ventricular tachyarrhythmia, patients taking lithium for bipolar disorder, and individuals with a family history of goiter or thyroiditis are all considered to be at risk for the development of iodine-induced hypothyroidism or hyperthyroidism either due to the possible excessive consumption of iodine or due to the presence of risk factors for thyroid dysfunction (Backer & Hollowell, 2000; Torremante & Rosner, 2011; Venturi et al, 1993). Such individuals must be excluded from studies looking into the effects of iodine supplementation.

Hypothyroidism may arise from iodine-induced inhibition of thyroid hormone synthesis or the development of thyroid auto-antibodies (Leung & Braverman, 2012). Iodine-induced thyroiditis has been observed in autoimmune-prone animals and is characterized by lymphocytic infiltrations in the thyroid, and increased cytokine secretion. Thyroid autoantibody titers are significantly upregulated along with the progression of lymphocytic infiltration (Xue et al., 2011). Iodine induces cytokine and chemokine-mediated lymphocytic infiltration in the thyroids of autoimmune-prone individuals, which is critical for the generation of thyroid autoantibodies and thyroiditis. Excess iodine-induced oxidative cell injury has been proposed as a potential trigger for this lymphocytic response. Several underlying mechanisms may explain its action: redundant reactive oxygen species (ROS) generated during trapping, oxidation, and organification of excessive iodine in thyroid follicular cells, due to a defect in the iodine processing machinery, can lead to elevated oxidative stress resulting in oxidative cell damage. This damage may stimulate thyrocytes to produce and secrete cytokines and chemokines, thus recruiting lymphocytes to the thyroid, where they meet major thyroid auto-antigens, including thyroglobulin (Kawashima, Tanigawa, Akama, Wu et al., 2011). Modification by excessive iodine may alter the conformation of the thyroglobulin molecule to facilitate its antigen presentation by antigen presenting cells.

Thus, excessive iodine may eventually lead to pathological intolerance to thyroid auto-antigens and the development of thyroiditis (Kawashima, Tanigawa, Akama, Yoshihara et al., 2011; Kawashima et al., 2013). However, these phenomena are more likely to occur in individuals with underlying thyroid dysfunction, rather than euthyroid individuals with no underlying thyroid pathology. Certain individuals, not all, with underlying or evident pathologies such as multinodular goiter or thyroiditis can develop hyper or hypothyroidism if they are exposed to high doses of iodine more than 1.5 mg/day (Bürgi, 2010). More recent studies reported that iodine supplements at low (1.5-8 mg/day) and intermediate doses (10-32 mg/day), ingested from a variety of sources such as potassium iodide supplements, kelp, and seaweed supplements, are well- tolerated in euthyroid subjects, maintaining levels of thyroid hormones T_3, T_4 and TSH within reasonable limits. Only very high doses (>30 mg/day) may generate hypothyroidism and goiter, which rapidly revert to normal with discontinuation of high dose iodine supplement. (Leung & Braverman, 2014; Michikawa et al., 2012; Rhee, Braverman, Pino, He, & Pearce, 2011). Therefore, excess iodine consumption through diet or supplementation is generally considered safe in euthyroid individuals without underlying thyroid pathology.

Thyroid Hormones in Energy Metabolism

Thyroid hormones play an essential role in regulating energy homeostasis by modifying the basal metabolic rate and by contributing to both obligatory and adaptive thermogenesis (Silva, 2003). The role of thyroid hormones in energy homeostasis is exemplified in patients with thyroid dysfunction. More than 85% of patients with thyrotoxicosis show weight loss despite markedly elevated energy intakes.

Hypothyroidism, on the other hand, is associated with decreased basal metabolic rate and weight gain despite reduced food intake (López, Alvarez, Nogueiras, & Diéguez, 2013). Adipose

tissue contains receptors for TSH and the thyroid hormones, T_4, and T_3. T_3 is a thermogenic hormone that increases energy expenditure (Silvestri, Schiavo, Lombardi, & Goglia, 2005).

Hyperthyroidism is characterized by suppressed serum TSH concentrations and elevated basal metabolism. Patients with hyperthyroidism or thyrotoxicosis show marked decrease in weight due to an increase in concentrations of free and bound T_3, leading to a depletion of fat and muscle mass (Duntas, 2001, 2002). Thyroid gland dysfunction affects both lipid and carbohydrate metabolism, with hypothyroidism causing weight gain due to alterations in body composition due to increased fat deposition (Kim, Tull, Talbott, Vogt, & Kuller, 2002). Typically the metabolic effects of thyroid hormones have been connected to their direct actions on metabolically active tissues, such as liver, adipose tissue, skeletal muscle and heart (Silva, 2006; Warner & Mittag, 2012). Thyroid hormones are involved in lipogenesis and lipolysis that are possibly mediated by affecting local norepinephrine levels and adrenergic post-receptor signaling (Nyrnes et al., 2006).

Thyroid Hormones and Body Composition

Many studies have looked at the association between serum TSH, thyroid hormones, and changes in BMI. A study that included 401 participants (361 females) with the presence of a thyroid nodule or goiter found no evidence for an association between thyroid status and BMI in euthyroid subjects. There was no difference in BMI when participants were categorized according to serum TSH or free T_4 (Manji et al., 2006). There was also no difference in serum TSH or free T_4 between lean and obese euthyroid subjects. A six-month study in collegiate rowers showed a direct correlation between thyroid volume, body weight, BMI, and lean body mass. Thyroid volume was directly regulated by amounts of TSH and iodine from the diet (Wesche & Wiersinga, 2001). It is possible that obese patients have a central thyrostat reset, which makes

them less sensitive to circulating thyroid hormones (Michalaki et al., 2006). A positive correlation between BMI and serum TSH and a negative association between BMI and free T_4 levels was observed in The Dan Thyr Study (Laurberg et al., 2006). A population-based study in Norway reported an association between BMI and serum TSH in non-smokers (Nyrnes et al., 2006). Serum TSH concentration is associated with BMI in obese euthyroid subjects. A positive correlation between weight gain during five years and a progressive increase of serum TSH has been found (Knudsen et al., 2005). Postulated causes for increased TSH concentration in obese individuals include subclinical hypothyroidism resulting from iodine deficiency or autoimmune thyroiditis, thyroid hormone resistance, derangement of the hypothalamic-pituitary axis, or an adaptation process to increase energy expenditure (Reinehr, 2010).

Thyroid Hormones and Resting Energy Expenditure

Overt hypothyroidism is associated with decreased resting energy expenditure (REE) and weight gain, whereas hyperthyroidism is associated with increased REE and weight loss. (de Moura Souza & Sichieri, 2011; Mansourian, 2010). REE decreased in obese patients with subclinical hypothyroidism with TSH > 6 mIU/L, but no association was observed between REE and TSH in euthyroid obese subjects (Tagliaferi et al., 2001). Thyroid hormones regulate hepatic gluconeogenesis and hyperglycemia is common among individuals with overt hypothyroidism (Crunkhorn & Patti, 2008). In a group of weight stable but post-obese women, RMR was significantly lower than a group of never obese women matched for age, fat mass, and fat-free mass. The post-obese group had significantly lower free T_3 concentrations (Astrup et al., 1996). Since thyroid hormones regulate REE through many metabolic pathways, any process that results in thyroid gland dysfunction may change body weight, and metabolic rate resulting in changes in body composition due to altered metabolism of macronutrients. There is a lack of consensus of

association between thyroid hormones, BMI and REE. The difference in the results could be due to variations in assessments, a cross-sectional sampling of subjects, the presence of active thyroid disease, and dietary factors such as iodine intake and energy intake that may impact thyroid function.

Other factors such as caloric intake and physical activity also influence BMI and need to be considered in conjunction with iodine metabolism and thyroid function. Total energy intake has an impact on BMI. When energy intake consistently exceeds the body's need for energy, excess energy is converted to fat and stored, resulting in weight gain (Whitney & Rolfes, 2005). Energy intakes in the U.S. have increased over the past several decades (Bauman & Crawford, 2003). The Economic Research Service of United States Department of Agriculture (USDA) reports that Americans consumed an average of 2,234 calories per person per day in 1970 and 2,757 calories in 2003, after adjusting for plate waste, spoilage, and other food losses. This shows an increase of 523 calories/day or 16% higher intake (Farah & Buzby, 2005). Energy intake is a significant contributor to increased BMI. Even though adjusted mean energy intake increased from 2003, they have since declined to 2195 kcal/d during NHANES 2009–2010. Significant decreases in energy intake from 1999 to 2010 were noted for participants aged 20–39 y, men, women, and participants with a BMI of 18.5 to <25 and ≥30 kg/m^2 (Ford & Dietz, 2013).

Physical activity also has an impact on BMI. The 2008 Physical Activity Guidelines, state that adults need at least 150 minutes (2.5 hrs) a week of moderate-intensity, or 75 minutes (1.15 hrs) a week of vigorous-intensity aerobic physical activity, or an equivalent combination of moderate- and vigorous-intensity aerobic activity plus two days a week of muscle-strengthening activities for all muscle groups. According to the American College of Sports Medicine (ACSM) guidelines regular physical activity plays a vital role in the maintenance of body weight and body

composition. ACSM exercise recommendations can be met through 30-60 minutes of moderate-intensity exercise (5 d/wk) or 20-60 minutes of vigorous- intensity exercise (3 d/wk) (Garber et al., 2011). Adults who are unable or unwilling to meet the exercise targets can still benefit from engaging in amounts of exercise less than recommended. In addition to exercising regularly, there are health benefits in concurrently reducing total time engaged in sedentary pursuits and also by interspersing frequent, short bouts of standing and physical activity between periods of sedentary activity, even in physically active adults (Ainsworth et al., 1993). Based on the National Health Interview Survey, 2015, conducted by the Centers for Disease Control and Prevention (CDC), 46% of U.S. adults met the 2008 federal guidelines for aerobic activity, and less than 20% met the guidelines for both aerobic and muscle-strengthening activities (Leisure-time physical activity, 2016). Decreased levels of physical activity are consistently associated with weight gain and changes in body composition favoring increased fat mass vs. lean mass.

This review of the literature has looked at iodine, its role in thyroid function, thyroid hormone insufficiency, and the association between thyroid hormones, body composition, and energy metabolism. This review has also looked at the current status of iodine nutrition among reproductive-age women in the U.S. Thus far, no studies have looked at an association between high dose iodine supplementation and assessment of iodine status in conjunction with thyroid function, resting metabolic rate and body composition among healthy, euthyroid, premenopausal women in the reproductive age group. It is possible that a simple iodine supplement provided to vulnerable populations, such as reproductive-age women, could improve iodine nutrition status resulting in correction of thyroid dysfunction, increased basal metabolism, and improved body composition.

CHAPTER III

METHODS

Informed Consent

This study was approved by the Institutional Review Board (IRB) at Texas Woman's University. See Appendix A for copies of IRB approval of the study. The contents of the Informed Consent form approved by the IRB were explained to all participants and signed forms were obtained before starting any screening or assessments for the study. See Appendix B for copies of the Informed Consent form approved by the IRB.

Research Design

The study followed a randomized, double-blind, placebo-controlled type of study design. Participants were randomized into either an experimental (iodine) group (IG) or control (placebo) group (PG). Researchers and participants were blinded to the experimental and control groups until all data collection was complete and samples analyzed. Study duration was six months.

Recruitment

An effort was made to recruit participants from the TWU campus community and the broader Denton community. Methods of recruitment included posting flyers approved by TWU's Center for Student Development on campus bulletin boards; advertisements for the study were printed in the TWU campus newspaper, "The Lasso" and the community newspaper, "The Denton Record-Chronicle." Recruitment advertisements were also disseminated through campus-wide emails to the entire Denton and Dallas faculty, students, and staff population. Announcements were posted on Blackboard in select courses and the Pioneer Portal home page

for faculty, students, and staff. Houston students and staff were excluded from the campus-wide emails to avoid scheduling difficulties. Also, local, primarily family practice physicians were contacted and asked to share the flyer with eligible, interested patients. Local gyms, clubs, and community organizations were also contacted and asked to share information about the study with their members. Instructors at the University of North Texas (UNT) were contacted as well and asked to share information about the study with their students. See Appendix C for a copy of the recruitment flyer, Appendix D for Email script and Pioneer Portal Announcement, and Appendix E for a copy of the study recruitment news article in the Denton Record-Chronicle.

Participants

This research study recruited women, 18 – 45 years old, as this group has been shown to have a propensity for iodine deficiency during the reproductive years (Hollowell & Haddow, 2007). Interested potential participants contacted the primary researcher by phone or e-mail. If the first contact was by phone, the researcher requested the potential participant's e-mail address to send information on study criteria and how to determine study eligibility. Researchers contacted interested potential participants to set a time to meet with the participant on campus, explain and sign the consent form, and complete the screening process. Following the completion of informed consent, participants were screened to see if they met study criteria. Only participants without significant medical histories were allowed to participate. After the screening was complete, participants were assigned a time to go to Clinical Pathological Labs (CPL) in Denton for baseline screening of thyroid function. Participants that cleared the baseline thyroid screening at CPL were contacted to schedule a time to come to the Institute for Women's Health (IWH), Pioneer Performance Clinic, TWU Denton, to collect the iodine loading kit and to be scheduled for baseline assessments. See Appendix F for a sample of the scripts for contact with the

researcher and Appendix G for a copy of the study screening checklist sent to potential new participants.

Responses were obtained from 483 individuals to the recruitment emails, posters, and print ad; 80 of these individuals did not qualify for the study based on the inclusion-exclusion criteria. Inclusion criteria included female, 18 – 45 years of age, serum TSH concentration between 0.5 – 4.0 mIU/mL, and generally healthy.

Exclusion criteria included males, females < 18 or > 45 years of age, abnormal serum TSH - <0.5 or > 4.0 mIU/mL, elevated antibodies for TPO (> 10 IU/mL), diagnosed hypothyroidism, hyperthyroidism, thyroiditis or Hashimoto's disease, previous history of iodine contamination, treatment with drugs known to alter thyroid function (ex: lithium, amiodarone), smoking within 1 year of study, pregnant or desiring to become pregnant during the study, self-reported history of chronic conditions such as arrhythmias, congestive heart failure, chronic obstructive pulmonary disease, liver disease, kidney disease, hypertension, hypercholesterolemia, diabetes, cancer, or metabolic disease.

See Appendix H for a copy of the inclusion-exclusion checklist provided to participants. Post inclusion-exclusion screening, 142 healthy female participants between the ages of 18 and 45 years were recruited from the Dallas Fort Worth Metropolis area, the majority of them being from the Texas Woman's University campus community. These individuals were determined eligible to participate in the study if they qualified based on the clinical assessment of thyroid function at CPL, Denton.

Baseline Inclusion Screening for Thyroid Function

Baseline screening for thyroid function was conducted at CPL, Denton. See Appendix I for a copy of the requisition form provided to participants to bring to CPL. All individuals who showed abnormal serum TSH concentrations, < 0.5 mIU/mL or > 4.0 mIU/mL, and/or had elevated serum TPOAb (> 10 IU/mL) were excluded from the study. These individuals were recommended to follow up with their primary care physician for evaluation of thyroid function. The screening process helped to exclude participants with previously undiagnosed thyroid dysfunction. Participants who had normal serum TSH concentrations (0.5- 4.0 mIU/mL) and had normal serum TPOAb (<10 IU/mL) satisfied the inclusion criteria. Of the 142 participants who were given requisition forms to be taken to any of the four CPL location in Denton, seven individuals had abnormal serum TPOAb levels, and one had low serum TSH level and were excluded from the study. 134 individuals qualified to participate in the study. Of this number, 103 volunteered to participate in the study and were referred to the TWU IWH Pioneer Performance Clinic for baseline assessments.

Assessments

All of the assessments detailed in the following sections were conducted at TWU IWH Pioneer Performance Clinic at the beginning and at the completion of the study at six months. The assessments conducted were anthropometric measurements, iodine status determination, thyroid function testing, body composition evaluation, resting metabolic rate determination, the completion of health, demographic and physical activity questionnaires, and a three-day dietary record. The methods used for all baseline and final assessments are described as follows.

Anthropometric Measurements

Participants reported to the IWH Pioneer Performance Clinic for anthropometric measurements. All anthropometric measurements were collected in triplicate, and participants wore light clothing without shoes for measurements. A portable stadiometer was used to measure height to the nearest one-quarter inch, and a beam scale was used to measure weight to the nearest 0.5 pound. Height and weight measurements were converted to centimeters and kilograms, and BMI was calculated in kg/m^2. Height, weight, and BMI were also measured in the same manner at study completion, at six months.

Iodine Status Determination

Iodine status determination included testing for 24-hr Urinary Iodine (24-hr UI) to determine current iodine status using a test developed by Optimox® Potentiometric R&D Lab and administered by Flechas Family Practice (FFP) Labs, North Carolina (Abraham, Flechas, & Hakala, 2002). Participants were instructed to consume a 50 mg loading dose of iodine (Iodoral®, Optimox® Corp.) a stable preparation of Lugol's solution containing KI and I^{2-} after their first-morning urine void. They were then instructed to collect all urine for 24 hours immediately following the iodine load. A 3-liter sterile container was provided to collect the 24-hr urine sample. Instructions were also provided via handouts to help participants with the urine collection at home. See Appendix J for a copy of the iodine loading regimen and 24-hr urine collection steps outlined by FFP labs.

Iodine status was determined by the percentage of the 50 mg iodine load that was excreted in the 24-hr sample. Individuals with iodine deficiency retained iodine and excreted lower amounts of urinary iodine than individuals who were iodine sufficient. An individual who

excreted >44 mg, which is more than 90% of the original 50 mg dose, was considered to be iodine sufficient. The 24-hr urine sample was also used to assess percent iodide saturation. Percent iodine saturation > 90% indicated iodine sufficiency (Abraham, 2004; Abraham et al., 2002; Abraham et al., 2005).

Sodium iodide symporter (NIS) function was also determined 24-hrs following the intake of a 50 mg loading dose of iodine. 10 ml of fasting blood was collected along with a 5 ml saliva sample. Serum and saliva samples were collected at the IWH Pioneer Performance Clinic and shipped to FFP Labs for testing. Iodide levels in serum and saliva were measured using an ion sensitive electrode technique. Saliva/serum iodide ratio was used as an index of the efficiency of the NIS transporter system. A ratio of 28-74 was considered normal, and <28 or >74 indicated a malfunctioning NIS (Abraham et al., 2005). NIS symporter estimation assisted in the evaluation of functional iodine deficiency of the thyroid.

Once all samples were collected, they were packaged into temperature sensitive storage containers and mailed via FedEx or U.S. Postal Services (USPS) to FFP Labs where all laboratory testing was completed for 24-hr urine, serum/saliva iodide, and NIS estimations. A pre-paid requisition form was included in the package (See Appendix K), and all results were faxed to the IWH Pioneer Performance Clinic. All iodine status assessments including 24-hr UI, % IS, NIS, and serum and saliva iodide were completed at baseline and six months.

Dual-Energy X-Ray Absorptiometry Scan

General Electric (GE)'s Lunar Prodigy (Madison, WI) Dual-Energy X-Ray Absorptiometry (DXA) system was used for the assessment of body composition. Participants were instructed to wear light clothing and remove all metal items such as jewelry before starting

the scan. The participant's name, date of birth, sex, and measured height and weight were entered into the DXA software. A certified DXA operator instructed the participant to lie supine according to guidelines on the DXA table with palms facing down next to the participant's hips, and feet strapped together. The 8-10 minute scan occurred without any bodily movement. DXA reports for the total body scan included evaluation of percent body fat, fat mass, lean mass, visceral adipose tissue (VAT) mass and VAT volume, android fat, gynoid fat, and android-gynoid (A-G) ratio (Martin et al., 2007). DXA assessments were conducted at the beginning of the study to establish a baseline and again six months later.

Resting Metabolic Rate

RMR was determined using a ventilated canopy-system and a Parvo Medics metabolic cart (TrueOne) calibrated according to the manufacturer's specifications. Participants reported to the IWH Pioneer Performance Clinic between 6:00 and 8:00 am after fasting for a minimum of 12 hours. They were instructed to not participate in any physical activity for a minimum of 48 hr before their assessment (Poehlman et al., 1989). Participants rested in a supine position in a darkened, quiet room for 30 minutes under the ventilated canopy. Values obtained for the last 10 minutes were averaged, and RMR was calculated according to Potteiger, Kirk, Jacobsen, and Donnelly (2008). RMR assessment was conducted at zero and six months.

Physical Activity Questionnaire

Physical activity was assessed using a validated physical activity questionnaire (Kohl, Blair, Paffenbarger, Macera, & Kronenfeld, 1988). See Appendix L for a copy of the physical activity questionnaire completed by all participants. Participants were strictly instructed to not change their physical activity levels for the duration of the study. Physical activity questionnaires

were completed by participants at initiation and completion of the study. Participants answered a series of questions based on their current physical activity and exercise habits performed regularly, at least once a week. These answers were then scored based on the guidelines outlined in the 2011 Adult Compendium of Physical Activities. Each task that an individual completed was assigned a Metabolic Equivalent Score (MET), defined as the ratio of work metabolic rate to the resting metabolic rate. METs reflect the energy cost of physical activities. One MET is defined as 1 Kcal/kg/hour and is roughly equivalent to the energy cost of sitting quietly. Alternatively, a MET is also defined as oxygen uptake in ml/kg/min with one MET equal to the cost of sitting quietly, equivalent to 3.5 ml/kg/min, which the Compendium considers a proxy value for RMR of 1 MET. Adult Compendium activities were classified by a five-digit code that identified the category (heading) as the first two digits and the type (description) of activity as the last three digits. MET values have been validated for use in surveillance and epidemiologic research and in settings where data are compared between groups. A list of all the physical activity categories included in the questionnaire and their scoring system based on the 2011 Compendium is provided in Appendix M.

MET scores for each activity were multiplied by the number of sessions per week, and minutes per session, resulting in a value for MET-minutes per week. The MET-minutes per week for each activity were calculated similarly; adding all MET-minutes per week provided values for total MET-minutes for all activities, which was then reported as MET calories.

Nutritional Assessment

Nutritional assessment was conducted by analyzing three-day dietary records. See Appendix N for a copy of the three-day dietary record provided to participants. Participants received nutrition education on the day of enrollment into the study, and were were instructed not

to alter daily eating and living patterns during the study. Participants were instructed how to assess food intake and make entries on the food record three days before the visit. They were instructed to include one weekend and two weekdays on their dietary record to account for a change in eating patterns during the weekends. Three-day dietary records were analyzed using Axxya Systems Nutritionist Pro™ (Stafford, TX). The three-day dietary records analyzed by Axxya Systems Nutritionist Pro™ measured caloric, macronutrient, and micronutrient intake. A sample dietary analysis report provided by the Axxya Systems Nutritionist Pro™ is attached in Appendix O. The software reported dietary analysis of energy (Kcals), macronutrients including total carbohydrates (g), proteins (g) and fat (g) content, and micronutrients including vitamins such as A (IU), C (mg), D (IU), folate (µg), iron (mg), zinc (mg), selenium (µg), and most importantly, dietary iodine(µg) intake. Each of these vitamins and minerals contributes to optimal iodine metabolism and thyroid function (Hess, 2010; Kazi et al., 2010; Thilly et al., 1992; Zimmermann, 2007; Zimmermann, 2009). All participants completed three-day dietary records at study initiation and completion.

Health and Demographic Questionnaires

All participants also completed general health history and demographic questionnaires. See Appendix P for a copy of the general health history questionnaire and Appendix Q for a copy of the demographic questionnaire completed by study participants.

Thyroid Function Evaluation

Fasting blood was obtained from an antecubital vein at the end of initial and final visits and collected into Becton Dickinson Serum Separator Tube (BD SST™) Vacutainer® at the IWH Pioneer Performance Clinic. Blood was then allowed to clot for 30 minutes and centrifuged at

3600 rpm for 15 minutes. Sera were separated into 0.5 ml aliquots and stored at -80 degree C until assayed. Serum concentrations of TSH, free T_4, and free T_3 were assessed using ultrasensitive Enzyme Linked Immuno- Sorbent Assay (ELISA) (ALPCO Diagnostics ™ Salem, NH) tests based on the principle of solid phase immuno-sorbent assay. See Appendix R for the methods used for the assessment of serum TSH concentration, Appendix S for the methods used for serum T_3 concentration, and Appendix T for the methods used for the assessment of serum T_4 concentrations.

Intervention

Iodine supplementation in the form of Iodoral®, 12.5 mg (Optimox®) pills, was provided to participants in the IG. 12.5 mg Iodoral® provides 5 mg iodine and 7.5 mg iodide as a potassium salt. This is equivalent to two drops of Lugol's iodine, which is the amount of iodine required per day to achieve whole body sufficiency; levels up to 50 mg/d iodine have been shown to have no significant adverse effects (Abraham, 2004). See Appendix U for an information sheet on Iodoral® /supplement provided to participants in the IG. It is for this reason that this specific dosage was chosen for consumption each day by participants in the IG. Placebo in the form of glucose tablets (5g glucose) was provided to the participants in the PG. See Appendix V for an information sheet on the glucose tablets provided to participants in the PG. Participants in both groups also received an Equate Brand multivitamin/mineral supplement along with either the iodine or placebo, for the duration of the study. The multivitamin supplement provided 150 µg/d, or the RDA for iodine, along with the RDA for other essential vitamins such as vitamins A, C, and D, and minerals such as iron, and selenium, zinc, and folate, among others. See Appendix W for the information sheet on Equate supplements. Supplements (12.5 mg Iodoral®) or placebo

(glucose) and the generic multivitamin supplement were to be consumed daily, preferably in the morning for the duration of the study, six months.

Supplement Delivery

Individuals that satisfied all inclusion criteria and had cleared the CPL thyroid screen were referred to the IWH Pioneer Performance Clinic where they completed baseline assessments for the study. After completing all baseline assessments, they were randomized to either a supplement or placebo group by a third party, who provided their packet of iodine or placebo and multivitamin supplements. Participants and researchers were blinded to which group the participants had been assigned. Once each month, additional supplements, placebo or iodine were provided to the participants at the IWH Pioneer Performance Clinic. Monthly visits continued for the duration of the study. Participants were required to bring in any leftover pills to these monthly visits to evaluate study compliance.

All participants who completed the study received signed a study completion consent form and received a $25.00 Walmart gift card at the end of the final visit. See Appendix X for a copy of the study completion consent form.

Timeline

A timeline for the assessments was provided to all participants at the beginning of the study, and participants were expected to complete the prescribed treatment as indicated and report for supplement delivery and data collection. See Appendix Y for a copy of the timeline handed to study participants.

Step 1: Recruitment of participants into the study

Step 2: Informed consent

Step 3: Screening for inclusion or exclusion

Step 4: Baseline screening for thyroid function at CPL Labs

Step 5: At first visit to the IWH Clinic, baseline assessments included- completion of health and demographic questionnaires, physical activity questionnaire, three-day dietary record, DXA, RMR testing, blood sample collection for thyroid function, and iodine status determination

Step 6: Randomization into IG or PG and supplement delivery

Step 7: Monthly visits for supplement pick-up, leftover pills drop-off for six months

Step 8: Final assessments at the IWH Clinic at six months included completion of health and demographic questionnaires, physical activity questionnaire, three-day dietary record, DXA, RMR testing, blood sample collection for thyroid function, and iodine status determination

Step 9: Study completion procedures, included signing completion forms, and collection of $25 Walmart gift card

Statistical Analyses

All statistical analyses were performed using IBM SPSS Statistics v23.0 software (IBM Corporation, Armonk), and all data are expressed as mean ± standard error of the mean (SEM). The significance level (alpha) for all statistical tests was set at $p \leq 0.05$ (Stevens, 2002). Based on power calculations, using G-Power© software, a minimum sample size of 62 was needed. This sample size was determined to be sufficient to detect differences between the groups, IG and PG. Descriptive statistics, including measures of central tendency for continuous variables and

frequencies for categorical variables were calculated. Preliminary analyses to determine the relationships between the demographic and dependent/independent variables included Pearson's Product Moment correlations for the continuous variables, χ^2 tests for the categorical variables, as well as independent t-tests and one-way analysis of variance (ANOVA) to test for differences between the levels of categorical variables on the continuous variables. Repeated measures ANOVAs were used to examine the outcome difference between IG and PG from baseline to six months. Significant effects were examined using pairwise comparisons. Non-parametric equivalent tests were conducted when basic assumption tests were not met for parametric testing. Non-parametric tests included Mann-Whitney U to compare changes between groups and Wilcoxon signed rank test to compare changes with time. A subgroup analysis was conducted by comparing individuals who had adequate iodine status to those who had deficient iodine status. Repeated measures ANOVA was conducted to compare the means of the two subgroups over time, and a two-way ANOVA was conducted to compare the main and interaction effects between supplement groups IG and PG, and matching groups, adequate versus deficient iodine, on the six month follow-up outcomes, and results were confirmed by non-parametric equivalents.

CHAPTER IV

AN ASSESSMENT OF IODINE STATUS OF REPRODUCTIVE-AGE WOMEN IN THE U.S.: IS THERE A NEED TO REVIEW THE RECOMMENDATIONS FOR DIETARY IODINE INTAKE?

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KEYWORDS

Iodine, iodine deficiency, iodine status, reproductive-age women, thyroid, urinary iodine

ABSTRACT

Iodine, an essential micronutrient, is required for the production of thyroid hormones. Iodine deficiency disorders (IDD) comprise a range of adverse maternal and fetal outcomes, with the most significant irreversible effect resulting from neurodevelopmental deficits in fetal brain caused by deficient iodine status during early pregnancy. Iodine deficiency is the leading cause of preventable intellectual impairment worldwide. Although the U.S. has been considered iodine sufficient, U.S. dietary iodine intakes have decreased drastically since the 1970s, with mild iodine deficiency reemerging in vulnerable population groups such as reproductive-age women. This review has looked at National Health and Nutrition Examination Surveys (NHANES) data and research studies examining iodine status among U.S. reproductive-age women. A majority of the data reviewed clearly demonstrates iodine deficiency in this population, indicating alarm for a public health concern needing immediate attention. The long-term socioeconomic impact of iodine deficiency in reproductive-age U.S. women cannot be overemphasized.

INTRODUCTION

Iodine, an essential micronutrient, is found in every tissue in the body. About 50% of iodine is heavily concentrated and utilized in skin and muscle. Thyroid, breast, ovaries, salivary glands, stomach, brain, pancreas, and thymus also have discernable concentrations of iodine. The thyroid is the primary storage site for iodine and may hold up to 50 mg alone (1). The only known function of iodine is its role in the production of thyroid hormones, although it also acts as an anti-oxidant, anti-inflammatory, apoptotic, antiviral, and antibacterial agent (2).

The thyroid hormones, thyroxine, T_4, and triiodothyronine, T_3, contain four and three molecules of iodine, respectively, and up to 65% of the weight of thyroxine molecules and 59%

of the weight of triiodothyronine. Dietary iodine is concentrated at the thyroid basal cell membrane by a sodium iodide symporter (NIS). Thyroglobulin (Tg) present in the gland is iodinated and the hormones, T_3 and T_4, are formed by the coupling of monoiodothyronine (MIT) and diiodothyronine (DIT) mediated by the enzyme, thyroid peroxidase (TPO). T_3 and T_4 are stored in the thyroid follicles until they are needed, at which time, thyroglobulin residues pass into lysosomes to release T_3 and T_4 into the circulation (3). Iodine deficiency leads to increased stimulation of thyrotropin-releasing hormone (TRH) from the hypothalamus, leading to increased TSH secretion by the pituitary gland, increased iodine uptake and turnover, decreased iodine excretion, and enhanced production of the active hormone, T_3.

Thyroid hormones play essential roles in regulating energy homeostasis by modifying basal metabolic rate and thermogenesis (4). A large number of people in the U.S. unknowingly have hypothyroidism and should be screened (5). Mild or subclinical hypothyroidism, defined as elevated TSH (4.0–10.0 mIU/L), with T_3 and T_4 levels within normal limits, is found in about 7.5% of females (6). Hypothyroidism causes weight gain and alterations in body composition with increased fat deposition. Adipose tissue contains receptors for TSH and the thyroid hormones, T_4, and T_3. Low thyroid function even within the clinically normal range may lead to obesity (7). Low levels of free T_4 or high levels of TSH in euthyroid individuals are associated with higher BMI (8, 9).

In the U.S., iodized table salt is the most common source of iodine in the diet. One gram of sodium chloride (NaCl) contains 74 µg of iodine (10). Less than half of one teaspoon of salt per day would meet an adult's needs for iodine. Iodine intake in the U.S. was considered to be sufficient since iodization of salt and other food products began in the 1920s. It is estimated that among Americans, 7-8% of sodium intake is contributed by table salt, but only 70% of consumers

choose iodized salt (11). Most salt ingestion is from processed foods that do not contain iodized salt. Commonly consumed foods and beverages have low iodine content, and only provide 3-80 µg iodine per serving. Seafood, such as fish, and seaweed, contain high iodine content because marine plants and animals concentrate iodine from seawater.

Other dietary sources of iodine in the U.S. are milk, meat, eggs, and bread (12). The diets of cattle and chickens are often supplemented with kelp, iodine-rich seaweed, which results in variable amounts of iodine in meats, milk, and eggs (13). Another source of iodine has been the use of iodophors in the dairy industry; iodophors are cleaning agents containing iodine used to sanitize the machinery in dairy operations and clean cow teats (14). They are used unevenly, and some of the iodine from teat dips is absorbed and found in milk and meat (15). There is little information whether iodophors are still widely used as there are other options for sanitizers including some that contain chlorine, an endocrine disruptor, and competitor of iodine. In the late 1950s, some bakeries were adding iodate to commercial bread mix as a dough conditioner or bread stabilizer (16), which provided a significant source of iodine in the average American diet. However, bread makers stopped using this iodide additive in the 1980s, possibly due to pressure exerted by health policymakers and the preference of brominated flour (17).

Historically, iodine deficiency in the U. S. was a matter of geographic location. Iodine is found in varying quantities in the oceans and on land masses. The "Goiter Belt" included states from the Pacific Northwest, the northern Rocky Mountains, to the Midwest, Great Lakes, and Appalachia. Areas prone to flooding and mountainous terrain are consistently iodine depleted. Food sources from these areas lack iodine, and therefore goiter was prevalent. Severe iodine deficiency manifests as a goiter. In the 1820's, J. F. Coindet, a Swiss physician, recognized iodine for its ability to reduce goiter size. By 1920, David Marine, M. D., from Cleveland, OH, in

the Goiter Belt region, brought iodine treatment to the forefront in the United States. Dr. Marine first studied iodine on animals and then on school girls with goiter. Sodium iodide supplementation among adolescent girls reduced thyroid enlargement, a result of iodine deficiency, from 21% to 0.2% (18). This study is what led to the iodization of salt in the United States. Goiters, a common problem in the early 20th century and the result of iodine deficiency, became a thing of the past with salt iodization. Salt iodization programs, including bread fortification, began as a result of the work of these men. Due to the iodization of salt in the 1920s, iodine deficiency and the symptoms associated with it were markedly reduced (19).

It is imperative to know how much iodine is necessary for optimal health to understand iodine sufficiency. The recommended dietary allowance (RDA) for iodine is determined by the Institute of Medicine's (IOM) Food and Nutrition Board of the U.S. National Academy of Sciences which in turn is derived from consensus statements from the International Council for Control of Iodine Deficiency Disorders (ICCIDD), the World Health Organization (WHO), and the United Nations International Children's Emergency Fund (UNICEF) (20). The recommended amounts are derived from the following: the calculated daily thyroid hormone turnover in euthyroidism, the iodine intake producing the lowest values for serum TSH and for serum thyroglobulin, the amount of thyroid hormone replacement necessary to restore euthyroidism to athyreotic subjects, the iodine intake associated with the smallest thyroid volumes in populations, and the lowest incidence of transient hypothyroidism in neonatal screening with blood spot TSH. The best way to determine iodine adequacy from the diet is by testing urine iodine concentration because 90% of dietary iodine is excreted in the urine. WHO, UNICEF, and ICCIDD determined that the *minimal* urinary iodine (UI) concentration for iodine sufficiency of a population would be set at 100 μg/L, with less than 20% of the population excreting < 50 μg/L (20). UI concentrations

of 100-200 µg/L, averaging 150 µg/L, corresponding approximately to a daily intake of 150 µg/d for adults. Therefore, the IOM recommendation for the recommended dietary allowance (RDA) of iodine of 150 µg/d for adults corresponds with 70-80% of daily iodine intake.

Dietary iodine consumption of 150 µg/d for adults may not provide enough iodine for tissues throughout the body, however. In an iodine deficient state, iodine intake may need to be adjusted higher than 150 µg/d for repletion. The American Thyroid Association recommends that women of reproductive age, planning to become pregnant, and pregnant and lactating women consume between 220-290 µg/day of iodine, to compensate for fetal and maternal requirement and losses (21). Iodine levels below these guidelines may lead to iodine deficiency disorders (IDD) in the mother and offspring. Women of childbearing age, 15-44 years, are at the highest risk for iodine deficiency. A Boston study reported that 49% of pregnant and lactating women were consuming iodine below the RDA of 150 µg/day (12). Iodine deficiency during fetal and infant developmental stages may cause mental retardation, autism, developmental delays, cretinism, goiter, and hypothyroidism in the offspring (21). Iodine deficiency in the mother may contribute to hypothyroidism, goiter, fibrocystic breast disease, breast cancer, cognitive decline, and fibromyalgia (22). Overall, iodine deficiency manifests in the thyroid gland as clinical or subclinical hypothyroidism, goiter, myxedema, and cretinism or it may present as non-specific conditions such as stunted growth, mental retardation, and decreased intelligence (1, 23).

Since an individual's iodine status is dependent on iodine consumption and excretion, it would be ideal to use dietary intake to measure iodine consumption. However, the USDA food database for iodine is incomplete; reliance on dietary recall will not provide accurate results (24). Deficiencies of micronutrients such as vitamin A, selenium, iron, and zinc, have also been shown to adversely affect iodine metabolism and thereby thyroid function (25). Vitamin A may

modulate central and peripheral thyroid gland metabolism and TSH production by the pituitary. Vitamin A deficiency has been postulated to reduce thyroidal iodine uptake, thereby reducing thyroid hormone synthesis (26). Selenium is necessary to synthesize thyroid hormones and reduces oxidative damage to the thyroid. If there is iodine deficiency, selenium status should be investigated as inadequate selenium may worsen iodine deficiency outcomes. Iron deficiency may impair TPO enzyme synthesis thereby reducing thyroid hormone synthesis, or may impact thyroid metabolism by lowering oxygen transport to thyroidal tissue (27). It is possible that zinc is essential for thyroid hormone homeostasis due to its effect on the synthesis and action of thyroid hormones due to its interactions with the thyroid hormone receptor (28).

Iodine intake is a crucial determinant of iodine status but is difficult to assess. Urinary iodine (UI) concentration is the method most commonly used to assess dietary iodine intake and is based on the assumption that >90% of iodine consumed from foods and supplements is absorbed. The amount of iodine excreted in the urine reflects recent iodine intake. UI content has been measured using spot UI collection for the Centers for Disease Control (CDC) National Health and Nutrition Examination Survey (NHANES) from 1972 to the present. This method provides information on iodine status based on one point in time and also has limitations to its validity (29). For population-based studies, only spot UI collection is logistically possible, but the data may not be reflective of an individual's iodine status; thus, when extrapolated to a population, the results should be interpreted with caution. Urinary iodine per gram creatinine ratio (UI/Cr) of a single voided urine sample is also considered a reliable method to quantify iodine in individuals. The determination of UI provides little information on the long-term iodine status of an individual. Measures of thyroid function also indicate iodine status. If serum TSH and T4 are

within normal limits, individuals are assumed to be iodine sufficient, since a decrease in T4 and increase in TSH only occurs when iodine deficiency is severe (30).

Recommended tolerable safe upper limit for iodine is 1100 µg/d. According to the Japanese Ministry of Health, the average Japanese person, living in Japan, consumes an average of 5.2-14.5 g/d of seaweed (31). This amount of seaweed yields an estimated 0.3% iodine (range 0.08-0.45%), which would approximate an average daily intake of iodine of 13.8 mg/d (32). The Japanese are known to have one of the lowest incidences of iodine deficiency and hypothyroidism, and breast cancer despite high iodine intakes (33). One of the explanations for the high iodine levels not reaching toxic levels is the high consumption of seafood in the Japanese diet. Many types of seafood are high in trace elements such as selenium, copper, and zinc, which could have a protective effect. More recent data indicate that the average iodine consumption, from all sources, in the Japanese population is 1200-5280 µg/d, whereas it is 209 µg/d in the U.S. (34, 35).

Since the landmark papers published by Wolff and Chaikoff in 1948 and 1949 (36, 37, 38, 39, 40, 41) clinical practice using iodine as a prophylactic has changed. In the 1948b paper, many assumptions were made regarding iodine. The first assumption was that the amount of iodine injected into male rats in the amounts of 10 µg, 50 µg, 100 µg, 200 µg, and 500 µg would yield the same results in humans, such that functioning of a rat's thyroid correlates with a human's thyroid. Thyroid function in the rats was not measured before or after the intervention to determine thyroid status, but assumption number two was iodine levels greater than 50 µg would impair the thyroid. Although no impairment was seen, the conclusion was made that because of iodine, thyroid hormone production decreased for 6-12 hours after injection. Even the highest doses induced no goiter or hypothyroidism. The third assumption was that iodine is taken up only

by the thyroid and has no significance to other tissues and therefore the lowest dose to prevent goiter is sufficient. The fourth assumption was that it is unhealthy to consume iodine above the lower doses. In the 1969 Wolff paper, arbitrary iodine levels were assigned as first, second, third, and fourth-degree excess. The Wolff-Chaikoff effect has been perpetuated in the medical literature and textbooks since 1948 and never thoroughly explained.

Levothyroxine is the second most prescribed drug in the US costing $12/month of prescription, second only to hydrocodone (42). Why is there such a plethora of thyroid dysfunction today? In the US, approximately 27 million Americans have thyroid disease, and about 13 million of them are undiagnosed, according to the American Association of Clinical Endocrinologists (43). The American Thyroid Association states there are 20 million Americans with thyroid disorders and 60 million are undiagnosed (44). These assumptions are based on the current evaluation of TSH levels and depending upon a "normal" range of values, accounts for the significant discrepancy in numbers. Nevertheless, this would mean ~20% of our population is at risk for thyroid disorders, a number not unlike the population observed in the Goiter Belt before salt iodization in the 1920s.

The Mayo Clinic states that "The good news is that accurate thyroid function tests are available to diagnose hypothyroidism, and treatment of hypothyroidism with synthetic thyroid hormone is usually simple, safe and effective once you and your doctor find the right dose for you." However, iodine deficiency is not even a thought because "iodine — found primarily in seafood, seaweed, plants grown in iodine-rich soil and iodized salt — is essential for the production of thyroid hormones. In some parts of the world, iodine deficiency is prevalent, but the addition of iodine to table salt has virtually eliminated this problem in the United States. Conversely, taking in too much iodine can cause hypothyroidism (45). Therefore, we have a

problem that we do not take in enough iodine because we are fearful of causing thyroid dysfunction but because of iodine deficiency, we have an epidemic of thyroid disorders that is currently only being treated by taking an over-prescribed drug that eliminates healthy thyroid functioning. Would it not be simpler to mandate iodization of salt or take iodine supplements rather than creating a levothyroxine-dependent population?

According to the WHO guidelines for iodine sufficiency, UI excretion of 150 µg/L is the standard for healthy adults, which includes women of reproductive age, 18-44 years (46). Iodine status differs in men and women, and iodine deficiency has more severe consequences for women as it will affect future generations (47). The downward trend from iodine sufficiency in the early 1970's to deficiency (<150 µg/d) has plateaued at a level below sufficiency for reproductive age women. These women may become pregnant during these years and will have iodine deficiency at the beginning of pregnancy and, without supplementation, throughout the pregnancy, and into lactation. Since this is a critical time for development in the life of the fetus and infant, this may be catastrophic. Iodine deficiency is the leading cause of preventable mental retardation in the world (48).

Unfortunately for women and children, low iodine intakes combined with pregnancy and lactation has dangerous health implications. Iodine disruptors further exacerbate the problem. According to the Iodine Global Network (IGN) Global Scorecard of Iodine Nutrition 2017, pregnant women in the U. S. have insufficient iodine intake. The median UI concentration was 320 µg/L in 1974, 164 µg/L in 2002 (49), and 144 µg/L in 2010, suggesting a more than 50% reduction in dietary intake (50). According to the Iodine Global Network, 17% of the U.S. population is iodine deficient, with a median urinary iodine concentration <150 µg/L. Median UI for all females was 134 µg/L, and for women of childbearing age was 124 µg/L (50). 36% of

women had low UI levels (<100 µg/L), and 16% of women had levels below 50 µg/L, which would mean 2.2 million women have low or deficient iodine intakes (51). These reports indicate that iodine intake in the US diet has steadily decreased over the past few decades.

NHANES data have been the only assessment utilized for iodine sufficiency in the U.S. In an NHANES study combining the data from 2005-2006 and 2007-2008, the median UI was 125 µg/L for pregnant women and 130 µg/L for non-pregnant women. The first, second, and third trimesters were divided into median UI, trimester I = 182 µg/L, trimester II = 154.6 µg/L and trimester III = 135.9 µg/L (52). Another study determined that percentage of reproductive age women with iodine deficiency (< 50 µg/L) increased by 6.9 times in the period between the 1971-1974 and 1988-1994 NHANES data collection (53). Women had lower UI than men in every NHANES data collection since 1971-1974.

Public awareness of the importance of iodine consumption in the prenatal period and the first trimester of pregnancy is severely lacking. Over 75% of obstetricians and midwives do not recommend iodine supplementation to patients planning to become pregnant, during pregnancy, or lactation despite the deleterious effects of iodine deficiency in reproductive-age women (54). Also troubling is the fact that as many as 50% of prenatal vitamins currently available on the market do not contain iodine at all and those that do may have inconsistent labeling (52, 55).

This review aims to investigate the iodine status of reproductive age women in the U. S. and address possible causes of iodine deficiency in this population. It is possible that a simple correction of iodine status may help prevent some of the hidden or overt adverse outcomes associated with iodine deficiency which may be unnoticed in reproductive age women in the U.S.

METHODS

The studies in the review included all those articles which analyzed NHANES data since the 1971-1974, NHANES I through the present, had specific analyses on women of reproductive age including those who may be pregnant, lactating, nulliparous, and multiparous. Other qualifications included using standardized IOM guidelines for iodine sufficiency, details on the method of iodine collection, and how iodine status was determined, such as using 24-hour urine iodine, spot urine iodine or iodine: creatinine ratio. NHANES data collection relies on spot UI due to the volume of participants. Spot UI gives *a recent*, one-time glimpse of the iodine in one void. Dietary iodine intake was not controlled in these studies. See Supplemental Table.

DISCUSSION

Women of reproductive age in the U.S. are iodine deficient according to the analysis of NHANES data. Since the original NHANES I (1971-1974) study, non-pregnant women have been below the WHO guidelines for UI of 150 μg/L, indicating inadequate iodine nutrition. The period between NHANES I and NHANES III captured the significant decline in iodine levels in about 20 years (52, 56). Analyzing data from 2001 through 2010 continued to show iodine deficiency in reproductive age women. Median UI levels may be stabilized; however, they are stabilized at deficient levels. The most plausible reason for this drop in UI levels is that women of reproductive age may not have sufficient dietary iodine intakes to support thyroid and metabolic health and the health of the developing fetus. The leading indicator of iodine consumption and thereby iodine status is dairy intake (57). Women who consume dairy have a lower risk of iodine deficiency than those who do not. Iodine supplementation also correlates with adequate iodine status in women of reproductive age (58). It then becomes imperative that women supplement with iodine, especially in the first and second trimesters, to allow for thyroid

hormone production for fetal brain development. Between 2001 and 2012, the percentage of women with < 50 µg/L UI or severe deficiency increased from 11.6% to 13.2% (59). Every study included in this review reported the median UI's of women to be lower than males.

All NHANES UI data was collected by spot samples. For obvious reasons, this is the only practical way to assess large populations and may reflect median iodine excretion. However, overall iodine status of an individual cannot be determined by spot UI. Spot samples are a one-time glance at the concentration of excreted iodine. This method has inherent problems as it does not take into consideration factors affecting iodine excretion. Possibly, recent consumption of foods with high iodine content, high fluid volume, and goitrogens could account for falsely high iodine excretion. Iodine correlates to estrogen variability of the menstrual cycle (60). Therefore, the day of UI collection may influence iodine excretion up or down.

Another way to assess iodine levels is by twenty-four hour UI collection, which represents an individual's single day concentration of iodine. Although this is a more accurate estimate of dietary iodine intakes, it is not feasible for a population study. In the study by König, et al., it would take ten spot UI tests to have any degree of accuracy, and even one 24-hour sample has only 20% reliability estimating iodine status (28).

For women of reproductive age, achieving total body iodine sufficiency should be a greater priority. The iodine loading protocol developed by Abraham et al. may be closer to the determination of existing iodine status (61). The basis for the test is to examine how much iodine is excreted compared to the load consumed. Individuals performing the iodine loading test will consume 50 mg of iodine/iodide and collect urine for 24 hours, a sample of which is then sent to the lab for iodine status determination. The reasoning is the body will clear whatever amount of

iodine/iodide is not absorbed. Excretion of 45 mg, or about 90%, means the individual is iodine sufficient (62).

Iodine deficiency may lead to hypothyroidism and permanent damage to thyroid function. The offspring of a woman with hypothyroidism and iodine deficiency may also experience hypothyroidism due to the iodine deficiency of the mother. Iodine deficiency in utero and during lactation could result in iodine deficiency and underdevelopment of the thyroid in the neonate. Another issue is the fact that it is still unknown how much iodine is necessary for iodine sufficiency beyond prevention of goiter, but actual health-promoting levels of iodine. The guidelines are the minimum for disease prevention, but not necessarily whole body health. The WHO established UI threshold goals for half the population to be > 100 μg/L, and less than 20 % of the population to be deficient at <50 μg/L. It is important to note that these numbers are population medians and do not take into account iodine sufficiency of particular groups such as women of reproductive age.

All potential factors involved in iodine uptake and excretion must be scrutinized to assess iodine sufficiency of a population. The lack of mandatory salt iodization programs in the U.S., changes in dietary patterns leading to a reduction in iodine intake, the decline in bread fortification, reduced salt guidelines may lead to iodine deficiency, especially in vulnerable populations such as reproductive-age women (63). Salt-iodization in the U.S. is voluntary and not mandatory unlike in many other countries, and consumers can still purchase either iodized or non-iodized salt. Although the Iodine Global Network estimates that the proportion of U.S. households with access to iodized salt now exceeds 90%, data regarding actual usage is limited, and the contribution of iodized salt to the overall iodine sufficiency of the U.S. population is uncertain. The USDA also does not mandate the listing of iodine content on food packaging. It is

assumed that the majority of salt consumption in the U.S. comes from processed foods, which primarily uses non-iodized salt during production (64). Many individuals in the U.S. consume restricted diets for various reasons and may have eliminated iodine-rich foods from their diets. Dairy consumption by adults and children, especially milk, has declined in the U.S. Consuming dairy products was the principal indicator of iodine status for both men and women (56). Milk has been limited or eliminated, in many diets for reasons such as lactose intolerance, allergies, or believing it is a high fat, high-calorie food. Eggs have been restricted from many people's diets due to the high cholesterol levels in egg yolk and its relationship to heart disease. Just a few decades ago, sodium was implicated as the cause of hypertension; doctors and dietitians were instructing patients to avoid using table salt leading to the elimination of salt from the diets of many Americans. The recent change in the dietary guidelines for reduction of salt intake from 3500 to 2300 mg/d further exacerbates the issue of reduced iodine intake.

Iodine deficiency may also be caused by substances called goitrogens which inhibit or block iodine uptake into the thyroid. Iodine is part of the halide group along with bromine, chlorine, and fluorine. These halides act as goitrogens and compete with iodine uptake into the thyroid, especially under iodine deficiency conditions (62). The thyroid requires iodine to produce thyroid hormones; however, if bromine is dominant, then iodine may not be utilized for thyroid hormone production. If goitrogens subvert iodine, it may be lost by excretion in the urine. It is not known if this could induce a falsely high urinary iodine excretion. Other goitrogens hurting iodine status are perchlorate, bisphenol A, oral contraceptives, amiodarone, and flavonoids, and polyphenols (62, 66).

Goitrogenic factors may have some role in declining iodine measurement; however, their role may be underestimated. In the U. S., the impact of iodine disrupting elements on the iodine

status of the population, especially on reproductive-age women is not known. Goitrogens found in cruciferous vegetables, and lima beans are commonly consumed in the U.S. The other halogens, especially, bromine as potassium bromate replaced iodine in bread in the 1970's over concern of excessive iodine consumption. Potassium bromate is a renal carcinogen and increases iodine excretion (67, 68). Perchlorate has been discovered in contaminated groundwater and has also been detected in vegetables and dairy products. Since perchlorate blocks iodine intake via the NIS, it has a strong goitrogenic effect (69, 70). In an analysis of the NHANES 2007-2008 dataset determined that perchlorate along with thiocyanate and low iodine reduce thyroid function exhibited as decreased circulating T_4 (71). Another highly toxic substance affecting thyroid hormones during low iodine status is serum perfluoroalkyl acids (PFAS). These chemicals are found in stain, carpet, food packaging, paints, and stains. When iodine levels are low, and TPO antibodies are present, PFAS may disrupt thyroid function and alter thyroid hormone levels (72). Flavonoids, primarily from soy and millets, may become goitrogenic if consumed excessively. Vegetables, fruit, and supplements contain flavonoids, such as quercetin, in significant amounts. Flavonoids are healthy substances possessing antioxidant, antiviral, apoptotic and anti-inflammatory qualities. However, excessive consumption coupled with iodine deficient conditions may disrupt thyroid function. Similarly, resveratrol found in grapes and berries is a polyphenol with health-promoting properties, but over-consumption may inhibit the NIS gene expression thus blocking iodine uptake (73).

Women's use of oral contraceptives began in the early 1960's; however widespread use did not take hold until after the 1972 Supreme Court decision to allow all citizens access irrespective of marital status. This is approximately the same time iodine levels were reduced in the U. S. The age group most utilizing oral contraceptives in this period were those in the early

years of reproductive age. By the 1990's, the breast cancer rates had increased 3.5 times the rate of the early 1970's. Thyroid cancer rates have also doubled since the early 1970's. Outside of the thyroid hormones, estrogen has the most widespread hormonal influence in women. Estrogen wields dominance over thyroid hormones and thyroid function (74). It affects iodine uptake and excretion (75).

Awareness of the importance of adequate iodine nutrition, particularly during pregnancy and lactation, among the U.S. populace is lacking (76) indicating cause for a significant public health concern that needs to be addressed. It is possible that all of the above-discussed factors could be contributing to iodine deficiency in the U.S. population, with reproductive age women being the most vulnerable to the effects of decreased iodine intakes.

CONCLUSIONS

Since the first NHANES in 1974, median UI concentrations for women of reproductive age have all been below the WHO recommendation of 150 μg/L. This simply means that over 50% of women in childbearing years are iodine deficient. Any degree of deficiency in women may have repercussions on her health and wellbeing, however, quite possibly the more significant question is what impact iodine deficiency has on her offspring. Even mild ID may cause impairment; however, severe ID may cause profound disability. It is a simple, inexpensive remedy of iodine supplementation that could be recommended to all women of reproductive age, but the concern over excess iodine outweighs the current, already dire situation. Almost all first-world countries have mandatory salt iodization programs, and iodine recommendations expressly promoted except the United States. This is not a new problem. It has been known since 1988 and little, if nothing, has been done to ameliorate the problem. Every study in this review clearly demonstrates there is iodine deficiency in this key population.

CONFLICT OF INTEREST DISCLOSURE

None of the authors had any personal or financial conflicts of interest**.**

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Study	Data	Subjects	Method	Results
Caldwell, et al. (2005)	NHANES 1988-1994, 2001-2002	Women 15-44 yrs, n=5405, 348 pregnant 5057 non- pregnant n=679, 126 pregnant 132 non- pregnant	Urine iodine spot test, urinary creatinine	NHANES 1988-1994 Overall Median UI =128 µg/L, 36.1%,100 µg/L Pregnant= 141 µg/L, 28.5%<100 µg/L, Non pregnant=127 µg/L, 36.5%<10µg/L NHANES 2001-2002 Overall Median UI=132.5 µg/L, 38%<100 µg/L Pregnant=172.6 µg/L, 37.7%<100 µg/L, Non pregnant=132.0 µg/L, 38%<100µg/L *When iodine adjusted for creatinine, >% of deficiency for 1988-1994 data
Caldwell, et al. (2008)	NHANES 2003-2004	Women 15-44 yrs	Urine iodine spot test, urinary creatinine	Pregnant and non-pregnant median UI=139 µg/L, UI<100=37.2±5.8%, UI<50µg/L=15.1±3.2%, UI<2 µg/L=4.0±2.6%
Caldwell, et al. (2011)	NHANES combined 2005-2006 2007-2008	Subgroup women 15-44 yrs	Urine iodine spot test, urinary creatinine	Non pregnant women=130 µg/L, Pregnant women=125µg/L Trimester I=182 µg/L, Trimester 2=154.6 µg/L, Trimester 3=135.9 µg/L 56.9%± 7.9% of all pregnant women UI< 150 µg/L (n= 184) 2005-2008 Non-pregnant women 15-45 yrs during NHANES 2005-2005, 29.5 million women <WHO recommendations, 76.2 million UI < 50 µg/L
Caldwell, et al. (2013)	NHANES 2005-10 NCS Vanguard Study 2009-2010	2233 Women 15-44 yrs 206 pregnant, 2027 non-pregnant, 501 pregnant women (NCS) 3rd trimester	Urine iodine spot test, urinary creatinine	Dairy was sig. correlated with median UI in women 15-44 yrs. Trimester I and II < adequate median UI.

Hollowell et al. (1998)	NHANES I, III	Women 18-44 yrs	Fasted, urine iodine spot test, urinary creatinine	Percentage of 15-44 yrs women with UIC<5µg/dL increased 3.8 times between studies, pregnant women UIC<5µg/dL increased 6.9 times
Hollowell & Haddow (2007)	NHANES I 1971-1974 NHANES III 1988-1994	5279 women, 15-44 yrs, 208 pregnant, 5405 women, 15-44 yrs, 312 pregnant	Urine iodine spot test, urinary creatinine	Median UI 294 µg/L, Pregnant 327 µg/L, Not pregnant 293 µg/L Median UI 128 µg/L, Pregnant 141 µg/L, Not Pregnant 127 µg/L
Lee, et al. (2016)	NHANES 2001-2004 2005-2008 2009-2012	12779, 20> yrs, 51% women	Urine iodine spot test	Prevalence of <50 µg/L increased each data set above the last. The highest sub-group increase was among women at 14.5% with <50 /L, with UIC lowest in 20-39 age women
Pan et al. (2013)	NHANES 2001-2010	3935 women 15-44 yrs, 415 pregnant women	Urine iodine spot test, urinary creatinine	Overall females (141.8 µg/L)<males (176.1 µg/L), women 15-44 UI with age Women 15-44 yrs median UI=131.1 µg/L, pregnant women median UI=145.0 µg/L. Pregnant women >35 yrs higher median UI than pregnant women <35 yrs 76.9% pregnant women took supplements, only 20.3% of supplements contained iodine
Pessah-Pollack, et al. (2014)	New York City Health Clinic	182 pregnant women	Urinary iodine spot test	Women offered 150 µg of KI. Two groups-supplemented vs non-supplemented. Overall median UI 152 µg/L, supplemented group median UI 169.8 µg/L, non-supplemented group median UI 128.4 µg/L, non-supplemented group 38.9% risk for ID vs 22.8% of supplemented group
Perrine et al. (2010)	NHANES 2001-2006	326 pregnant 15-44 yrs 53 lactating, 1437 non- pregnant/ non- lactating	Urine iodine spot test	Pregnant women median UIC = 153µg/L, lactating = 115µg/L non pregnant, non-lactating = 130µg/L. Those who consumed dairy had significantly higher median UIC than those who did not.

CHAPTER V

RESULTS

The purpose of this study was to determine the impact of a 12.5 mg Iodoral® (iodine) supplement (IG) vs. placebo (glucose tablet) (PG) on iodine status, thyroid function, resting metabolic rate, and body composition in reproductive-age women, 18-45 years of age in a parallel, randomized, double-blind, placebo-controlled trial over six months.

Hypotheses

This study examined the following null hypotheses at significance levels of $p \leq 0.05$:

1. Iodine supplementation for six months will not improve iodine status by increasing concentrations of 24-hr UI excretion, percent iodine saturation, and sodium iodide symporter (NIS) ratio in a group of reproductive-age women, 18–45 versus a placebo.

   FAILED TO REJECT: There was no significant increase in 24-hr UI excretion, % IS, and NIS ratio in the IG.

2. Iodine supplementation for six months will not improve thyroid function by increasing serum concentrations of thyroid hormones, free thyroxine (T_4), free triiodothyronine (T_3), and decreasing thyroid stimulating hormone (TSH) in a group of reproductive-age women, 18–45 versus a placebo.

   FAILED TO REJECT: There was no significant increase in serum free T_4, and free T_3 concentrations or decrease in serum TSH concentrations in the IG.

3. Iodine supplementation for six months will improve resting metabolic rate (RMR) in a group of reproductive-age women, 18–45 versus a placebo.

FAILED TO REJECT: There was significant increase in RMR in both the groups IG and PG.

4. Iodine supplementation for six months will not improve body composition by decreasing BMI (kg/m^2), percent body fat, and increasing percent lean mass in a group of reproductive-age women, 18–45 versus a placebo.

   FAILED TO REJECT: There was no significant decrease in BMI, and percent body fat or increase in percent lean mass in the IG.

A total of 103 participants were recruited into the study. As outlined in Chapter III, along with the informed consent forms, participants completed demographic, health history, physical activity questionnaires, and three-day dietary records. Participants were randomized into either the iodine group (IG) or placebo group (PG). Participants in IG received Iodoral® pills containing 12.5 mg iodine, along with Equate multivitamin supplements for the duration of the study. Individuals in PG received glucose pills containing 5mg glucose, along with Equate multivitamin supplements for the duration of the study.

As indicated previously in Chapter III, all participants and researchers were blinded to the group assignment of participants. After analyses were completed, information on blinding was released to the researchers. Of the 103 participants, 65 participants were assigned to the IG at the beginning of the study, and 38 were assigned to the PG. At the end of six months, 64 (62% of original) participants completed the study. Of those who completed the study, there was 100% compliance to consuming either iodine (n=32) or placebo (n=32) measured by counting left over pills. The majority of the individuals who dropped out of the study did not provide any reason. Three individuals reported scheduling conflicts for data collection, and two individuals reported

adverse reactions to the iodine supplement, such as the development of skin rash and metallic aftertaste, and dropped from the study for these reasons.

This chapter will explore the demographics of the study participants, analysis of their three-day dietary records, and physical activity assessment by analyzing physical activity questionnaires. This chapter will also present the effects of the iodine supplement versus placebo on iodine status, thyroid function, body composition, and RMR, at baseline and six months. All values will be reported as Mean ± SEM unless noted otherwise.

Demographic Variables

Per the inclusion criteria, all participants were female and between 18-45 years of age. Participants were classified based on ethnicity, employment status and level of education. Table 5.1 shows the frequencies for the demographic variables as reported for IG and PG at baseline.

At baseline, 65 individuals were recruited into the IG. Mean age of the IG participants was 27.1 ± 0.8 years. Classification of the ethnicity of this population is as follows: 39 (60%) Caucasian (non-Hispanic), 12 (18.5%) Hispanic, 8 (12.3%) African American, 3 (4.6%) Asian/ Pacific Islanders, 1 (1.5%) American Indian, and 2 (3.1%) classified themselves as other. Of the 65 individuals, 39 (60%) were employed either full time or part-time, 20 (30.8%) were students, 6 (9.2%) were unemployed. The educational level varied with 43 (66.1%) pursuing or had completed high school, an associate's degree or equivalent, bachelor's degree, or a graduate program, and 20 (30.7%) classified themselves as having some college or technical training, and 2 (3.1%) reported no education-related data.

Table 5.1.

Demographic Variables of the Study Population at Baseline

	IG, N=65	Percent of N	**PG**, N=38	Percent of N
Ethnicity				
African American	8	12.3	5	13.2
American Indian	1	1.5	0	0
Asian/ Pacific Islanders	3	4.6	2	5.3
Caucasian (non-Hispanic)	39	60	26	68.4
Hispanic	12	18.5	5	13.2
Other	2	3.1	0	0
Employment Status				
Full time or part-time	39	60	27	71
Students	20	30.8	10	26.3
Unemployed	6	9.2	1	2.6
Education Level				
Pursing or had completed- high school, associate's degree or equivalent, bachelor's degree, or a graduate program	43	66.1	28	73.7
Some college or technical training	20	30.7	10	26.3
No data	2	3.1	0	0

Legend: IG, iodine group; PG, placebo group

Out of 103 participants, 38 individuals were assigned into the PG. Mean age of the participants in the PG was 28.3 ± 1.3 years. Classification of the ethnicity of this population is as follows: 26 (68.4%) Caucasian (non-Hispanic), 5 (13.2%) African American, 5 (13.2%) Hispanic, and 2 (5.3%) Asian/ Pacific Islanders. Of the 38 individuals, 27 (71%) were employed either full time or part-time, 10 (26.3%) were students, and 1 (2.6%) was unemployed. With regard to the education, 28 (73.7%) were pursuing or had completed high school, an associate's degree or

equivalent, a bachelor's degree, or a graduate program, and 10 (26.3%) classified themselves as having some college or technical training.

Mean age of the participants differed by race when the participants were classified as Caucasians (n = 65) vs. non-Caucasians (n = 38). Non-Caucasians included African Americans, American Indians, Asian/ Pacific Islanders, Hispanics, and individuals classifying themselves as other. Mean age for Caucasians was 28.9 ± 1 years versus 25.1 ± 1 years for non-Caucasians (p = 0.007).

Age of the participants differed by education status ($p < 0.001$). Mean age of participants who had a high school degree was 23.6 ± 2.5 years, associated degree or equivalent was 26.4 ± 1.4 years, some college or technical training was 28.6 ± 1.1 years, bachelor's degree was 28.6 ±1.1, and graduate degree was 35 ± 1.5years.

Age of the participants also differed by employment status ($p < 0.001$). Mean age of participants who were unemployed was 24.5 ± 3.2 years, employed full time was 33.2 ± 1.5 years, employed part-time was 25.7 ± 1.0 years, homemakers was 35.7 ± 4.6 years, self-employed was 20.0 ± 4.6 years, and students was 26.5 ± 1.2 years.

Dietary Analysis

All three-day dietary records for participants collected at baseline and final assessment were analyzed using the Axxya Systems Nutritionist Pro™ software. Dietary data analysis reported values for total energy consumed (Kcals), the amount of proteins (g), carbohydrates (g), and fats (g), and amounts of vitamins A (IU), C (mg), and D (IU), and minerals, iodine (µg), selenium (µg), folate (µg), zinc (mg), iron (mg), and calcium (mg), consumed during the three-day time period.

Of 65 subjects, 57 (87.7%) participants in the IG provided completed three-day dietary records at baseline. Of the 32 participants that completed the study in this group, 25 (78.1%) provided completed three-day dietary records. Of 38, 32 (84.2%) participants in the PG provided completed three-day dietary records at baseline. 32 participants completed the study in this group of whom 25 (78.1%) provided complete three-day dietary records. Data from the remainder of the participants in the study that either did not provide dietary records or provided incomplete records, were considered as missing data.

A comparison of dietary intake based on the nutrient analysis of the three-day dietary records between IG and PG at baseline and six months are shown in Table 5.2. There were no significant differences in any macro or micronutrient intakes between IG and PG, at baseline and six months. Dietary iodine intake for IG and PG was significantly below the RDA of 150 µg /d both at baseline and six months.

Table 5.2.

Dietary Intake between IG and PG at Baseline and Six Months

		IG			PG		
		n	Mean	SEM	n	Mean	SEM
Energy (Kcals)	Baseline	57	1929.3	85.1	32	1870.0	103.0
	Final	25	1940.9	149.1	25	1851.5	121.2
Carbohydrates (g)	Baseline	57	214.7	10.3	32	212.6	14.2
	Final	25	220.6	16.3	25	210.9	15.4
Proteins (g)	Baseline	57	87.8	4.6	32	85.6	6.1
	Final	25	90.7	7.5	25	85.0	6.7
Fats (g)	Baseline	57	81.3	4.4	32	77.7	4.9
	Final	25	83.0	7.0	25	76.8	5.4
Vitamin A (IU)	Baseline	56	6713.0	1278.1	32	7930.2	1912.5
	Final	25	5480.1	1558.6	25	8061.7	2397.1
Vitamin C (mg)	Baseline	57	149.0	48.1	32	106.2	23.7
	Final	25	79.6	16.5	25	82.8	26.0
Calcium (mg)	Baseline	57	887.9	98.8	32	873.4	77.1
	Final	25	846.7	117.4	25	770.8	70.1
Iron (mg)	Baseline	57	14.1	1.1	32	12.0	0.9
	Final	25	11.8	1.0	25	13.4	1.4
Vitamin D (IU)	Baseline	57	152.2	36.7	32	132.4	24.0
	Final	25	161.6	36.3	25	115.9	21.9
Folate (µg)	Baseline	57	341.2	57.2	32	292.5	34.4
	Final	25	327.2	39.2	25	343.8	62.0
Iodine (µg)	Baseline	[§]56	*10.2	6.5	32	*12.6	5.0
	Final	25	*7.4	3.2	25	*7.7	4.2
Zinc (mg)	Baseline	57	8.2	1.0	32	7.1	0.7
	Final	25	8.1	0.7	25	7.8	0.9
Selenium (µg)	Baseline	57	73.2	5.9	32	68.9	7.1
	Final	25	80.8	10.1	25	75.3	8.6

Note. *Dietary iodine intake was significantly below RDA for both IG and PG at baseline and six months.

Note. [§]n=56 to exclude outlier (3120 µg/d); Mean baseline dietary iodine (n=57, IG) = 65.6 ± 55.8 µg/d

Legend: IG, iodine group; PG, placebo group; Kcals, kilocalories; g, grams; mg, milligrams; µg, micrograms; IU, international units; SEM, standard error of mean

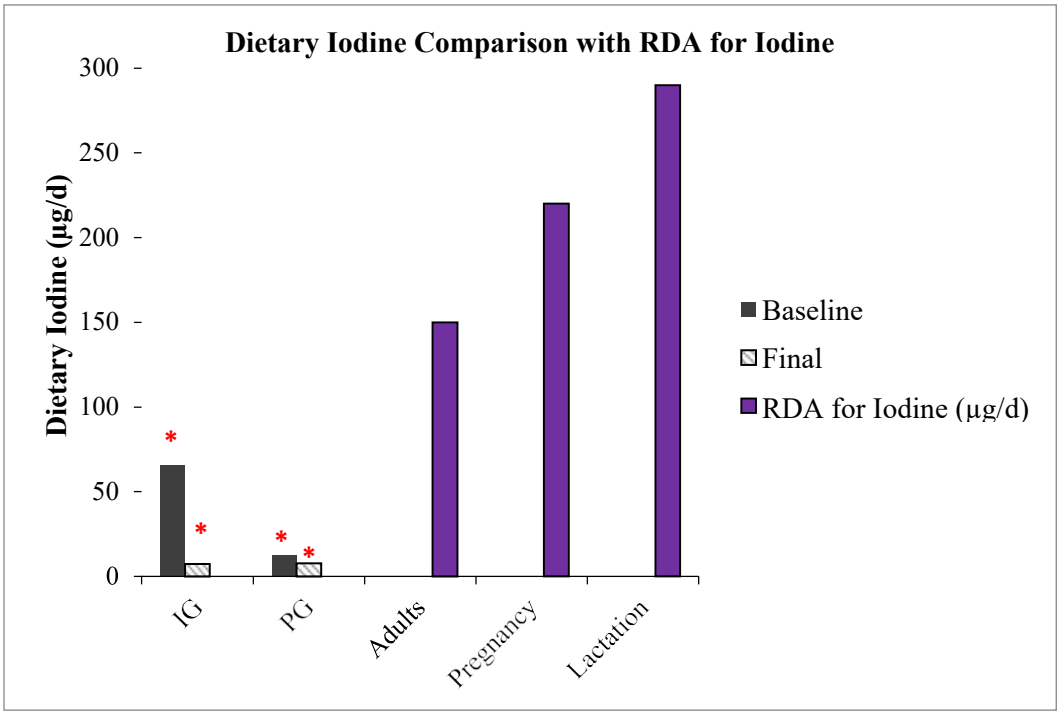

Figure 5.1. Dietary iodine intake of study population versus expected RDA for iodine

Note. * Dietary iodine intakes significantly below RDA in both IG and PG at baseline and six months

Legend: IG, iodine group; PG, placebo group; RDA, recommended dietary allowance; µg/d, micrograms per day

Dietary iodine intake in the study population at baseline was 10.22 ± 6.5 µg /d in IG (n=56) and 12.6 ± 5.0 µg /d in PG (n=32). Mean dietary iodine intake at six months was 7.4 ± 3.2 µg /d in the IG (n=25) and 7.7 ± 4.2 µg /d in the PG (n=25). Figure 5.1 shows the dietary iodine intake of the study population at baseline and six months in comparison with the RDA for iodine in adults which includes women of reproductive-age and the increased requirements during pregnancy and lactation. Supplemental iodine intake from the iodine pills for IG or multivitamin supplements for both IG and PG was not factored into the dietary analysis to evaluate the dietary records exclusively based on dietary iodine intake.

Physical Activity

Physical activity questionnaires were completed by participants in both the IG and PG groups at baseline and six months. Participants answered a series of questions based on their current physical activity and exercise habits that they performed regularly, at least once a week. These answers were then scored based on the guidelines outlined in the 2011 Adult Compendium of Physical Activities. A list of all the physical activity categories included in the questionnaire and their scoring based on the 2011 Compendium is provided in Appendix M.

MET scores were reported as MET calories at baseline and six months for participants in IG and PG. Of the 65 participants in IG, 63 provided usable physical activity questionnaires at baseline and 29 provided completed physical activity questionnaires at the end of the study from IG. Of the 38 participants in PG, 29 provided usable physical activity questionnaires at baseline and 29 provided completed physical activity questionnaires at the end of the study from PG.

MET calories calculated at baseline and six months for participants in IG and PG are shown in Table 5.3. MET calories calculated from reported physical activity questionnaires decreased from baseline to six months in both IG and PG. Mean MET calories decreased significantly from 3011.6 ± 287.4 Kcals/day at baseline to 2281.0 ± 256.5 Kcals/day at six months in IG ($p = 0.022$), and from 3497.8 ± 405.5 Kcals/day to 2598.2 ± 273.3 Kcals/day at six months in PG ($p = 0.006$). No significant differences were observed in physical activity levels between IG and PG at baseline or six months.

Table 5.3.

MET Calories (Kcals/d) for IG and PG at Baseline and Six Months

	IG			PG		
	n	Mean	SEM	n	Mean	SEM
MET Calories						
Baseline	29	*3011.6	287.4	29	**3497.8	405.5
Final	29	*2281.0	256.5	29	**2598.2	273.3

Note. *p<0.05 from baseline to six months in IG; ** p<0.01 from baseline to six months in PG

Legend: IG, iodine group; MET, Metabolic Equivalent Score; PG, placebo group; SEM, standard error of the mean; Kcals/d, kilocalories per day

Baseline Comparisons between Iodine (IG) and Placebo (PG) Groups

Independent samples t-tests and one –way ANOVAs were conducted to examine the differences in baseline variables between IG and PG. As shown in Table 5.4, baseline saliva iodide was significantly higher in PG, 42.3 ± 4.9 mg/L, than IG, 31.1 ± 2.6 mg/L (p = 0.050). Serum iodide was also significantly higher in PG at 1.6 ± 0.1 mg/L than IG, 1.2 ± 0.1 mg/L (p = 0.031) at baseline. However, these values did not impact the NIS (saliva to serum) ratio, which showed no significant difference between IG and PG at baseline.

Table 5.4.

Means and SEMs for Baseline Variables between IG and PG

	IG			PG		
	n	Mean	SEM	n	Mean	SEM
Age	32	27.2	1.1	32	29.3	1.5
RMR (Kcals/d)	32	1616.7	32.7	32	1571.9	39.8
TSH (μIU/mL)	32	1.8	0.2	32	2.2	0.3
FT_3(pg/mL)	30	2.7	0.3	32	3.3	0.4
FT_4 (ng/dL)	32	1.5	0.1	32	1.5	0.1
% Fat	31	36.8	1.6	32	35.5	1.7
Fat Mass (lbs)	31	60.7	4.9	32	55.2	5.2
Lean Mass (lbs)	31	97.6	1.9	32	93.5	2.4
BMI (Kg/m^2)	29	26.0	1.0	29	26.1	1.1
VAT-Volume (in^3)	22	25.2	5.1	21	21.2	5.3
VAT-Mass (lbs)	22	0.9	0.2	21	0.7	0.2
Android Fat (lbs)	30	37.0	2.5	32	34.7	2.5
Gynoid Fat (lbs)	30	41.4	1.6	32	40.3	1.7
A-G ratio	30	0.9	0.04	32	0.8	0.03
Weight (lbs)	32	162.2	6.3	32	154.3	6.8
24-hr UI (mg/24-hr)	32	35.4	1.4	32	34.0	1.5
% IS	32	70.6	2.7	32	67.9	2.9
NIS ratio (saliva/serum)	32	30.1	3.1	32	31.0	3.6
Saliva Iodide (mg/L)	32	*31.1	2.6	32	*42.3	4.9
Serum Iodide (mg/L)	32	*1.2	0.1	32	*1.6	0.1

Note. *$p \leq 0.05$, saliva, and serum iodide differed between IG and PG at baseline

Legend: IG, iodine group; PG, placebo group; MET, metabolic equivalent score; SEM, standard error of mean; RMR, resting metabolic rate; TSH, thyroid stimulating hormone; FT_3, free T_3; FT_4, free T_4; BMI, body mass index; VAT, visceral adipose tissue; A-G ratio, android gynoid ratio, 24-hr UI, 24 hour urinary iodine; NIS, sodium iodide symporter; Kcals, kilocalories per day; lbs, pounds, kg/m^2, kilograms per meter squared; in^3, inches cubed; μIU/mL, micro international units per milliliter; p/mL, picograms per milliliter; ng/dL, nanograms per deciliter; mg/L, milligrams per liter; mg/24-hr, milligrams per 24 hour

Iodine Supplementation and Iodine Status

Iodine status evaluated by 24-hr UI and % IS indicated iodine deficiency in the study population irrespective of grouping. Mean 24-hr UI for the study population was significantly below the expected normal, as was % IS at baseline. Per the protocol developed by Abraham

(2004), individuals are considered iodine sufficient if their 24-hr UI is >44mg/24-hr, and % IS >90%. According to this classification, individuals in both IG and PG were iodine deficient at baseline and also at six months. Median 24-hr UI was 35.0 mg/24-hr for IG and 42.0 mg/24-hr for PG and showed no significant improvement at six months in either IG or PG.

Repeated measures ANOVA was used to test each of the measures for iodine status, 24-hr UI, % IS, NIS ratio, saliva iodide, and serum iodide. Measures were examined for both changes over time between baseline and six months, and also for differences between groups, IG and PG, as well as interactions between time and group. As shown in Table 5.5 and Figure 5.2, 24-hr UI increased from 35.7 ± 1.4 mg/24hr to 38.9 ± 1.7 mg/24hr in IG (n = 31), and from 34.0 1.5 to 38.5 ± 3.2 mg/24hr in the PG (n = 31). % IS increased from 71.1 ± 2.7 to 77.7 ± 3.4 in IG (n = 31), and from 67.9 ± 2.9 to 76.3 ± 6.4 in PG (n = 31). NIS ratio decreased from 30.1 ± 3.2 to 28.6 ± 2.9 in IG (n = 31), and increased from 31.00 ± 3.6 to 32.6 ± 3.6 in PG (n = 31). Saliva iodide increased significantly from 32.2 ± 2.8 mg/L to 46.0 ± 6.4 mg/L in IG (n = 30; p = 0.041), and from 42.3 ± 4.9 mg/L to 59.8 ± 7.2 mg/L in the PG (n = 30; p = 0.013). Serum iodide increased from 1.2 ± 0.1 mg/L to 1.5 ± 0.1 mg/L in IG (n = 30), and increased significantly from 1.6 ± 0.1 mg/L to 2.1 ± 0.3 mg/L in PG (n = 30; p = 0.041). Serum and saliva iodide values differed significantly between IG and PG at baseline (p = 0.013). Even though 24-hr UI and % IS for both IG and PG showed a trend toward increase from baseline to six months, significance was not observed.

Table 5.5.

Iodine Status between IG and PG at Baseline and Six Months

	IG			PG		
	n	M	SEM	n	M	SEM
Iodine Status						
24-hr UI (mg/24hr)						
Baseline	31	35.7	1.4	32	34.0	1.4
Final	31	38.9	1.7	32	38.5	3.2
% IS						
Baseline	31	71.1	2.7	32	67.9	2.9
Final	31	77.7	3.4	32	76.3	6.4
NIS Ratio (saliva/serum)						
Baseline	31	30.1	3.2	32	31.0	3.6
Final	31	28.6	2.9	32	32.6	3.6
Saliva Iodide(mg/L)						
Baseline	30	*32.2	2.8	32	*42.3	4.9
Final	30	§46.0	6.4	32	§59.8	7.2
Serum Iodide(mg/L)						
Baseline	30	*1.2	0.1	32	*1.6	0.1
Final	30	1.5	0.1	32	§2.1	0.3

Note. *p<0.05 between IG and PG; § p<0.05 from baseline to six months

Legend: IG, iodine group; PG, placebo group; 24-hr UI, 24-hour urinary iodine; mg/24-hr, milligrams per 24 hours; % IS, percent iodine saturation; NIS, sodium iodide symporter; mg/L, milligrams per liter.

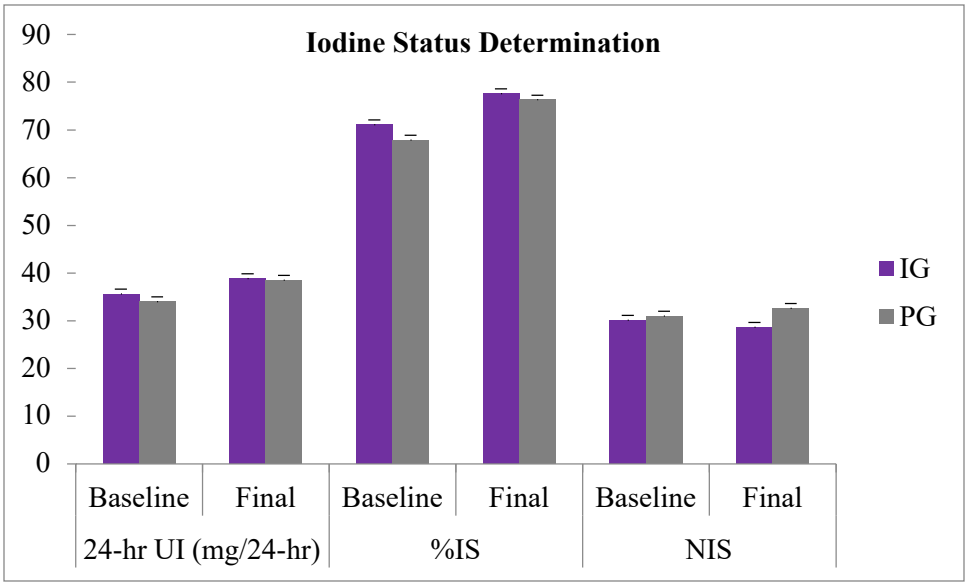

Figure 5.2. Iodine status at baseline and six months in IG and PG

Legend: IG, iodine group; PG, placebo group; 24-hr UI, 24-hour urinary iodine; mg/24-hr, milligrams per 24 hours; % IS, percent iodine saturation; NIS, sodium iodide symporter; mg/L, milligrams per liter.

Iodine Supplementation and Thyroid Function

Repeated measures ANOVA was used to test each of the measures for thyroid function, serum TSH, free T_4, and free T_3. Measures were examined for both changes over time at baseline and six months, and also for differences between groups, IG and PG, as well as interactions between time and group. Table 5.6 shows the changes in the measures of thyroid function between IG and PG at baseline and six months.

Table 5.6.

Thyroid Function between IG and PG at Baseline and Six Months

	IG			PG		
	n	M	SEM	n	M	SEM
Thyroid Function						
TSH (μIU/mL)						
Baseline	32	1.8	0.2	31	2.2	0.3
Final	32	1.6	0.2	31	2.3	0.3
F-T_3(pg/mL)						
Baseline	29	*2.6	0.2	31	*3.3	0.4
Final	29	*2.6	0.3	31	*3.2	0.3
F-T_4(ng/dL)						
Baseline	32	1.5	0.1	31	1.5	0.1
Final	32	§2.0	0.1	31	§2.2	0.1

Note. * $p<0.05$, free T_3 significantly differed between IG and PG at baseline and six months

§ $p<0.001$, free T_4 increased significantly from baseline to six months in both IG and PG

Legend: IG, iodine group; PG, placebo group; SEM, standard error of mean; TSH, thyroid stimulating hormone; FT_3, free T_3; FT_4, free T_4; μIU/mL, micro international units per milliliter; p/mL, picograms per milliliter; ng/dL, nanograms per deciliter.

As shown in Table 5.6, serum free T_4 increased significantly from 1.5 ± 0.1 ng/dL to 2.0 ± 0.1 ng/dL in IG (n=32), and from 1.5 ± 0.1 ng/dL to 2.2 ± 0.1 ng/dL in the PG (n=31) ($p<0.001$). Mean serum TSH decreased from 1.8 ± 0.2 μIU/mL to 1.6 ± 0.2 μIU/mL in IG (n=32), versus 2.2 ± 0.3 to 2.3 ± 0.3 in PG (n=31). While TSH showed a trend toward decrease from baseline to six months in IG, significance was not observed. Serum free T_3 remained unchanged in IG (n=29), (2.6 ± 0.2 pg/mL to 2.6 ± 0.3 pg/mL) and in PG (n=31), (3.3 ± 0.4 pg/mL to 3.2 ± 0.3 pg/mL) from baseline to six months.

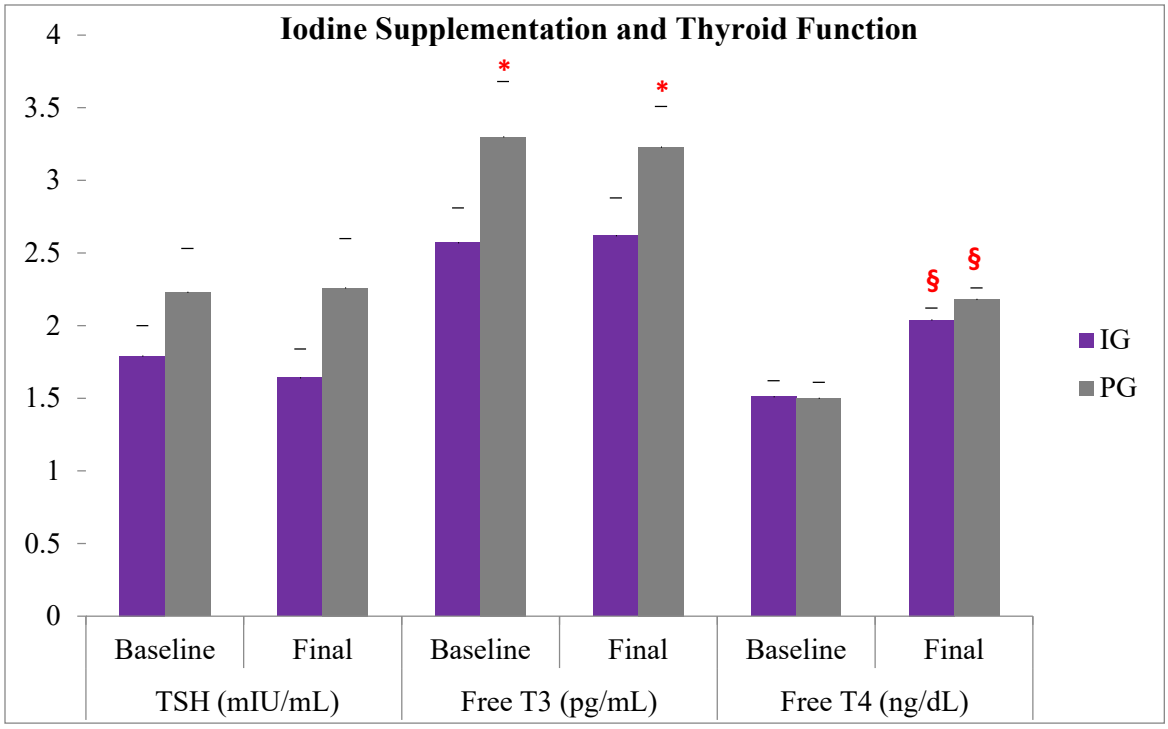

Figure 5.3. Thyroid function at baseline and six months between IG and PG
Note. § p<0.001, increase in free T_4 from baseline to six months in both IG and PG
* p<0.05 difference in free T_3 between IG and PG at baseline and six months

Legend: IG, iodine group; PG, placebo group; TSH, thyroid stimulating hormone; FT_3, free T_3; FT_4, free T_4; µIU/mL, micro international units per milliliter; pg/mL, pico grams per milliliter; ng/dL, nano grams per deciliter.

Figure 5.3 shows the changes in thyroid function between IG and PG at baseline and six months. Free T_4 increased significantly from baseline to six months in both IG and PG ($p <$ 0.001). Free T_3 differed significantly between IG and PG ($p < 0.05$) both at baseline and six months, with higher values in PG. However, Free T_3 did not show a significant increase from baseline to six months in PG. Free T_4 differed by race ($p = 0.038$) when participants were classified as Caucasians vs. non-Caucasians at baseline. Mean free T_4 was 1.9 ± 0.1 ng/dL for Caucasians ($n = 43$) and 1.6 ± 0.1 ng/dL for non-Caucasians ($n = 20$) at baseline.

Iodine Supplementation and Resting Metabolic Rate

The effect of iodine supplementation on RMR between IG and PG at baseline and six months is shown in Table 5.7, and Figure 5.4. RMR significantly increased from 1616.7 ± 32.7 Kcals/d to 1664.4 ± 33.4 Kcals/d in IG (n = 32), and from 1571.9 ± 39.8 Kcals/d to 1606.9 ± 34.5 Kcals/d in PG (n = 32; $p < 0.001$). However, there was no difference between IG and PG at either time point.

Table 5.7.

RMR between IG and PG at Baseline and Six Months

	IG			PG		
	n	M	SEM	n	M	SEM
Resting Metabolic Rate						
RMR (Kcals/d)						
Baseline	32	1616.7	32.7	32	1571.9	39.8
Final	32	*1664.4	33.4	32	*1606.9	34.5

Note. * p = 0.005, increased RMR in IG and PG from baseline to six months

Legend. IG, iodine group; PG, placebo group; RMR, resting metabolic rate, Kcals/d, kilocalories per day; SEM, standard error of mean

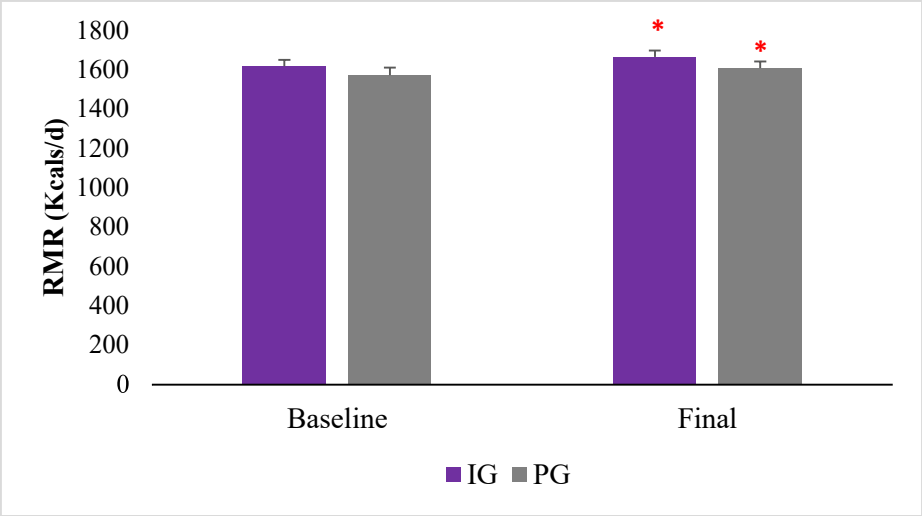

Figure 5.4. RMR at baseline and six months between IG and PG

Note. *Indicates significant increase in RMR from baseline to six months in IG and PG ($p < 0.01$)
Legend: RMR, resting metabolic rate; Kcals/d, kilocalories per day

Iodine Supplementation and Body Composition

Body composition outcomes included body weight, BMI, percent body fat, fat mass, lean mass, VAT mass, VAT volume, android fat, gynoid fat, and A-G ratio. These variables were measured at baseline and six months for participants in IG and PG. A comparison of body composition variables for IG and PG at baseline and six months are shown in Table 5.8.

Table 5.8.

Body Composition between IG and PG at Baseline and Six Months

	IG			PG		
	n	M	SEM	n	M	SEM
Body Composition						
% Body Fat						
Baseline	31	36.8	1.6	32	35.5	1.7
Final	31	36.9	1.7	32	35.6	1.7
Fat Mass (lbs)						
Baseline	31	60.7	4.9	32	55.2	5.2
Final	31	61.1	5.1	32	56.5	5.4
Lean Mass (lbs)						
Baseline	31	*97.6	1.9	32	*93.5	2.4
Final	31	97.3	1.9	32	95.2	2.8
BMI (kg/m^2)						
Baseline	29	26.0	1.0	29	26.1	1.1
Final	29	26.1	1.1	29	26.6	1.2
Weight(lbs)						
Baseline	32	162.2	6.3	32	154.3	6.8
Final	32	162.4	6.3	32	157.2	7.0
VAT- Volume (in^3)						
Baseline	17	*28.3	5.7	18	*16.3	4.6
Final	17	*§34.0	6.6	18	*16.4	4.4
VAT-Mass (lbs)						
Baseline	17	*1.0	0.2	18	*0.5	0.1
Final	17	*§1.2	0.2	18	*0.6	0.1
Android Fat (lbs)						
Baseline	30	37.0	2.5	31	35.2	8.3
Final	30	37.9	2.7	31	34.9	8.3
Gynoid Fat (lbs)						
Baseline	30	41.4	1.6	31	40.8	1.7
Final	30	41.4	1.7	31	41.1	1.7
A-G Ratio						
Baseline	30	0.9	0.04	31	0.8	0.03
Final	30	0.9	0.04	31	0.8	0.03

Note. *$p < 0.05$ between IG and PG; §$p < 0.05$ from baseline to six months

Legend: IG, iodine group; PG, placebo group; SEM, standard error of mean; BMI, body mass index; VAT, visceral adipose tissue; A-G ratio, android gynoid ratio; lbs, pounds, kg/m^2, kilograms per meter squared; in^3, inches cubed

Lean mass was significantly different between IG and PG ($p = 0.034$) at baseline. VAT volume was significantly different between IG and PG at baseline ($p = 0.040$). VAT volume increased significantly from 28.3 ± 5.7 in^3 to 33.95 ± 6.6 in^3 in IG ($p = 0.035$) from baseline to six months. Similarly, VAT mass was also significantly different between the groups IG and PG at baseline ($p = 0.038$) and increased from 1.0 ± 0.2 lbs to 1.2 ± 0.2 lbs ($p = 0.036$) in IG from baseline to six months. No other significant differences were observed between the two groups at either baseline or six months. There were also no other significant changes over time in either group.

Adequate Iodine versus Deficient Iodine

Among the 103 participants that started the study, only 13 participants had adequate iodine status, as measured by 24-hr UI excretion and % IS. Of these 13 participants, 7 completed the study at six months and were matched on demographics and baseline measures, including outcomes for iodine status, thyroid function, RMR, and body composition with seven individuals who were iodine deficient. These subgroups were labelled adequate iodine ($n = 7$), and deficient iodine ($n = 7$) groups. A repeated measure ANOVA was conducted to compare the means of the adequate iodine group with the deficient iodine group over time.

Each of the outcomes for iodine status, thyroid function, RMR, and body composition was examined for both changes over time from baseline to six months, and differences between groups, adequate iodine and deficient iodine, as well as interactions between time and matching groups. Regardless of supplement groups IG or PG, RMR was significantly increased after six months as compared to the baseline. Significant time by matching interactions were detected in free T_3 concentration ($p = 0.017$), 24-hr UI ($p < 0.001$), and % IS ($p < 0.001$). For those who had adequate iodine, free T_3 concentration showed an increased tendency from baseline (2.47 ± 0.53

pg/mL) to six-month follow-up (3.47 ± 0.62 pg/mL), 24-hr UI dropped significantly from baseline (46.43 ± 0.48 mg/24hr) to six-month follow-up (39.00 ± 2.60 mg/24hr), and % IS dropped significantly from baseline (92.43 ± 1.04) to six-month follow-up (77.86 ± 5.18). These results indicate a significant increase in free T_3 concentrations and significant decrease in 24-hr UI and % IS in the adequate iodine group from baseline to six months. For those who were deficient in iodine, free T_3 concentration showed a decreased tendency from baseline (3.80 ± 0.91 pg/mL) to six month follow-up (1.81 ± 0.31 pg/mL), 24-hr UI increased significantly from baseline (28.67 ± 2.43 mg/24hr) to six month follow-up (39.83 ± 2.05 mg/24hr), and % IS increased significantly from baseline (57.50 ± 4.91) to six month follow-up (79.83 ± 4.05). These results demonstrate a significant decrease in free T_3 concentrations and increase in 24-hr UI and % IS in the deficient iodine group from baseline to six months follow-up.

Each of the outcomes were examined for both changes over time, from baseline to six months, and differences between groups, adequate iodine vs. deficient iodine, as well as interactions between time and supplement groups, IG and PG. Regardless of supplement groups, IG or PG, RMR was significantly increased from baseline (1676.13 ± 77.77 Kcals/d) to six month follow-up (1744.13 ± 86.58 Kcals/d). Specifically, RMR was significantly increased from baseline (1595.40 ± 86.23 Kcals/d) to six-month follow-up (1680.85 ± 45.27 Kcals/d) in the placebo group, PG. RMR was also increased from baseline (1655.86 ± 77.77 Kcals/d) to six-month follow-up (1723.29 ± 86.58 Kcals/d) in the iodine supplement group, IG.

Two-way ANOVA was conducted to compare the main and interaction effect between supplement groups, IG and PG, and matching groups, adequate iodine vs. deficient iodine, on the six-month follow-up outcomes. There was no interaction effect of supplement and matching groups on any outcomes. However, with regard to the main effect, 24-hr UI ($p = 0.031$) and % IS ($p = 0.022$) significantly differed between IG and PG groups. After six months, IG demonstrated

a higher concentration in 24-hr UI and % IS as compared to PG. In addition, free T_3 concentration was significantly higher in the participants who had adequate iodine (3.47 ± 0.62 pg/mL) than did those who had deficient iodine (1.81 ± 0.31 pg/mL). These results indicate that not only did the adequate iodine group show an improvement in iodine status outcomes over time when compared to the deficient iodine group, and but adequate iodine group also exhibited increased concentrations of active thyroid hormone free T_3 with iodine supplementation.

Correlations between Demographics and Outcomes

Pearson's product-moment correlations were used to test the correlations between age and body composition variables. The age of participants was positively correlated with the following variables at baseline: % body fat ($p = 0.003$), fat mass ($p = 0.004$), BMI ($p = 0.002$), VAT volume ($p = 0.003$), VAT mass ($p = 0.003$), android fat ($p = 0.004$), gynoid fat ($p = 0.008$), A-G ratio ($p = 0.024$), and weight ($p = 0.035$). Age of participants was also correlated with the following variables at six months: % body fat ($p = 0.014$), fat mass ($p = 0.012$), BMI ($p = 0.001$), android fat ($p = 0.017$), gynoid fat ($p = 0.045$), A-G ratio ($p = 0.035$), and weight ($p = 0.025$). Older participants were associated with greater body weight, higher body fat, and higher BMI.

Age of participants was also positively correlated with vitamin C ($p = 0.041$) and folate ($p = 0.014$) intake at baseline, indicating that older participants consumed higher amounts of vitamin C and folate in their diets. However, no correlations were observed between age and final dietary variables.

Total energy intake (Kcals) was positively correlated with lean mass ($p = 0.004$) at baseline and negatively correlated with % body fat ($p = 0.013$), positively correlated with lean mass ($p = 0.012$), and negatively correlated with both android fat ($p = 0.027$) and gynoid fat ($p = 0.010$) at six months, indicating that increased consumption of dietary Kcals

was associated with higher lean mass and lower body fat content. Protein intake was negatively correlated with % body fat ($p = 0.009$), android fat ($p = 0.025$), and gynoid fat ($p = 0.002$) at six months, showing that an increased consumption of dietary proteins was associated with lowered body fat content. CHO intake was positively correlated with lean mass at baseline ($p = 0.012$) and six months ($p = 0.021$), indicating that increased dietary CHO intake was associated with higher lean mass. Fat intake was positively correlated with lean mass at baseline ($p = 0.005$), and with the following at six months: negatively correlated with % body fat ($p = 0.037$), positively correlated with lean mass ($p = 0.021$), and negatively correlated with gynoid fat ($p = 0.040$) indicating that higher dietary fat content was associated with higher lean mass and lowered body fat.

Vitamin A intake was correlated with the following at baseline: negatively with % body fat ($p = 0.027$), and negatively with gynoid fat ($p = 0.005$). Vitamin A intake was correlated with the following at six months, negatively with % body fat ($p = 0.005$), positively with lean mass ($p = 0.028$), negatively with android fat ($p = 0.036$), and negatively with gynoid fat ($p < 0.001$). These results indicate that higher dietary vitamin A intake was associated with lowered body fat and higher lean mass. Vitamin D intake was correlated negatively with % fat mass ($p = 0.050$), negatively with android fat ($p = 0.044$), negatively with gynoid fat ($p = 0.046$) and negatively with A-G ratio ($p = 0.026$) at baseline. Vitamin D intake was correlated negatively with A-G ratio ($p = 0.023$) at six months, indicating that higher dietary vitamin D intake was associated with decreased body fat content.

Dietary iodine intake was negatively correlated with % fat mass ($p = 0.038$), negatively correlated with android fat ($p = 0.040$), negatively correlated with gynoid fat ($p = 0.036$), and negatively correlated with A-G ratio ($p = 0.032$) at baseline. Iodine intake was also negatively correlated with % body fat ($p = 0.016$), negatively correlated with android fat

($p = 0.012$), negatively correlated with gynoid fat ($p = 0.023$), and negatively correlated with A-G ratio ($p = 0.006$) at six months. Overall, decreased dietary iodine intake was associated higher body fat content. Dietary iodine intake was significantly correlated with RMR ($p = 0.005$), indicating that a decrease in iodine consumption is associated with a reduction in RMR. Other dietary variables and physical activity variables were not significantly related to thyroid function.

RMR was positively correlated ($p < 0.01$) with all body composition variables including % body fat, fat mass, lean mass, BMI, VAT volume, VAT mass, android fat, gynoid fat, and A-G ratio at baseline and six months, indicating that higher RMR was associated with increased BMI, body fat and lean mass.

Pearson's product-moment correlations ($r > 0.8$) between body composition variables showed positive correlations at a significance of $p < 0.01$ between the following: % body fat with fat mass, BMI, android fat, gynoid fat, and A-G ratio; fat mass with BMI, VAT volume, VAT mass, android fat, gynoid fat, and body weight; BMI with VAT volume, VAT mass, android fat, gynoid fat, and body weight; VAT volume with VAT mass and android fat, VAT mass with android fat; and android fat with gynoid fat and A-G ratio. These results indicate that body fat, BMI, and body weight are highly correlated.

Physical activity levels measured by MET calories were significantly negatively correlated with the following variables: with % body fat ($p = 0.040$), gynoid fat ($p = 0.022$) at baseline. MET Calories were significantly negatively correlated with the following variables: % body fat ($p = 0.002$), fat mass ($p = 0.027$, BMI ($p = 0.026$), android fat ($p = 0.006$), gynoid fat ($p = 0.005$), and A-G ratio ($p = 0.027$) at six months. These results indicate that decreased physical activity levels are associated with greater body fat and higher BMI.

CHAPTER VI

DISCUSSION

The purpose of this study was to examine the impact of an iodine supplement versus placebo on measures of iodine status, thyroid function, resting metabolic rate (RMR), and body composition in reproductive-age women, 18-45 years of age. Significant iodine deficiency was observed in the population at study initiation. The present randomized-double-blind-placebo-controlled trial demonstrated no significant improvement of iodine status as measured by 24-hr urinary iodine (UI) concentration, percent iodine saturation (% IS), and sodium iodide symporter (NIS) ratio at the end of six months. This study showed no significant improvement in thyroid function, as measured by serum thyroid stimulating hormone (TSH), free triiodothyronine (T_3), and free thyroxine (T_4) concentrations with iodine supplementation at the end of six months. This study also showed no significant improvement in RMR with iodine supplementation or outcomes for body composition, measured by body mass index (BMI), percent body fat, and percent lean mass at the end of six months. Even though statistical significance was not observed between the main outcomes measured at baseline and six months, encouraging trends were observed in serum TSH, 24-hr UI, and % IS with iodine supplementation. Serum TSH showed a trend toward decrease and 24-hr UI and % IS showed a trend toward increase in the iodine group (IG) at the end of six months, indicating that longer term iodine supplementation may be beneficial in improving iodine status and thyroid function in this group of iodine deficient reproductive-age women. A subgroup analysis revealed significant improvement in iodine status and thyroid function in the group with adequate iodine status as compared to the group with deficient iodine status over a period of six months. This discussion will primarily focus on the on main finding of observed iodine deficiency and lack of knowledge of iodine's importance in reproductive age

women observed in the study population, and an examination of the study outcomes relative to the research hypotheses.

Iodine supplementation in the form of iodized oil and salt iodization had been studied extensively; however, research on the effect of high dose iodine supplementation on biomarkers most susceptible to iodine intake such as iodine status, thyroid function, RMR, and body composition is lacking. The present study was the first of its kind to study the effect of high dose iodine supplementation in reproductive-age women on biomarkers of iodine status, thyroid function, RMR, and body composition over duration of six months. In this study, participants randomized to the IG received 12.5mg Iodoral®, an iodine supplement that is readily available over the counter. All participants, irrespective of randomization to iodine group (IG) or placebo group (PG), received a generic multivitamin supplement, Equate brand, which provided the 150 µg/d recommended dietary allowance (RDA) for iodine to ensure that the minimum dietary requirements may be met for iodine and other micronutrients that may impact iodine metabolism (Hess et al., 2010). One of the main limitations of this study was the dropout rate (37.87 %), which significantly reduced the sample size for final analyses. However, a power analysis conducted before data collection required a minimum sample size of 62. A total of 103 participants were recruited into the study, 64 of whom completed at six months, which satisfied the sample size requirement indicated in the power analysis.

The most significant finding of this study was the extent of iodine deficiency observed in the study population. Iodine status was tested by the iodine loading test developed by Abraham, 2004, and administered at Flechas Family Practice (FFP) Labs, North Carolina. As per the iodine loading protocol, participants were expected to excrete >44 mg of the 50 mg loading dose of iodine/iodide in a 24-hour urine void, indicating >90% iodine sufficiency (Abraham, 2004).

However, of the 103 participants at baseline, only 13 (12.6%) had 24-hr urinary iodine (UI) levels >44mg/24-hr, and % iodine saturation (IS) >90%, indicating that 87.4% of the study population was iodine deficient at baseline iodine status assessment. When stratified by group, mean 24-hr UI was 35.7 ± 1.4 mg/24-hr in iodine group (IG) and 34.0 ± 1.5 mg/24-hr in the placebo group (PG), indicating iodine deficiency in both groups at baseline. Percent IS was 71.1 ± 2.7 in IG and 67.9 ± 2.9 in PG at baseline. 24-hr UI and % IS showed a trend toward increase at six months. However, the increase was sustained in both IG (38.9 ± 1.7 mg/24-hr) and PG (38.5 ± 3.2 mg/24-hr). This trend toward increased UI was also reflected in the non-statistical increase in the % IS to 77.7 ± 3.4 in IG and 76.3± 6.4 in PG. The majority of individuals recruited into the study were significantly iodine deficient to start with, and a longer duration of supplementation may be needed to study the effect of supplement versus placebo on iodine status determinants. To test this assumption, participants were divided based on their iodine status determinants, 24-hr UI and % IS, into two subgroups, adequate iodine group (>44 mg/24-hr UI, and >90% IS) and matched on baseline variables to a deficient iodine group (<44 mg/24-hr UI, and <90% IS). A subgroup analysis (n = 14) conducted to compare these two groups, adequate vs. deficient iodine, showed that participants with adequate iodine status at the beginning of the study (n = 7) demonstrated greater improvements in 24-hr UI and % IS with iodine supplementation at the end of the six month study period than those who were deficient (n = 7). This indicates that baseline adequacy of iodine may be a major determining factor of iodine status. Participants with adequate iodine status not only maintained their iodine status throughout the study with iodine supplementation, but they also showed a significant ($p < 0.05$) increase in 24-hr UI and % IS compared to the placebo. Another significant finding was that the iodine deficient group showed a significant increase in 24-hr UI ($p < 0.001$) from baseline (28.67 ± 2.43 mg/24hr) to six-month follow-up (39.83 ± 2.05 mg/24hr), and % IS ($p < 0.001$) from baseline (57.50 ± 4.91) to six-month follow-up

(79.83 ± 4.05) with iodine supplementation showing that even short-term iodine supplementation is helpful in improving iodine status in this group of iodine deficient reproductive-age women.

Another, often ignored, determinant of iodine status is the activity or functioning of the NIS, which transports dietary iodide into the thyroid follicular epithelial cell for its incorporation into tyrosine residues of thyroglobulin resulting in the formation of thyroid hormones T_3 and T_4. Impaired activity of the NIS results in decreased transport of dietary iodide into the thyroid follicle, resulting in decreased serum free T_3 and T_4 concentrations. It is therefore crucial to test the functioning of NIS to possibly rule it out as a cause for iodine deficiency. The method of NIS testing used in this study is a relatively new technique, also developed by Abraham (2004). This method determines if a functional deficiency of the NIS causes a decrease in saliva and serum iodide transport across the NIS, thereby leading to iodine deficiency. Expected values for NIS ratio are 24-74, and values below or above this range indicate a functional deficiency of the symporter. Saliva iodide levels increased significantly from 32.2 ± 2.8 mg/L to 46.0 ± 6.4 mg/L in IG (n = 30; p = 0.041), and from 42.3 ± 4.9 mg/L to 59.8 ± 7.2 mg/L in the PG (n = 30; p = 0.013). The saliva/serum iodide ratio measures the ability of the salivary glands to concentrate peripheral iodide, and increased saliva iodide levels are consistent with the increase in iodine uptake post loading. Serum iodide increased from 1.2 ± 0.1 mg/L to 1.5 ± 0.1 mg/L in IG (n = 30), and increased significantly from 1.6 ± 0.1 mg/L to 2.1 ± 0.3 mg/L in PG (n = 30; p = 0.041). Serum and saliva iodide values differed significantly between IG and PG at baseline (p = 0.013), but not at six months. NIS ratio was 30.1 ± 3.2 in IG versus 31.0 ± 3.6 in PG at baseline, indicating normal functioning of the NIS. NIS ratio decreased from 30.1 ± 3.2 to 28.6 ± 2.9 in IG (n = 31), and increased from 31.00± 3.6 to 32.6 ± 3.6 in PG (n = 31). These changes were not significant between IG and PG and within the groups from baseline to six months. Although

saliva and serum iodide concentrations varied between IG and PG from baseline to six months, NIS ratio remained unchanged and indicates a normal functioning NIS in both groups, IG and PG. One of the advantages of this method of iodine sufficiency testing is that it indicates whole body sufficiency of iodine, and the efficiency of the NIS system which could impact iodine uptake by the thyroid gland (Abraham et al., 2005). Although this is an inherent advantage of this method of testing, it is also a disadvantage due to the inability to compare the 24-hr UI, % IS levels, and NIS ratio obtained from this iodine testing protocol to the WHO/UNICEF/ICCIDD guidelines for iodine sufficiency based on UI excretion of school-aged children (see Table 2.2; WHO, 2007). At this juncture, it is important to remember that the WHO guidelines for iodine deficiency are based on median UI concentrations in school-age children, which are extrapolated to population groups such as adults, including reproductive-age women (Benoist, 2004). As stated by Eastman and Li (2017), what we need to know is the iodine status of individuals with reference to EAR or RDI based on age, gender, reproductive status and possibly ethnicity to ensure optimal iodine nutrition and prevent adverse effects of iodine deficiency. Possible directions for future research could be testing for thyroglobulin levels, since it is a long-term biomarker for iodine status, and including measures of assessment of thyroidal volume using ultrasonography to detect the long-term effects of iodine nutrition status and possible iodine deficiency in populations that are generally considered iodine sufficient.

24-hr UI concentrations are a good indicator of recent iodine intake, and therefore it is likely that the decrease in 24-hr UI and % IS is reflective of the dietary habits of these individuals. Also, evaluating iodine intake by considering all dietary sources may provide a better and complete understanding of the population prevalence of iodine deficiency and excess than the use of UI data alone (Juan et al., 2016). Nutrient analysis of the three-day dietary records

collected at baseline in IG and PG demonstrated that the dietary iodine intake in the study population was significantly below the RDA for iodine of 150 µg/d. All study participants were women of reproductive-age, 18-45 years, and adequate dietary iodine intake in this demographic group is important not only for the optimal thyroid functioning of these individuals but also to provide for adequate iodine stores to prepare for pregnancy and lactation. The WHO and IOM requirements for iodine intake during pregnancy and lactation are 220 and 290 µg/d, respectively (IOM, 2001; WHO/UNICEF, 1994). Mean dietary iodine intake for IG (n = 57) was 65.6 ± 55.8 µg/d. One data point was identified as an outlier, as this participant was consuming 3710 µg iodine/d mostly from seaweed. On excluding this data point, mean dietary iodine intake at baseline for IG (n = 56) fell to 10.2 ± 6.5 µg/d, and iodine intake in PG was 12.6 ± 5.0 µg/d, indicating significantly reduced dietary iodine intake in the study population when compared to the expected RDA of 150 µg/d. Dietary iodine levels from three-day dietary records remained unchanged at 7.4 ± 3.2 µg/d in IG and 7.7 ± 4.2 µg/d in PG at study completion at six months. That the entire study population was not consuming even 10% of the expected RDA for iodine seems implausible.

The most likely reason for dietary iodine intakes to be significantly lower than the RDA is an underestimation of dietary iodine intake by the Axxya Systems Nutritionist Pro™ software used for analysis of the three-day dietary records. This software uses the references used in the USDA's National Food and Nutrient Analysis Program (NFNAP) database to analyze macronutrient and micronutrient levels from dietary records. Data on the nutrient composition of the >8700 distinct foods and food components are reported in the National Nutrient Database for Standard Reference, however, currently, iodine is not among the nutrients analyzed by the NFNAP (Phersson et al., 2016; USDA, 2017). Analyzing dietary records using a different

database or system, containing more comprehensive information about the iodine content of commonly consumed foods may provide different and possibly, higher values for dietary iodine intake. It is highly unlikely that the values obtained in such a manner would be close to the RDA for iodine for this group of women considering the current trends for reduced dietary iodine intake in the U.S. population (Dasgupta et al., 2008).

Dietary assessment methods also often tend to underestimate iodine intake for various reasons, most important of those being the difficulty in quantifying the contribution of iodized salt to total iodine intake (Ma & Skeaff, 2014). However, over 95% of participants reported limited knowledge of dietary sources of iodine or significance of iodine in reproductive-age women; over 90% reported reduced or no salt intake in their diets. A majority of these women were ignorant of the fact that iodine could be obtained from table salt, and unaware of the difference between iodized vs. non-iodized salt. It is therefore unlikely that the reduction in iodine intake was due to a difficulty in quantifying the amount of iodine consumed from iodized salt, as these individuals were consuming little to no iodized salt. A careful analysis of the three-day dietary records also showed that a majority of dietary intake was comprised of processed foods or foods from fast food chains such as McDonald's, Arby's, and Whataburger. It is important to note that the salt used in processed foods, which is the primary source of salt for most Americans, typically does not contain iodine (Zelmon, 2015). The majority of the fast food chains in the U.S. also do not use iodized salt in their food preparations (Lee, Leung, He, Braverman, & Pearce, 2010).

Participants were questioned about their dietary practices and knowledge of iodine at the baseline visit. Some of the typical responses noted when questioned about iodine were as follows: "What is iodine?" "Why do you need iodine?," "Where do you get it from?," "I know it is present

in salt," "Is there a difference between salt and iodized salt?," "Is it present in sea salt?," and "My thyroid is fine, so, why do I need iodine?." These responses coupled with the data from the analysis of the three-day dietary records indicate a potential etiology for the significant reduction in dietary iodine intake from the expected RDA of 150µg/d. Dietary iodine intake did not increase at six months, despite the participants receiving information about dietary iodine sources, and the importance of iodine in the everyday functioning of the thyroid. The trend of eating less table salt, dairy, bread, and increased consumption of processed foods, along with a lack of knowledge of the significance of iodine nutrition, more likely results in iodine deficiency in this group of reproductive-age women, which could have serious public health implications. Neurological development of the infant's brain is critically dependent on maternal iodine before the development of the fetal thyroid (de Escobar, Obregón, & del Rey, 2007; Gordon et al., 2009). In the U.S., where mild to moderate iodine deficiency during pregnancy is emerging, women in early pregnancy constitute the population subgroup of greatest concern (Pearce, Lazarus, Moreno-Reyes & Zimmermann, 2016); it is important to note that iodine deficiency in non-pregnant women of reproductive-age is also of concern because of the possibility of pregnancy (Ershow, Goodman, Coates, & Swanson, 2016).

Concurrent deficiencies of micronutrients selenium, iron, or vitamin A may exacerbate the effects of iodine deficiency. Deiodinases are selenoenzymes required for the conversion of inactive T_4 to active T_3. Glutathione peroxidases are another set of selenoenzymes that protect the thyroid follicular cells from peroxide induced damage during thyroid hormone synthesis (Schomburg, 2011). Certain randomized controlled intervention trials have also shown that correcting only selenium deficiency may have a deleterious effect on thyroid hormone metabolism in school-age children with co-existing selenium and iodine deficiency (Contempre et

al., 1991; Contempre et al., 1992). Iron deficiency can impair thyroid metabolism by altering the TSH response of the pituitary, reducing the activity of TPO enzyme, and limiting the conversion of T_4 to T_3 in the liver, thereby increasing T_3 turnover, and decreasing T_3 binding to nuclear receptors (Zimmerman, 2006). Correcting iron deficiency improves the efficacy of iodine supplementation and decreases goiter rates, thereby resulting in an improvement of thyroid function (Hess, 2010; Hess, Zimmermann, Adou, Torresani, & Hurrell, 2002). Vitamin A may impact iodine metabolism by increasing the synthesis and secretion of TSH by the pituitary gland, increasing the size of the thyroid, reducing iodine uptake by the thyroid and impacting the synthesis and iodination of thyroglobulin, and increasing circulating concentrations of thyroid hormones (Zimmermann, 2007). Concurrent Vitamin A deficiency and iodine deficiency are endemic in school-aged children in West Africa, and a 10 month randomized controlled trial conducted in these children showed that a combination of vitamin A (200,000 IU) and iodine supplement in the form of iodized salt significantly decreased serum TSH and thyroid volume compared to placebo (Zimmermann, Wegmüller, Zeder, Cahouki, & Torresani, 2004). Assessment of deficiency states for the above mentioned vitamins and minerals in iodine deficient individuals can provide a possible etiology for the iodine deficiency when dietary iodine intakes meet the RDA. Although it would have been ideal to evaluate these micronutrient deficiencies and exclude these individuals to rule out possible biases, it was outside of the scope of this study to conduct assessments other than the ones reported in the methods. Dietary analyses included the status of micronutrients such as vitamins A, C, and D, selenium, zinc, copper, and iron, and the possible effect of deficiencies of any of these micronutrients on iodine status determinants and thyroid function were not tested for the same reason. However, it is important to note that all participants, irrespective of grouping, received a multivitamin supplement for the duration of the study to ensure that all participants received at least the RDA for all major

vitamins and minerals, to reduce the possible impact of dietary micronutrient deficiencies on iodine status determinants and thyroid function.

The present study examined the impact of a high dose iodine supplement versus a placebo on measures of thyroid function including serum TSH, free T_3 and free T_4 concentrations since iodine is critical for normal thyroid functioning. Serum TSH, and thyroid hormones, T_3 and T_4 were measured for changes between and within groups, IG and PG, over six months. Iodine deficiency is one of the leading causes of thyroid dysfunction. Since iodine status is an important determinant of thyroid function, many studies have looked at the effects of iodine supplementation in the form of iodized oil, iodine fortification, and salt iodization on thyroid function (Zimmerman et al., 2008). In a randomized-double- blind-placebo-controlled trial of 248 euthyroid Chinese adult males and females, between 19-25 years, subclinical hypothyroidism, measured by an increase in serum TSH concentration ($p < 0.05$) was observed in those supplemented with doses >400 μg iodine/d for four weeks, than in those given placebo (Sang et al., 2012). Another iodine supplementation program conducted in Bangladesh showed no differences in thyroid function measured by serum TSH, T_3 and T_4 concentrations between 200 subjects consuming iodized salt versus non-iodized salt (Parveen, Latif, Kamal, & Uddin, 2007). A double-blind, placebo-controlled trial in 111 women with fibrocystic breast disease, reported that molecular iodine at doses of 1.5, 3, or 6 mg/d for six months showed significant improvement in pain measures. This study also demonstrated no statistically significant changes in thyroid function measured by serum total T_3, free T_3, total T_4, free T_4 uptake, and TSH concentrations over a period of six months in any of the groups (Kessler, 2004). Similar to these two studies that did not show any significant changes in thyroid function with high dose iodine supplementation, the key finding of the present study was that thyroid function, as measured by

serum TSH, free T_3, and T_4 concentrations remained unchanged between IG and PG at baseline and six months even with high dose iodine supplementation. Concentrations of serum TSH, free T_3, and T_4 also stayed within their expected normal range without showing a significant change from the expected normal. Although serum TSH concentration showed an increasing trend from baseline to six months in the IG, no significance was observed. Serum free T_4 concentration increased significantly in both the groups IG and PG from baseline to six months, Specifically, serum free T_4 increased significantly ($p < 0.001$), from 1.5 ± 0.1 ng/dL to 2.0 ± 0.1 ng/dL in IG ($n = 32$), and from 1.5 ± 0.1 ng/dL to 2.2 ± 0.1 ng/dL in the PG ($n = 31$). All participants in IG and PG received a multivitamin supplement providing 150 µg iodine/d, and participants in IG received 12.5 mg Iodoral®/d. Iodine from diet and supplements is taken up by the thyroid gland, and incorporated into thyroglobulin, resulting in the formation of thyroid hormones. The increase in free T_4 observed is consistent with this mechanism of thyroid hormone production. Thyroid hormones are also highly regulated, and concentrations of free T_3 and free T_4 fall out of range only with prolonged severe iodine deficiency, resulting in hypothyroidism and goiter (Eastman & Zimmermann, 2017; Li & Eastman, 2012). Another significant result observed in subgroup analysis in comparing adequate iodine and deficient iodine groups of this study is the significant increase in free T_3 concentration ($p < 0.05$) from baseline to six months in the adequate iodine group as compared to the deficient iodine group. For those who had adequate iodine free T_3 concentrations significantly increased from baseline (2.47 ± 0.53 pg/mL) to six months (3.47 ± 0.62 pg/mL), when compared to the deficient iodine group who showed a significant decrease in free T_3 concentration from baseline (3.80 ± 0.91 pg/mL) to six months (1.81 ± 0.31 pg/mL). This decrease in free T_3 concentration in the deficient iodine group as compared to the adequate iodine group clearly demonstrates the significance of dietary iodine intake and adequate iodine status for the normal functioning of thyroid gland, and resulting thyroid hormone production.

One of the significant concerns with high dose iodine supplementation or excessive dietary iodine consumption is the risk of iodine-induced hypothyroidism (Markou, Georgopoulos, Kyriazopoulou, & Vagenakis, 2001), or iodine-induced hyperthyroidism (Roti & Uberti, 2001; Stanbury et al., 1998). High iodine intake can lead to iodine-induced hyperthyroidism in susceptible individuals, especially those with underlying thyroid disease. Development of iodine-induced thyroid dysfunction is also influenced by prior iodine status and is more likely to develop endemic iodine-deficient areas. The exact mechanisms for iodine-induced thyroid dysfunction remain unclear. The acute inhibitory effect described by Wolff and Chaikoff (1948) is usually transient, and intrathyroidal iodine decreases within a few days despite high plasma iodine concentration resulting in normalization of thyroid hormone synthesis. This phenomenon described as the "escape" indicates that hypothyroidism will not develop in most individuals despite excessive iodine intakes (Nobukuni, 2009). A majority of the individuals in IG did not report any significant side effects of the high dose iodine supplement, indicating that the iodine supplements were generally well-tolerated. Most importantly, iodine supplementation in the 12.5 mg/d dosage for six months did not significantly alter serum TSH, free T_3, and T_4 concentrations in IG, when compared with PG, indicating that thyroid function remained unchanged in these individuals despite the high dosage of iodine consumed for six months. It is possible that iodine intake over the recommended levels, at least for such a short duration of six months, may not be as harmful as it has been purported to be. Care should, however, be exercised, in extrapolating these results to the general population.

Certain studies have reported a possible alteration in thyroid function and the development of autoantibodies to thyroid peroxidase (TPO) and thyroglobulin (Tg) with exposure to high doses of iodine (Teng et al., 2006; Teng et al., 2009). Excessive iodine consumption post-

salt iodization was also associated with an increase in the prevalence of thyroid peroxidase autoantibodies (TPOAb) in young Danish women (Pedersen et al., 2011). Therefore, individuals with a history of thyroid disorders such as Hashimoto's thyroiditis, Graves' disease, family history of thyroid disease, those undergoing treatment with thyroid hormone replacement, amiodarone, lithium, history of exposure to radioiodine, and those with a history of chronic diseases were therefore carefully excluded from the study at recruitment. Care was also taken to screen participants for sub-clinical thyroid dysfunction, and to screen individuals who may never have been diagnosed with thyroid dysfunction by screening them at CPL labs for serum TSH and TPOAb concentrations, and all individuals with abnormal lab values, seven for serum TPOAb, and one for serum TSH, were excluded from the study. One of the limitations of this study was the non-inclusion of assessments for thyroid autoantibodies, TPOAb and TgAb along with other measures of thyroid function to evaluate for changes in concentrations of autoantibodies before and after iodine supplementation. Future research should focus on including the evaluation of thyroid autoantibodies in studies evaluating effects of iodine supplementation, in a previously euthyroid group of individuals, to provide a broader understanding of thyroid function and development or lack thereof of thyroid autoantibodies with excessive iodine intakes.

Dietary iodine is directly taken up by the thyroid gland and used for the production of thyroid hormones T_3 and T_4, which exert their effects on resting metabolism, and energy expenditure. Overt hypothyroidism is associated with decreased resting energy expenditure (REE) and weight gain, whereas hyperthyroidism is associated with increased REE and weight loss (Mansourian, 2010). A moderate increase of TSH, which is associated with normal T_4 values and T_3 values in or slightly above the upper normal range, should not be treated with thyroxine under the "diagnosis" of subclinical hypothyroidism since these alterations in thyroid hormones

are instead a consequence than a cause of overweight or obesity (Reinehr, 2010). Given the association between iodine, thyroid hormones, and energy metabolism, it is imperative that iodine status also be evaluated in individuals with excessive weight gain, as it could indicate subclinical hypothyroidism as a consequence of iodine deficiency. It is possible that iodine deficiency is a contributing factor to the increasing overweight and obesity rates, due to its effects on resting metabolism and thyroid hormone production, and more studies are warranted to explore this connection.

This present study was the first of its kind to examine the impact of high dose iodine supplementation on resting metabolic rate and body composition variables over six months. RMR significantly increased in both IG and PG from baseline to six months; however, no significant differences were observed between IG and PG in RMR and a majority of body composition variables from baseline to six months. A few other studies have investigated whether there is an association between iodine supplementation, serum TSH and BMI. Short term iodine supplementation in the form of kelp supplements given to 36 euthyroid subjects in a randomized- placebo-controlled clinical trial for four weeks showed a significant increase in serum TSH concentration in the kelp supplementation group, but no changes in basal metabolic rate (Clark, Bassett, & Burge, 2003). A positive association was observed between serum TSH concentration within the normal range and BMI among non-smokers in the Tromsø study that included 6164 subjects (Nyrnes et al., 2006). In the present study no similar correlations were observed between serum TSH or other thyroid function determinants and body composition variables. However, significant associations were observed between dietary iodine intake and body composition variables for the first time in this study. Dietary iodine intake was negatively correlated with % fat mass ($p = 0.038$), android fat ($p = 0.040$), with gynoid fat ($p = 0.036$), and

A-G ratio (p = 0.032) at baseline. Iodine intake was also negatively correlated with the same variables, % body fat (p=0.016), android fat (p = 0.012), gynoid fat (p = 0.023), and A-G ratio (p = 0.006) at six months. This study was also the first of its kind to explore the effect of a high dose iodine supplement on RMR. Dietary iodine intake was significantly correlated with RMR (p = 0.005), indicating that a decrease in iodine consumption is associated with a reduction in RMR. RMR significantly increased from 1616.7 ± 32.7 Kcals/d to 1664.4 ± 33.4 Kcals/d in IG (n = 32), and from 1571.9 ± 39.8 Kcals/d to 1606.9 ± 34.5 Kcals/d in PG (n = 32; $p < 0.001$), showing no significant differences between the groups. Serum free T_4 concentrations were significantly elevated in both IG and PG from baseline to six months, and this increase in free T_4 activity could explain the increased RMR in both these groups from baseline to six months. Unfortunately, RMR at baseline and six months in IG and PG did not show any significant correlation with either thyroid function or iodine status parameters in this study. A lack of association between REE and TSH has been reported in euthyroid subjects with severe obesity (Tagliaferri et al., 2001). The findings of the present study are consistent not only with the findings of Tagliaferri et al. (2001), but also with the observation that resting metabolism is not associated with TSH in euthyroid, non-obese subjects (Spadafranca et al., 2015). A cross-sectional study of 140 otherwise healthy hypothyroid subjects receiving chronic replacement therapy with levothyroxine demonstrated that serum free T_3 levels were directly correlated with resting energy expenditure, BMI, body fat mass, and visceral fat mass (Samuels et al., 2017). Similar to these findings, the present study showed that RMR was positively correlated ($p <$ 0.01) with all body composition variables including % body fat, fat mass, lean mass, BMI, visceral adipose tissue (VAT) volume, VAT mass, android fat, gynoid fat, and android gynoid (A-G) ratio at baseline and six months. In a recent MRI study of 282 sedentary participants, adiposity parameters were positively associated with total REE, and increased VAT proportion was independently associated with higher REE (Serfaty et al., 2017). However, the mechanisms

behind these associations are not clearly understood and need to be further investigated. Thyroid hormone plays a critical role in determining energy expenditure, body mass, and body composition, and therefore clinically relevant variations in these parameters may occur across the normal range of thyroid function. Free T_3 levels in the present study were found to be significantly higher in the adequate iodine subgroup than the deficient iodine group, and these associations need to be explored further. As dietary iodine intake directly impacts thyroid hormone production, it is essential to examine the impact of iodine deficiency on these measures of thyroid function, resting energy expenditure and body composition. Future directions for research should include exploring the association between body composition variables, dietary iodine intake, iodine status parameters, and thyroid function, even within the normal range, in larger populations.

In summary, although high dose iodine supplementation did not show significant beneficial effects on biomarkers of iodine status, thyroid function, resting metabolism and body composition, encouraging trends and associations were observed needing additional research. Subgroup analysis further revealed that adequacy of iodine nutrition is associated with better outcomes for iodine status and thyroid function. The key finding was that thyroid function remained unchanged with high dose iodine supplementation indicating that short term iodine supplementation may be beneficial in improving iodine status and may not impact thyroid function in euthyroid individuals. Significant iodine deficiency was observed in the study population coupled with a severe lack of awareness of iodine nutrition or its significance in reproductive-age women, indicating cause for public health concern that needs to be addressed.

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APPENDIX A:

Institutional Review Board Study Approval

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
940-898-3378 Fax 940-898-3416
e-mail: IRB@twu.edu

August 7, 2009

Dr. Nancy M. DiMarco
Institute for Women's Health

Dear Dr. DiMarco:

Re: Effect of an Iodine Supplement on Biomarkers of Thyroid Function, Body Composition, and Resting Metabolic Rate in Women, 18-45 Years of Age

The above referenced study has been reviewed by the TWU Institutional Review Board (IRB) and appears to meet our requirements for the protection of individuals' rights.

If applicable, agency approval letters must be submitted to the IRB upon receipt PRIOR to any data collection at that agency. A copy of the approved consent form with the IRB approval stamp and a copy of the annual/final report are enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. The signed consent forms and final report must be filed with the Institutional Review Board at the completion of the study.

This approval is valid one year from July 17, 2009. According to regulations from the Department of Health and Human Services, another review by the IRB is required if your project changes in any way, and the IRB must be notified immediately regarding any adverse events. If you have any questions, feel free to call the TWU Institutional Review Board.

Sincerely,

Dr. David Nichols, Chair
Institutional Review Board - Denton

enc.

cc.

INSTITUTIONAL REVIEW BOARD

940-898-3378 (Denton & Dallas)
713-794-2480 (Houston)

http://www.twu.edu/research/irb.asp

RECEIVED
APR 11 2013
RESEARCH & SPONSORED PROGRAMS
TEXAS WOMAN'S UNIVERSITY

STUDY MODIFICATION REQUEST

Principal Investigator: DiMarco, Nancy **Protocol #:** 15648 **Campus:** Denton

Title of Study:
Effect of an Iodine Supplement on Biomarkers of Thyroid Function, Body Composition, and Resting Metabolic Rate in Women, 18-45 Years of Age

Description of Modification Requested:

Addition of Margaret Basiliadis, DO, and Pallavi Panth, MS to the research team. Both certificates are attached. Addition of one thyroid function test to be measured using the same amount of blood collected as previously requested, thyroid peroxidase antibodies and it has been added to the consent form. We have also updated our flyer and it also is attached.

List of Attachments:

No Attachments

Approved Rhonda R. Buckley 4/11/13

INSTITUTIONAL REVIEW BOARD

940-898-3378 (Denton & Dallas)
713-794-2480 (Houston)

http://www.twu.edu/research/irb.asp

RECEIVED
SEP 2 6 2013
RESEARCH & SPONSORED PROGRAMS
TEXAS WOMAN'S UNIVERSITY

STUDY MODIFICATION REQUEST

Principal Investigator: DiMarco, Nancy **Protocol #:** 15648 **Campus:** Denton

Title of Study:
Effect of an Iodine Supplement on Biomarkers of Thyroid Function, Body Composition and Resting Metabolic Rate in Women, 18 - 45 Years of Age.

Description of Modification Requested:

WE HAVE SHORTENED THE STUDY FROM ONE YEAR TO 6 MONTHS because we were not attracting any participants, we have added the Dallas campus as a recruitment site, we have added Clinical Pathological Laboratories in Denton to order initial blood draws and we added a WalMart gift card as an incentive for participants. All protocols and procedures are the same.

List of Attachments:

No Attachments

Approved Rhonda R Buckley 9/27/13

APPENDIX B:

Institutional Review Board Informed Consent Form

TEXAS WOMAN'S UNIVERSITY
CONSENT TO PARTCIPATE IN RESEARCH

Title: Effect of an iodine supplement on biomarkers of thyroid function, body composition, and resting metabolic rate in women, 18 – 45 years of age.

Principal Investigator: Nancy DiMarco, Ph.D., R.D., C.S.S.D.
Co-Principal Investigator: Larry Petterborg, Ph.D.
Graduate Research Assistant: Pallavi Panth, M.S.

Explanation and Purpose of the Study

You are being asked to take part in a research study at Texas Woman's University. This study will look into how iodine supplements in women (who still have a monthly period) with low iodine levels (subclinical hypothyroidism or underactive thyroid gland) might improve thyroid function, decrease body fat, and increase the energy used when resting or Resting Metabolic Rate or RMR. Under normal medical care, patients who are told they have an underactive thyroid gland would not receive any drug therapy but would be watched for any changes. This study is based on the Pioneer Project at Texas Woman's University (a longitudinal, observational health study for women), where ~60% of women, 18 to 60 years of age, had a daily dietary iodine intake below the recommended 150 micrograms. Although, low iodine intake and being overweight can lead to an increased risk for disease, this was not found in the longitudinal study. However, it was noted that those who were overweight did have a lower iodine intake. This study will see if an iodine supplement can decrease body weight and increase energy used while resting by changing thyroid function. We will see if even a small change in iodine intake can help to decrease obesity in the United States.

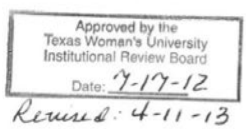
Research Procedures

You have already been told, by your physician, that you have a low functioning thyroid gland. Your total bone density and body composition will be measured in the bone densitometry lab located at the Institute for Women's Health at Texas Woman's University. We ask that those in the study give a health, physical activity, and dietary history.

Without bias, you will be placed in one of two study groups: (1) control group (multivitamin + placebo) or (2) iodine supplement + multivitamin group. The study will take 12 months. After six months and 12 months, all tests will be run again but no blood will be taken at the six-month test. Total time invested in the study for each study member will be about 9 - 12 hours.

Baseline, 6-month, and Follow-up procedures for all participants:

1. If you choose to take part in the study, you will go to the Exercise and Sports Nutrition (ESN) Clinic in the Institute for Women's Health, HDB 011, on the Texas Woman's University Denton campus. You will be given an iodine-loading test. The iodine naturally lost from your body will be measured through a urine sample. You will collect your first morning urine then immediately take a 50 mg dose of iodine supplement (Iodoral™). You will collect your urine throughout the day for one day. Supplements (12.5 mg Iodoral™ plus generic multivitamin supplements not to go above 150% USRDA) or control (generic multivitamin plus placebo; not to go above 150% USRDA) will be taken each day (best in the morning). The placebo is a tablet of 4 grams of glucose.



Participant initials_____

Then once each month, additional supplements or placebo will be given at the ESN clinic. You will take these tablets every day for one year. The iodine supplement, Iodoral ®, has both iodine and potassium iodide and can be bought over-the-counter in any pharmacy in 12.5 mg tablets. One 12.5 mg tablet will give the same amount of iodine as in food eaten each day in Japan. The iodine-loading test will be done on your own time.

2. Height and weight, seven skin-fold measures of the triceps, subscapular, biceps, iliac crest, suprailiac, abdominal, thigh and calf, and waist and hip circumference measurements will be done with skin-fold calipers and tape measure with accepted practices. This will take about 30 minutes.

3. Measurement of fat-to-lean body make-up and total body bone density will be done by Dual Energy X-ray Absorptiometry (DXA). In measuring bone density of the total body, the DXA will also measure fat-to-lean body make-up plus the amount of muscle and bone. To do the test, you will lie flat on a padded scan table for about 20 minutes.

4. Serum levels of TSH, free T4 and free T3 hormones and Thyroid Peroxidase (TPO) antibodies that are released to or from the thyroid gland, will be measured from your blood sample (25 ml) taken from a vein in your arm, collected at the start of the study and one year later as a follow-up, in the ESN clinic by a phlebotomist. Both the serum and plasma will be frozen in 0.5 ml amounts for later use. The blood draw will take about 10 minutes each time.

5. Resting Metabolic Rate or RMR: You will come to the ESN clinic between 6:00 am and 8:00 am after fasting for 12 hours from the night before and after not exercising for at least 48 hr. This is done because exercise may increase RMR. You will rest on your back in a darkened, quiet room for 30 minutes. RMR will be measured using a ventilated, clear canopy-system and a ParvoMedics metabolic cart set to manufacturer's specifications. You will breathe normally and the upper part of your body will be covered with a see-through canopy so that oxygen or carbon dioxide cannot leak in or out. This test will be used to see how much energy your body uses at rest for 24 hours. It will take about one hour to do this test.

6. Questionnaires: You will answer questions about health and demographic history and physical activity. This will take about 15 minutes. Also, you will complete a 3-day dietary intake survey that you will do on your own time.

   - The health history will be used to ensure safety while taking part in the study. The demographic history will be used to characterize who is participating in the program. The physical activity questionnaire will be used to see how much each member of the study normally exercises and will be used to set a baseline and a one-year follow-up estimation of physical activity.

   - *3-day Dietary Intake:* A 3-day nutrition record will measure, specifically; calories; intake of carbohydrates, proteins, and fats; iodine intake; and intakes of riboflavin, niacin, selenium, chloride, and vitamin C. Your dietary records will be evaluated using Nutritionist Pro™ (Axxya Systems, Stafford, TX).

Participant Initials_____

7. At 6-months and 12-months, the same tests will be run as at the beginning of the study, but no blood will be taken at the 6-month test. You will be asked to keep doing your normal activities for the length of the study.

Potential Risks of the Study

1. Risk of Loss of Confidentiality – to help prevent this, all data will be coded and names of those in the study will not be used. The Principal Investigator (PI, NDiMarco) will keep hard copies of all data and personal information in a folder, locked in a filing cabinet, in the PI's office. Only the PI will have entry to the cabinet. Only research personnel will use the coded data. A master list with names related to codes will be kept in a separate filing cabinet until the study is done. The master list will be destroyed at the end of the study. Bone density data will be collected using a computer and the data will be kept on a password secured network in the IWH. Once the study is done, all private data will be shredded within 5 years. De-identified computer data will be kept indefinitely. It is possible that the results of the study may be in peer-reviewed journals; however, your private data will not be used. There is a chance that privacy may be lost in all email, internet and downloading information. Confidentiality will be protected to the extent that is allowed by law.

2. Risk of Hypoglycemia – Hypoglycemia or low blood sugar can occur when fasting for a long time before RMR testing. If any signs of hypoglycemia are seen during a test session, the test will be stopped. Signs of hypoglycemia include tremors, cold sweat, low body temperature, headache, confusion, hallucinations, unusual behavior, convulsions, and coma. If this happens, you will be given a glass of orange juice and watched until the signs of hypoglycemia stop.

3. Risk of Bruising - The risk of bruising from taking blood is small since trained personnel will do this. Accepted precautions will be used when blood is taken. To minimize bruising, pressure will be used at the stick site for about five minutes after each blood draw.

4. Risk of Infection - The risk of infection from drawing blood is small since trained personnel will do this. Accepted precautions will be used when blood is taken. Sites for drawing blood will be cleaned with alcohol immediately before each stick. New needles will be used for each blood draw and will be placed in biohazard boxes right after use as well as any opened needles.

5. Risk of Latex Allergy - The phlebotomist will wear latex gloves when drawing blood. Before each blood draw, you will be asked if you are allergic to latex. If you are, please tell the phlebotomist and a non-latex glove and tourniquet will be used.

6. Risk of Emotional Distress - When collecting personal information, you may feel emotionally uneasy. To decrease emotional discomfort, you may choose to share this information with a research team member of the same sex.

7. Risk of Embarrassment - During the measurement of body composition, or height, and weight, you may feel embarrassed. To reduce embarrassment, you may choose to be measured by a research team member of the same sex. To ensure privacy, body composition, height, and weight measurements will be taken in a small private room in the Exercise and Sport Nutrition Clinic (HDB 011).

.....

Participant Initials_____

8. Risk of Radiation Exposure - During body composition and bone density testing, with the Lunar Prodigy dual energy X-ray absorptiometer (DXA), you will be exposed to a small amount of radiation. The total amount received is 0.26 mrem (whole body using the DXA scan). This is about the same amount received during a 2-hour airplane flight and less than that from normal background exposure in a 24-hour period.

9. Risk of Loss of Time - All procedures will be done as efficiently as possible. Parking spaces will be arranged for you. Building access, to the Institute for Women's Health Exercise and Sports Nutrition Clinic (HDB 011), is at street level. Research assistants will be there at all training sessions to help you. At any time, you may feel free to withdraw from the study.

10. Risk of Coercion - The possibility of coercion exists for patients of physicians. The PI will explain why the study is to be done and supply informational flyers for physicians to enlist those who can be in the study. Every effort will be made to ensure proper treatment of participants by physicians. When you come to the clinic for your first visit, the study will be explained to you and you will have a chance to ask questions before signing the consent form.

11. Risk of Iodine toxicity - Iodine toxicity is rare but it may occur in sensitive people. Even with large amounts of iodine, the thyroid can still function with no problem. Those who take very large amounts may have a brassy taste in the mouth, increased saliva, GI upset, and/or acne. If at any time you have any of these signs, your doctor will be called immediately and they will decide if you should stay in the study. You have the choice to withdraw from the study at any time.

The researchers will work to avoid any problems that might come up during the study. You should let the researchers know, at once, if you are having a problem and they will help you. Since you are a volunteer, TWU does not provide medical or financial aid for injuries that might happen during the study.

Participation and Benefits

Your participation in the study is voluntary. Even if you start the study, and later decide to withdraw, you may do so without penalty. During the study, you will receive a total body bone mineral density scan, body make-up and resting metabolic rate assessments, thyroid screening and dietary intake analysis, iodine supplements and multivitamins for those in the iodine group, and multivitamins plus a placebo for those in the placebo group.

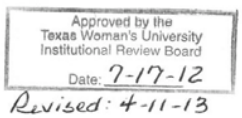
Results of all assessments may be obtained by request.*

Questions about the Study

You will be given a copy of the signed and dated consent form to keep. If you have any questions about the study, please ask the researchers. Contact phone numbers are at the top of this form. If you have questions about your rights as a member of the study or the way the study has been conducted, you may contact the Texas Woman's University Office of Research and Sponsored Programs at 940-898-3378 or via e-mail at IRB@twu.edu.

_____ _____

Signature of Participant Date



Participant Initials_____

*If you want a copy of your results, please provide a name and address on the lines below where they can be sent:

_____

_____

_____

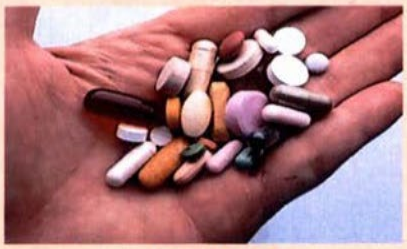
APPENDIX C:

Iodine Study Flyer

Are you a female between 18 and 45 years of age?

No Cost!

Free Supplements!



No Cost!

Free Supplements!

YES?

You may be eligible to participate in a nutrition research study.

Purpose: To determine if an Iodine Supplement can improve thyroid function, reduce body fat, and increase metabolic rate in women, 18-45 years of age

Required criteria: Generally healthy women between 18-45 years of age who are neither pregnant nor wanting to become pregnant for 6 months (or the duration of the study)

You will receive: An analysis of body fat and bone density, metabolic rate measurement, dietary assessment, thyroid function assessment, and multivitamins or iodine supplement, and a $25 Wal-Mart Gift card

The study will be conducted at:
The Institute for Women's Health,
Human Development Building [HDB], 013
Texas Woman's University
Denton, TX 76204

CSD Approved 2013-2014

Contact Dr. Nancy DiMarco @ [redacted] *or*
Pallavi Panth, MS @ [redacted]

Participation is voluntary.

There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions.

APPENDIX D:

E-mail Script/Pioneer Portal Announcement

TWU seeks volunteers for thyroid study

DENTON — The Texas Woman's University Department of Nutrition and Food Sciences is seeking volunteers for a study of the effects of an iodine supplement on improving thyroid function to reduce body fat and increase metabolic rate.

The study is open to women between the ages of 18 and 45, who are neither pregnant nor wanting to become pregnant for one year – the duration of the study. Participants must otherwise be healthy and mobile and must be willing to consume multivitamins or iodine supplement for six months. Participants also will receive an analysis of body fat and bone density, measurement of metabolic rate, a dietary assessment and thyroid function assessment.

Participants will receive a $25 Wal-Mart gift card at the end of the study.

For more information, contact Dr. Nancy DiMarco, professor in the TWU Department of Nutrition and Food Sciences, at [redacted] or Pallavi Panth, doctoral student in the TWU Department of Nutrition and Food sciences, at [redacted] or [redacted].

APPENDIX E:

Denton Record Chronicle Advertisement

Friday in the Louisiana Tech Student Center as part of the university's 2013 homecoming festivities.

TWU seeks volunteers for thyroid study

The Department of Nutrition and Food Sciences at Texas Woman's University is seeking women between 18 and 45 to volunteer for a study of the effects of an iodine supplement on improving thyroid function to reduce body fat and increase metabolic rate.

Participants cannot be pregnant or wanting to become pregnant for six months, the duration of the study. They must otherwise be healthy and mobile and willing to consume multivitamins or the iodine supplement for six months.

To participate, volunteers will receive an analysis of their body fat and bone density, measurement of metabolic rate, a dietary assessment and a thyroid function assessment. They will also receive a $25 Walmart gift card at the end of the study.

For more information, contact Dr. Nancy DiMarco at [redacted] or [redacted]

APPENDIX F:

Information Email to Potential Participants

Hello

This is Pallavi; I will be conducting the Iodine supplement research at the Institute for Women's Health, along with Dr. Nancy DiMarco. Thank you for your interest in the Iodine Study! Basically what we plan to do is provide a multivitamin supplement along with iodine or a placebo and see its effects on body composition and thyroid hormone panel.
The study duration is 6 months.

As a part of the study you will receive the following:

- Multivitamin supplements for duration of study
- Iodine or a placebo for the duration of the study
- A total body composition analysis which includes a body fat and lean mass evaluation, and a bone scan
- Resting Metabolic rate evaluation- which will tell you exactly how many calories you need to be consuming per day (these tests are worth at least 300-500 dollars when done outside)
- Blood tests for thyroid hormones before and after the study
- Nutrient evaluation of your 3 day food diary

You will only come to the clinic for evaluations at the beginning and at the end of the study, and once during the mid-line period to get the supplements.

If you have any other questions or concerns, please do not hesitate to contact me at [redacted] If you have any friends that may be interested, please let them know as we are actively recruiting and are in need of female participants.

We look forward to hearing from you and hope that you will decide to participate!

APPENDIX G:

Screening Checklist

IODINE SUPPLEMENTATION STUDY- SCREENING

NAME:

Date of Birth:

1. Normal period every month (yes/no)
2. Current medications-

3. Supplements
   - Multivitamins-
   - Herbal supplements-

4. Present thyroid problems
5. Past thyroid problems
6. Allergies
7. Plans for pregnancy in next 6 mo-
8. Smoking in past year
9. Diabetes
10. Hypertension
11. History of Cancer
12. Issues with blood draw
    - Fainting/ dizziness
    - Difficult draw
    - Thin veins making it difficult to draw blood

APPENDIX H:

Iodine Study Inclusion-Exclusion Checklist

Iodine Supplementation Study- Inclusion and Exclusion Criteria Checklist:

Inclusion Criteria:

- ☐ Female
- ☐ 18-45 years of age
- ☐ Healthy
- ☐ Normal Serum TSH levels
- ☐ Negative TPO antibody levels
- ☐ Not pregnant or desiring to become pregnant during the study

Exclusion Criteria:

- ☐ Males
- ☐ <18 or > 45 years of age
- ☐ Subnormal TSH - <0.5 or > 4.0 µU/ml
- ☐ Diagnosed hypothyroid
- ☐ Diagnosed hyperthyroid
- ☐ Thyroiditis
- ☐ Elevated Antibodies for TPO
- ☐ Past history of thyroid disease
- ☐ Past history of thyroid surgery
- ☐ Treatment with Lithium or other drugs known to increase thyroid growth
- ☐ Iodine contamination
- ☐ Arrhythmias
- ☐ Congestive heart failure
- ☐ COPD
- ☐ Renal dysfunction
- ☐ Smoking within 1 year of study
- ☐ Pregnant or desiring to become pregnant during the study

APPENDIX I:

Clinical Pathological Labs Requisition Form

PLEASE PRINT WITH BLUE OR BLACK INK

Clinical Pathology Laboratories Inc. ■ V3982181

Clinical Acsn Label

Patient Name - Last First M.I. [redacted]

940 898 2763
TWU-INSTITUTE FOR WOMENS HLTH
011 HUMAN DEV BLDG; OLD MAIN CIRCLE
DENTON, TX 76204
[redacted]

Patient I.D. Room # Phone/Add'l ID

Date of Birth (M M - D D - Y Y Y Y) Sex Date (M M D D Y Y) Time Collected (H H : M M) AM PM

Requesting Physician Fasting (Y N) Urine Volume STAT CALL Same Day

BILL TO: MEDICARE MEDICAID HMO ACCOUNT PATIENT PPO / POS

ST B U OP GR SC KO
L SE GP SW PR UC M4
GY F HC AF OT CU PE

920 Venipuncture CPL 925 Finger / Heel Stick PSC ID
919 Venipuncture 922 Ur Vol Meas
997 Verbal Diagnosis 996 Standing Order Phleb ID
9999 Verbal Order ABN Attachments

PLEASE COMPLETE INFORMATION BELOW

Responsible Party Address City, State, Zip Phone

Medicare Number (Include Prefix/Suffix) Provider Number Medicaid Number State Insured SSN Ordering Physician UPIN Ordering Physician Medicaid TPI or Provider Number

Primary Insurance Name Member I.D. Group Date of Injury or Onset of Illness ■ R 11

Primary Insurance Address City, State, Zip Phone

Secondary Insurance Name Member I.D. Group Secondary Insurance Address City, State, Zip Phone

ICD-9 Code REQUIRED

Physicians (or other individuals authorized by law to order tests) should only order tests that are medically necessary for the diagnosis or treatment of the patient. Physician or physician's staff shall provide ICD-9 codes, rather than narrative diagnoses, for each test or panel. @ = Medicare Limited Coverage Tests. ICD-9 Code(s) Required. See reverse page for additional Medicare limited coverage tests.

AMA Defined Panels See reverse for components

9329	General Health Panel •	L, ST
142	Basic Metabolic Panel	ST
9179	Comp Metabolic Panel	ST
115	Electrolyte Panel (Na, K, Cl, CO2)	ST
173	Lipid Panel @ #	ST
9175	Liver Function Panel	ST
514	Obstetric Panel * @	2L, ST
9324	Kidney Function Panel	ST
9325	Acute Hepatitis Panel @	ST
3270	Drug Abuse Screen II *	CU

TESTS Specimen Type

3800	ABO & Rh Type	L
3550	ANA (Anti-Nuclear Abs) *	ST
2025	Amylase	ST
2208	BUN ■	ST
2209	Calcium	ST
1000	CBC with Differential with Platelets * @	L
4824	Ca 125 @	FS

3025	Carbamazepine (Tegretol)	PR
2645	CEA @	ST
2210	Cholesterol, Total @ #	ST
2075	CK	ST
3545	CRP (C-reactive Protein) #	ST
5083	CRP - High Sensitivity •	ST
2214	Creatinine	ST
3034	Digoxin (Lanoxin) @	ST
2675	Estradiol	ST
2090	Ferritin @	ST
2695	Folic Acid @ #	FS
2700	FSH ■	ST
2216	Gamma GT @	ST
2217	Glucose @ #	ST
2713	HCG Quantitative @	ST
2220	HDL Cholesterol @ #	ST
1041	Hemogram w/o Differential with Platelets @	L
1025	Hemoglobin @	L
1030	Hematocrit @	L

2708	Hgb A1C (Glycohemoglobin) @	L
4565	H. pylori, IgG Ab, Qualitative	ST
2725	Hepatitis A Antibody *	ST
2739	Hepatitis Bs Antigen	ST
2737	Hepatitis Bs Antibody	ST
4675	Hepatitis C Antibody	ST
3540	HIV-1 Antibody @	ST
4288	Homocysteine @ #	PE
2222	Iron @	ST
2118	Iron + IBC @ ■	ST
2128	Lithium	ST
2224	LDH	ST
2776	Luteinizing Hormone (LH)	ST
2130	Magnesium @	ST
3510	Mono Screen	ST
3064	Phenytoin (Dilantin)	PR
2228	Potassium	ST
2714	Pregnancy Test Serum HCG Qualitative	ST
2800	Prolactin	ST

2608	PSA, Total (Medicare Screen) #	ST
2606	PSA, Total (eg Medicare Diagnostic) @	ST
1425	PT (Prothrombin Time) @	B
1430	PTT Activated @	B
3505	Rheumatoid Factor	ST
1055	Sedimentation Rate (ESR)	L
3500	RPR w/Reflex Titer *	ST
2218	SGOT (AST) ■	ST
2219	SGPT (ALT)	ST
2830	Testosterone	ST
2817	T3, Uptake @ #	ST
4273	T3, Free	ST
2819	T4, Total @ #	ST
2823	T4, Free @ #	ST
2835	TSH @ #	ST
2233	Uric Acid	ST
1501	Urinalysis, Reflex Microscopic *	U
3094	Valproic Acid (Depakene)	ST
2840	Vitamin B-12 @ #	FS

2618	TAST-MS @	ST
5375	QUAD-MS @	ST
2621	AFP-MS @	ST

(Circle): Black White Hispanic Other Insulin dependent: Yes No Maternal weight: _____ Number of fetuses: _____
(Circle): NTD History: Yes No Gestational Age: _____ wks _____ days on date of US ___/___/___ Date of LMP ___/___/___
on date of Exam ___/___/___ (Circle) Initial or Repeat Test

MICROBIOLOGY Please Provide Source as indicated

6007	Culture, Routine *	Source	
6070	Culture, Acid Fast *	Source	
6010	Culture, Anaerobe *	Source	SW
6013	Culture, Blood *		BC
	Culture, Fungal *	Source	
6074	Culture, Fungus - Blood *		IS
6052	Culture, Genital-Routine *	Source	SW
6069	Culture, Herpes Simplex	Source	HS
6040	Culture, Sputum *		SP
6042	Culture, Stool *		SC
6047	Culture, Strep A *	Source	SW
6079	Culture, Strep B-Pregnancy *	Source	SW
6046	Culture, Throat *		SW
6049	Culture, Urine * @ ■		UC
4102	Chlamydia DNA Probe @	Source	GP
4100	GC DNA Probe @	Source	GP
5398	Chlamydia by PCR @	Source	M4
5396	GC by PCR @	Source	M4
7000	Ova & Parasites + Trichrome		OP
7015	Occult Blood Screen (1-3) #		HC
7017	Occult Blood Medicare Diagnostic (1-3) @		HC

* If indicated, reflex testing will be performed with additional charge(s) @ Medicare Limited Coverage # Medicare Frequency Limit • Not Covered by Medicare

ADDITIONAL TESTS (include ICD-9 Codes) / SPECIAL INSTRUCTIONS: 206

Add 4513 TPO Antibody

ST=SERUM SEPARATOR TUBE L=LAVENDER TOP TUBE PR=PLAIN RED TOP TUBE U=URINE CU=URINE CUP PE=EDTA PLASMA SW=SWAB HS=HERPES CULTURE SWAB M4=TUBE+SWAB GP=GENPROBE

Rev 01/2005

APPENDIX J:

Iodine Loading Regimen Handout, Flechas Family Practice Labs

FFP LABORATORY

Instructions for Collecting the 24-hour Loading Test

Kit Contents:

1 red Solo cup to catch urine in
2 small specimen bottles with pink labels
1 3-liter orange collection container
1 USPS bag and a zip lock bag to put samples in for shipping
1 Test Requisition Form (must be filled out and sent back with samples)

Things you should know:

Do not wash out preservative dye in specimen bottles!! This is not mold!!
Do not eat seaweed, seafood, or take vitamins with milligrams of iodine for 24 hours prior to doing this test or in the process of this test.
Do not do this test if you have blood in your urine unless it's a small amount. The test requisition form and indicated "Total Volume" is very important to send back.

FFP Lab does not accept insurance or insurance claims, nor do we give out Tax ID numbers or information to insurance companies. Results will be given to your Ordering Physician only, and please allow 3-5 business days after we receive your sample.

Collection for 24-hour Loading Test:

1.) Discard your first morning urine. (DO NOT COLLECT)

2.) After your first morning urine, take the four (12.5 mg) *Iodoral* tablets (at the same time or one 50 mg tablet) given to you by your health care provider, with or without food. This will begin your 24-hour period. Please collect every urination after taking these pills for 24 hours including the following morning's first urine of the day using the red Solo cup and pouring urine into the orange container. This orange container with urine in it must be refrigerated at all times!

3.) If you get close to filling up the orange collection container before your 24-hour period is up, turn the container up on its side to view the measuring lines, and make note of the amount of urine in the container (to the nearest 50 ml), and write this amount on one of the pink-labeled specimen tubes. Then gently shake container to stir urine, and then pour some urine into one of the two pink-labeled specimen tubes provided, and place the tube in the refrigerator. Then discard the remaining urine from the orange collection container, and begin collecting again for the remainder of the 24-hour period. When the 24-hour period is up, again note the amount of urine in the orange collection container (to the nearest 50 ml), and write this amount on the second of the two pink-labeled specimen tubes, and then fill the tube with urine from the orange collection container. NOTE: It is very important to write the two different volumes on each tube, and on your test requisition form you will see that if you have collected in two parts, to add the volumes together to reach a "total volume".

4.) If you did not fill up the orange container before your 24-hour period is up, you will only need to fill *one* of the pink-labeled specimen bottles. Record your volume on your test requisition form and on your pink specimen bottle(s). This concludes your test. Put all specimen bottles in the plastic bag, and package in white box with form. You may ship the kit back however you would like using any carrier, but we need to receive it in about seven days. Thank you.

Revised 1-22-14 Find: Revised Kit Forms/Desktop Title: 24 hour Load Instructions

Delivery:
Your Specimen's will be fine if you ship on any day of the week including Friday, we use a preservative dye in the bottles to preserve your urine during shipment. Once the urine is in the specimen bottles it is preserved for 14 days at room temperature. You are responsible for the cost of shipping your kit back whatever way you would like, United States Postal, UPS, or FedEx. **The shipping bag in your kit is not pre-paid.**

Your Test Requisition Form and Side Effects Reporting form:
Your test requisition form is **very** important. It must be filled out completely and sent back with your samples. It is very important to indicate what your total volume is. If you are unsure of how to measure your volume, call the lab and ask. Please print neatly so there will be no confusion in information that may cause a delay in testing. If you are responsible for paying FFP LABORATORY please make sure that your payment information sheet is with your samples and your requisition form unless you pre paid at the time of ordering. The side effects reporting form is for major side effects from the Lugo Tabs, if you did not experience any, than you do not need to indicate anything.

Toxic Metals and Bromide Spot and Provocation Testing:
We can do both of these tests from the same spot and 24-hour urine specimen you sent in for Iodine Testing. On your requisition form you will see the option to order these 2 tests. In order to have the Toxic Metals Test and the Bromide Test you must have approval from your doctor. If you are using Dr. Flechas as your physician we will get the signature for you. You **do not** need a signature for the Spot or 24 hour Loading Iodine Test.

Results:
Results will be sent to the Physician Only, we will not send them to patients. Please allow 3-5 business days from the day we receive your samples for Iodine Test, 14 days for Bromide test, and 3-4 weeks for Toxic Metals. If your doctor has stated that they have not received the results, have them call our lab and request for the results to be faxed at any time.

Insurance:
You, your physician or FFP Lab CANNOT submit this test to insurance companies. We do NOT give out Tax Id numbers to insurance companies or accept insurance. You are responsible for all fees. If you need a receipt for personal reasons, you must call and request for one, but please do not request for our Tax Id number, it will not be given out. We can only give out the CPT Code. You may have a receipt to take to your insurance company if they reimburse you, but we do NOT accept Insurance.

Our Contact Information:
If at any point in time you have a question or are unsure about something, please contact your Health Care Provider. If they cannot help you, please contact us with the following information:

[redacted]

FFP LABORATORY
576 Upward Rd., Suite 8
Flat Rock, NC 28731
[redacted]

NIS Testing Instructions

Check Your Kit

- Clinician Instructions and Patient Requisition Form
- 2 lg (10ml) Red Top Tubes
- 1 Transfer Tube (for serum)
- 1 Transfer Tube (for saliva)
- 2 Transfer Pipettes (1 for saliva, 1 for serum)
- 1 White Kit Box
- 1 White Carrier Box
- 1 Zip lock biohazard bag with absorbent towel
- 1 White Styrofoam Box for returning samples in carrier box
- Medicine Cup for saliva collection

If you are missing any of these items or if the tubes are expired please contact us for replacement. Keep the large white kit box for shipping specimens to FFP Lab.

Before the Collection

- This is a time-critical test. All procedures for this test must be scheduled so that the entire test will be completed Monday-Thursday only. The completed kit must be sent to FFP Lab the day blood is drawn to ensure delivery within 48hrs for lab processing.
- Arrange shipping prior to collection. (See shipping and packaging instructions)

Required

- Patient must ingest 50mg of Iodine 24hrs prior to collection of specimens. (Iodine is to be provided by Health Care Provider)
- All paperwork MUST be filled out and returned with the specimens. Please make sure that all labels and forms are filled out completely and neatly to ensure that there is no delay in processing your test. Please put your name on the transfer tubes that contain the serum/plasma and Saliva
- If the Patient is paying directly, and if your account is set up as "Patient Pay" please have patient provide all billing information with provided payment sheet.

Things you need to know

- Ship specimens to FFP Lab on the day of collection
- The transfer tubes contain a #3 green preservative dye that is NOT to be washed out so your sample can be sent by any carrier.
- The turn-a-round time for the test results is 5-7 business days.
- Dr. Flechas will provide consultation regarding test results if needed.
- We CANNOT process this test without proper paperwork and specimen collection

Serum Collection

1. Completely fill out Requisition Form and all labels.
2. Patient should ingest 50mg of Iodine 24hrs prior to collection.
3. Fill the 2 red top tubes using standard venipuncture technique.
4. Mix blood gently inverting both tubes 5-10 times.
5. Allow blood in the tubes to clot for 15 minutes while standing in a rack.
6. Centrifuge red top tubes for 10 minutes at 3,000 RPM.
7. Transfer all of the serum from both tubes into the screw cap red top tube and verify that the tube is labeled as "serum".
8. Make sure the tops on the tubes and serum transfer (screw top) are tightly closed and taped to avoid leakage and that they are labeled correctly. Place tubes into the Styrofoam box securely.

Saliva Collection

1. If the patient is a smoker, have him/her stop smoking 2 hrs before saliva collection.
2. Have the patient rinse mouth vigorously before collection.
3. Collect 5 to 8ml of saliva in the medicine cup provided.
4. Decant in plastic green screw top tube labeled saliva.
5. Make sure the top is closed tightly and taped to avoid leakage and that the tube it is labeled correctly.

Packaging and Shipping

- Once the specimens have been collected remove all contents from the Kit box.
- Place the sample tubes in position in the Styrofoam box.
- Place the Styrofoam box inside the small White Carrier box.
- Place the White Carrier box inside the provided Biohazard bag.
- Place the Biohazard bag (leave absorbent towel in the bag) with samples in it inside the White Kit Box.
- You are responsible for shipping. You can ship US Postal, FedEx or UPS.

It is very important that samples are collected properly and packaged properly. Please double check all return packaging and requisition forms to ensure everything has been done correctly and properly. If you have any questions about how this should be done please contact FFP Laboratory at 877-900-5556.

APPENDIX K:

Iodine Status Determination Requisition Forms

FFP Laboratory
576 Upward Rd. Suite 8
Flat Rock, NC 28731

Ship Date: 12-1-14

[redacted]

CTTAO: Dimarco

NIS REQUISTION FORM

Please print and fill out completely and return with your serum/saliva samples to FFP Laboratory. No urine is necessary for this test, serum and saliva only.

Last Name: _____M: _____ First Name: _____

Address: _____City _____

St: _____ Zip: _____Home Phone:_____/_____

*D.O.B: _____ Weight: _____ Height: _____ Smoker _____y _____n

Male: _____ Female: _____ History of Cancer: _____ yes _____ no

* Currently on Iodine Supplementation: _____yes _____no
If currently on Iodine Supplementation please indicate below how much and for how long:

Health Care Provider:

Name: _____

Address: _____ City: _____

St: ____Zip Code: _____Ph: _____ Fax: _____

Date Serum Collected: _____

Date Saliva Collected:_____

Health Provider Signature: _____ Date:_____

- Please sign that you approve this testing for your patient.

Revised 10-15-14 Find: Revised Kit Forms/Desktop Title: NIS Requisition Form

FFP Laboratory
576 Upward Rd. Suite 8
Flat Rock, NC 28731
[redacted]

24-Hour Loading Test Requisition Form

Patient/Client Information: **(PLEASE PRINT AND FILL OUT ALL INFORMATION)**

Last Name: _____ First Name: _____ Middle Initial: _____

Address: _____

City: _____ State: _____ Zip: _____ Phone: _____

Date of Birth: _____ Height: _____(in) Weight: _____

Sex: Male_____ Female____ **Currently on Iodine Supplement prior to this test _____ yes ____ no**
Iodine Supplement: _____ Dose: _____
How long have you been on this supplement: _____

Health Care Provider:

Name: _____

Address: _____

City: _____ State: _____ Zip: _____

Phone: _____ Fax: _____

Please check which or all tests you are sending your samples in for:

____ 24hr Loading Iodine _____ Toxic Metals _____ Fluoride Loading Only

____ Bromide Loading Only

Date Sample Collected: _____ **Total Volume**: _____

If Collected in 2 parts: 1st Volume collected: _____ 2nd Volume collected: _____
If you have collected in 2 parts, please indicate both collection volumes, add them together and then put the TOTAL Volume collected above, where it says "Total Volume".

For Health Care Provider ONLY:
To have a Toxic Metals/ Bromide/ or Fluoride Test you must sign this form:
I approve for my patient to have this testing done and for it to be charge to my account if my account is set up as CTTAO.
Health Provider Signature **ONLY**: _____ Date: _____
If samples are being sent in for Iodine ONLY you **do not** need to sign this form!

SHIPPED DATE: 11-4-14 **CHARGE TO THE ACCOUNT OF:** N. Dimarco/pre-pay

Revised 7-9-14 Revised Kit Forms/Desktop Title: 24 Hr. Load Requisition Form

FFP Laboratory
576 Upward Rd. Suite 8
Flat Rock, NC 28731

[redacted]

Side Effects Reporting Form (IOD-01)

If a serious side effect from Lugo Tabs Iodine/Iodide Supplement should occur, such as something that would require hospitalization, please fill out this report.

Date: _____ **if you do not experience any side effects, you do not have to fill out this form**.

Side effects that may occur but do not have to be recorded are: headache, brassy taste, acne like skin rash, and or gastric distress. These side effects are common with detoxification of Bromide.
Client/Customer Information:

Last Name: _____ First Name: _____ Middle Initial: _____

Date of Birth: _____ Weight: _____ Height: _____ Sex: Male ___ Female ___

Phone: _____

Product Information: Put the number stamped on the cellophane packet of your 4 tablets here:

Batch/Lot # _____ If you do not have a lot number on your pack, then disregard.
Side Effects Information:

A) Describe Symptoms: _____
_____
_____
B) Time Intervals between ingestion and symptoms:
Months: _____ Days: _____ Hours: _____ Minutes: _____
C) Number of tablets ingested at one time: _____
D) Number of tablets taken per day: _____
E) Date tablet was first ingested: _____

Examining Physician Information:

Name: _____ Address: _____

City: _____ State: _____ Zip: _____

Date of Examination: _____
Findings: _____
_____
_____

Recommendations: _____
_____

Revised 10-15-14 Find: Revised Kit Forms/Desktop Title: Side Effects Reporting Form (IOD-01)

APPENDIX L:

Physical Activity Questionnaire

Physical Activity Questionnaire

Most individuals find that the questionnaire can be completed in approximately 20-30 minutes. Replies are important from all Pioneer Project participants, regardless of health or exercise status. Be as accurate as possible, but provide your best estimate if you do not remember precisely. All responses will be kept strictly confidential like your other Pioneer Project records.

This questionnaire is used with the permission of The Cooper Institute for Aerobics Research.

In this section we would like to ask you about your current physical activity and exercise habits that you perform regularly, at least once a week. Please answer as accurately as possible. Circle your answer or supply a specific number when asked.

EXERCISE/PHYSICAL ACTIVITY

For the last **three months**, which of the following moderate or vigorous activities have you performed **regularly?** (*Please fill in the bubble for **YES** for all that apply and **NO** if you do not perform the activity; provide an estimate of the amount of activity for all marked **YES**. Be as complete as possible.*)

1. Walking

- ⬭ Yes
- ⬭ No **(Skip to Question 2)**

1.1 How many sessions per week?

☐☐

1.2 How many miles (or fractions) per session?

☐☐.☐☐

1.3 Average duration per session?

☐☐☐ min.

1.4 What is your usual pace of walking?

- ⬭ Casual or Strolling (< 2 mph)
- ⬭ Average or Normal (2-3 mph)
- ⬭ Fairly Brisk (3-4 mph)
- ⬭ Brisk or Striding (4 mph +)

2. Stair Climbing

- ⬭ Yes
- ⬭ No **(Skip to Question 3)**

2.1 How many flights of stairs do you climb **UP** each day? (1 flight = 10 steps)

☐☐

3. Running or Jogging

- ⬭ Yes
- ⬭ No **(Skip to Question 4)**

3.1 How many sessions per week?

☐☐

3.2 How many miles (or fractions) per session?

☐☐.☐☐

3.3 Average duration per session?

☐☐☐ min.

4. Treadmill
   - ○ Yes
   - ○ No **(Skip to Question 5)**

   4.1 How many sessions per week? ☐☐

   4.2 Average duration per session? ☐☐☐ min.

   4.3 Speed? ☐☐.☐☐ (mph)

   4.4 Grade? ☐☐.☐☐ (%)

5. Bicycling
   - ○ Yes
   - ○ No **(Skip to Question 6)**

   5.1 How many sessions per week? ☐☐

   5.2 How many miles per session? ☐☐.☐☐

   5.3 Average duration per session? ☐☐☐ min.

6. Swimming Laps
   - ○ Yes
   - ○ No **(Skip to Question 7)**

   6.1 How many sessions per week? ☐☐

   6.2 How many miles per session?
   (880 yds = 0.5 miles) ☐☐.☐☐

   6.3 Average duration per session? ☐☐☐ min.

7. Aerobic Dance/Calisthenics/Floor Exercise
   - ○ Yes
   - ○ No **(Skip to Question 8)**

   7.1 How many sessions per week? ☐☐

   7.2 Average duration per session? ☐☐☐ min.

8. Moderate Sports (e.g. Leisure volleyball, golf (not riding), social dancing, doubles tennis)
   - ○ Yes
   - ○ No **(Skip to Question 9)**

   8.1 How many sessions per week? ☐☐

   8.2 Average duration per session? ☐☐☐ min.

9. Vigorous Racquet Sports (e.g. Racquetball, singles tennis)

- ○ Yes
- ○ No **(Skip to Question 10)**

9.1 How many sessions per week?

☐☐

9.2 Average duration per session?

☐☐☐ min.

10. Other Vigorous Sports or Exercise Involving Running (e.g. Basketball, soccer)

- ○ Yes Specify:_____
- ○ No **(Skip to Question 11)**

10.1 How many sessions per week?

☐☐

10.2 Average duration per session?

☐☐☐ min.

11. Other Activities

- ○ Yes Specify:_____
- ○ No **(Skip to Question 12)**

11.1 How many sessions per week?

☐☐

11.2 Average duration per session?

☐☐☐ min.

12. Weight Training (Machines, free weights)

- ○ Yes
- ○ No **(Skip to Question 13)**

12.1 How many sessions per week?

☐☐

12.2 Average duration per session?

☐☐☐ min.

13. Household Activities (Sweeping, vacuuming, washing clothes, scrubbing floors)

- ○ Yes
- ○ No **(Skip to Question 14)**

13.1 How many hours per week?

☐☐.☐☐

14. Lawn Work & Gardening

- ○ Yes
- ○ No **(Skip to Question 15)**

14.1 How many hours per week?

☐☐.☐☐

15. How many times a week do you engage in vigorous physical activity long enough to work up a sweat?

☐☐☐

APPENDIX M:

Physical Activity Questionnaire MET Scoring

MET SCORING for PA QUESTIONNAIRE- IODINE STUDY

ACTIVITY	MET SCORE	MET CODE
1. WALKING		
Casual/ Strolling (<2mph)	2.0	17151
Average/ Normal (2-3mph)	3.0	17170
Fairly Brisk(3-4mph)	5.0	17220
Brisk /Striding (4mph=+)	7.0	17230
2. STAIR CLIMBING		
Slow Pace	4.0	17133
Fast Pace	8.8	17134
3. JOGGING	7.0	12020
3. RUNNING	8.0	Taylor Code 200
4mph	6.0	12029
5mph	8.3	12030
6mph	9.8	12050
7mph	11.0	12070
8mph	12.8	12110
9mph	14.5	12120
10mph	16.0	12130
11mph	19.0	12132
12mph	19.8	12134
13mph	23.0	12135
14mph		
4. TREADMILL	9.0	02065
5. BICYCLING	7.5	01015
6. SWIMMING		
Leisurely, Not Laps	6.0	18310
Laps, Vigorous	9.8	18230
7. CONDITIONING EXERCISE		
AEROBIC DANCE	7.3	03015
CALISTHENICS	6.0	02050 (Taylor Code 210)
FLOOR EXERCISE	3.5	02030 (Taylor Code 150)
8. MODERATE SPORTS		
Leisure Volleyball	3.0	15720
Golf (not riding)	4.8	15255
Social Dancing	7.8	13031
Doubles tennis	4.5	15685
9. VIGOROUS RACQUET SPORT		
Racquet Ball	7.0	15530
Singles Tennis	8.0	15690 (Taylor Code 420)
10. OTHER VIGOROUS SPORTS OR EXERCISE INVOLVING RUNNING		
Basket Ball	6.5	15055
Soccer, Casual/General	7.0	15610 (Taylor Code 540)
Soccer, Competitive	10.0	15605
11. OTHER ACTIVTIES		
12. WEIGHT TRAINING		
Machines	5.0	02061
Free Weights	6.0	02050 (Taylor Code 210)
13. HOUSEHOLD ACTIVITIES		
Sweeping	3.3	05010
Vacuuming	3.3	05043
Washing clothes	2.0	05090

MET SCORING for PA QUESTIONNAIRE- IODINE STUDY

Scrubbing floors (moderate effort)	3.5	05130
14. LAWN Work and GARDENING		
Gardening, general	3.8	08245
Lawn mowing	5.5	08095

SCORING:

1. Assign MET Score to activity
2. Multiply # of workouts/week **BY** # of minutes/workout
3. GET: MET minutes/week for each activity

MET min/wk for each activity = #workouts/wk times #min/workout

APPENDIX N:

Three-day Dietary Record

3-Day Dietary Record
Day 1

Breakfast	Serving Size	Lunch	Serving Size	Dinner	Serving Size
Morning Snacks	**Serving Size**	**Afternoon Snacks**	**Serving Size**	**Evening/Bed time Snacks**	**Serving Size**

3-Day Dietary Record
Day 2

Breakfast	Serving Size	Lunch	Serving Size	Dinner	Serving Size
Morning Snacks	**Serving Size**	**Afternoon Snacks**	**Serving Size**	**Evening/Bed time Snacks**	**Serving Size**

3-Day Dietary Record

Day 3

Breakfast	Serving Size	Lunch	Serving Size	Dinner	Serving Size
Morning Snacks	**Serving Size**	**Afternoon Snacks**	**Serving Size**	**Evening/Bed time Snacks**	**Serving Size**

APPENDIX O:

Sample Three-day Dietary Record Analysis Report Axxya Systems Nutritionist Pro™

Client Diet Record Nutrition Summary

First: [redacted]
Middle:
Last:
Company:

Female
Identification Number: [redacted]
Date of Birth: [redacted]
Height: 5 ft. 6 in. **Weight:** 129.80 lb.

Total Days: 3 **Total Foods:** 34
Avg. Daily Kcals: 2978.091 **Diet Name:** Baseline 3-day

Macronutrients	Value	Unit	Goal	%
Kilocalories	2978.091	kcal		*
Protein	138.545	g		*
Carbohydrate	245.271	g		*
Fat, Total	150.330	g		*
Alcohol	19.675	g		*
Cholesterol	512.634	mg		*
Saturated Fat	52.635	g		*
Monounsaturated Fat	12.483	g		*
Polyunsaturated Fat	3.272	g		*
MFA 18:1, Oleic	11.718	g		*
PFA 18:2, Linoleic	2.346	g		*
PFA 18:3, Linolenic	0.349	g		*
PFA 20:5, EPA	0.143	g		*
PFA 22:6, DHA	0.305	g		*
Dietary Fiber, Total	19.498	g		*
Sugar, Total	48.433	g		*

Vitamins	Value	Unit	Goal	%
Vitamin A (RE)	218.500	RE		*
Beta-Carotene	179.137	µg		*
Vitamin C	33.736	mg		*
Vitamin D (ug)	0.538	µg		*
Vitamin E (mg)	0.155	mg		*
Alpha-Tocopherol	2.638	mg		*
Thiamin	0.219	mg		*
Riboflavin	0.624	mg		*
Niacin	6.816	mg		*
Pyridoxine (Vitamin B6)	0.813	mg		*
Folate (Total)	63.420	µg		*
Cobalamin (Vitamin B12)	0.767	µg		*
Biotin	4.817	µg		*
Pantothenic Acid	2.421	mg		*
Vitamin K	22.914	µg		*

Amino Acids	Value	Unit	Goal	%
Tryptophan	214.632	mg		*
Threonine	778.079	mg		*
Isoleucine	868.522	mg		*
Leucine	1532.908	mg		*
Lysine	1551.563	mg		*
Methionine	485.940	mg		*
Cystine	197.522	mg		*
Phenylalanine	796.225	mg		*
Tyrosine	653.258	mg		*
Valine	1035.064	mg		*
Histidine	574.181	mg		*

Minerals	Value	Unit	Goal	%
Sodium	4489.564	mg		*
Potassium	1476.430	mg		*
Calcium	290.621	mg		*
Iron	3.610	mg		*
Phosphorus	389.381	mg		*
Magnesium	114.192	mg		*
Zinc	1.703	mg		*
Copper	0.353	mg		*
Manganese	1.962	mg		*
Selenium	28.623	µg		*
Chromium	0.007	mg		*
Molybdenum	2.292	µg		*

(No Goal Value)*

Exchanges

Bread/Starch	12.00
Fat	18.00
Fruit	2.00
Meat-Lean	10.00
Meat-Medium Fat	1.00
Meat-Very Lean	3.00
Milk-Skim	0.00
Other Carbohydrate	3.00
Vegetable	2.00

Percentage Of Kcals

Client Diet Record Nutrient Analysis

First: [redacted]
Middle:
Last:
Company:

Female
Identification Number: [redacted]
Date of Birth: [redacted]
Height: 5 ft. 6 in. **Weight:** 129.80 lb.

Total Days: 3
Total Foods: 34
Avg. Daily Kcals: 2978.091

Diet Name: Baseline 3-day

Nutrient	Value	Unit	Goal	%
Weight	2638.281	g		
Kilocalories	2978.091	kcal		
Protein	138.545	g		
Carbohydrate	245.271	g		
Fat, Total	150.330	g		
Alcohol	19.675	g		
Cholesterol	512.634	mg		
Saturated Fat	52.635	g		
Monounsaturated Fat	12.483	g		
Polyunsaturated Fat	3.272	g		
MFA 18:1, Oleic	11.718	g		
PFA 18:2, Linoleic	2.346	g		
PFA 18:3, Linolenic	0.349	g		
PFA 20:5, EPA	0.143	g		
PFA 22:6, DHA	0.305	g		
Trans Fatty Acid	2.982	g		
Sodium	4489.564	mg		
Potassium	1476.430	mg		
Vitamin A (RE)	218.500	RE		
Vitamin A (IU)	1477.992	IU		
Vitamin A (RAE)	169.280	µg		
Beta-Carotene	179.137	µg		
Alpha-Carotene	76.996	µg		
Lutein (+ Zeaxanthin)	1126.031	µg		
Beta-Cryptoxanthin	1.571	µg		
Lycopene	0.018	µg		
Vitamin C	33.736	mg		
Calcium	290.621	mg		
Iron	3.610	mg		
Vitamin D (ug)	0.538	µg		
Vitamin D (IU)	21.537	IU		
Vitamin E (mg)	0.155	mg		
Vitamin E (IU)	0.231	IU		
Alpha-Tocopherol	2.638	mg		
Thiamin	0.219	mg		
Riboflavin	0.624	mg		
Niacin	6.816	mg		
Pyridoxine (Vitamin B6)	0.813	mg		
Folate (Total)	63.420	µg		
Folate (DFE)	63.412	µg		
Cobalamin (Vitamin B12)	0.767	µg		
Biotin	4.817	µg		
Pantothenic Acid	2.421	mg		
Vitamin K	22.914	µg		

Nutrient	Value	Unit	Goal	%
Phosphorus	389.381	mg		
Iodine		µg	150.000	
Magnesium	114.192	mg		
Zinc	1.703	mg		
Copper	0.353	mg		
Manganese	1.962	mg		
Selenium	28.623	µg		
Fluoride	1718.693	µg		
Chromium	0.007	mg		
Molybdenum	2.292	µg		
Dietary Fiber, Total	19.498	g		
Soluble Fiber	0.412	g		
Insoluble Fiber	1.096	g		
Crude Fiber	0.951	g		
Sugar, Total	48.433	g		
Glucose	6.045	g		
Galactose	0.031	g		
Fructose	5.974	g		
Sucrose	3.619	g		
Lactose	2.142	g		
Maltose	0.096	g		
Sugar Alcohol	0.000	g		
Other Carbohydrate	0.000	g		
Tryptophan	214.632	mg		
Threonine	778.079	mg		
Isoleucine	868.522	mg		
Leucine	1532.908	mg		
Lysine	1551.563	mg		
Methionine	485.940	mg		
Cystine	197.522	mg		
Phenylalanine	796.225	mg		
Tyrosine	653.258	mg		
Valine	1035.064	mg		
Arginine	993.617	mg		
Histidine	574.181	mg		
Alanine	983.942	mg		
Aspartic Acid	1973.131	mg		
Glutamic Acid	3182.428	mg		
Glycine	781.824	mg		
Proline	917.595	mg		
Serine	820.185	mg		
Moisture	1460.169	g		
Ash	6.887	g		
Caffeine	195.387	mg		

Nutrient Goal Template
(Client)

Analyzed by
Baseline 3-day

Exchanges

Bread/Starch	12.00
Fat	18.00
Fruit	2.00
Meat-Lean	10.00
Meat-Medium Fat	1.00
Meat-Very Lean	3.00
Milk-Skim	0.00
Other Carbohydrate	3.00
Vegetable	2.00

APPENDIX P:

Health History Questionnaire

Texas Woman's University Health Questionnaire

Name _____ Date _____
(Last) (First) (Middle) PH/Home (____) _____
Address _____ City _____ ST ___ Zip _____ PH /Work (____) _____
Birth Date ____ / _____ / _____ Sex _____ Height _____ Weight _____ Occupation _____
Ethnicity: Caucasian _____ Hispanic _____ African American _____ Asian _____ Other _____
Closest Relative or Friend _____ PH (____) _____
Dentist _____ PH (____) _____ Doctor _____ PH (____) _____
Date, Type & Number of Last Dental X-Rays _____ Date & Type of Last Medical X-Rays _____

		Circle One	Notes
(1)	Have you been hospitalized or had a serious illness?	Yes No	
(2)	Have you been under the care of a physician during the past 2 years?	Yes No	
(3)	Have you taken any kind of medicine or drugs during the past 2 years?	Yes No	
(4)	Are you allergic to penicillin, any drugs, medicine, or latex?	Yes No	
(5)	Have you ever had excessive bleeding which required special treatment?	Yes No	
(6)	Women: Is there a chance you might be pregnant?	Yes No	
(7)	Women: Are you taking any birth control medication?	Yes No	
(8)	Have you had adverse reaction to local anesthetics?	Yes No	

(9) Date of last medical exam _____

(10) What prescribed medications, herbs, vitamins or over the counter medications are you taking?

_____ reason _____ _____ reason _____
_____ reason _____ _____ reason _____
_____ reason _____ _____ reason _____

(11) Do you use recreational drugs?_____ If yes, what?_____ Have you ever been in drug rehab or counseling? _____

(12) Do you use tobacco? _____ If so what form? _____

(13) Circle Yes to any of the following which you have had or have at present. Circle No to those that you have not had.

Yes	No	High Blood Pressure	Yes	No	Tuberculosis (TB) Emphysema
Yes	No	Stroke	Yes	No	Emphysema
Yes	No	Heart Pacemaker	Yes	No	Asthma
Yes	No	Heart Failure	Yes	No	Hay Fever
Yes	No	Heart Disease or Attack	Yes	No	Allergies or Hives
Yes	No	Angina Pectoris	Yes	No	Sinus Trouble
Yes	No	Fen-phen Redux use	Yes	No	Cancer
Yes	No	Artificial Heart Valve	Yes	No	Leukemia or Lymphoma
Yes	No	Congenital Heart Lesions	Yes	No	Radiation or Chemotherapy
Yes	No	Mitral Valve Prolapse	Yes	No	Anemia
Yes	No	Heart Surgery	Yes	No	Bruise Easily
Yes	No	Lupus	Yes	No	Bleeding Disorders
Yes	No	Rheumatic Fever	Yes	No	Sickle Cell Disease
Yes	No	Scarlet Fever	Yes	No	Alcoholism
Yes	No	Heart Murmur	Yes	No	Drug Addiction
Yes	No	Artificial Joints	Yes	No	Blood Transfusion
Yes	No	Kidney Dialysis	Yes	No	Liver Disease
Yes	No	Kidney Disease	Yes	No	Yellow Jaundice
Yes	No	Eating Disorders	Yes	No	Hepatitis
Yes	No	Rheumatoid Arthritis	Yes	No	AIDS /HIV Infection
Yes	No	Arthritis	Yes	No	Cold Sores /Fever Blisters
Yes	No	Pain in jaw /TMJ Dysfunction	Yes	No	Psychiatric Treatment
Yes	No	Chronic head, neck, or back pain	Yes	No	Depression /Bipolar
Yes	No	Diabetes	Yes	No	Nervousness /Anxiety
Yes	No	Hypoglycemia	Yes	No	Fainting or Dizzy Spells
Yes	No	Thyroid Disease	Yes	No	Epilepsy or Seizures
Yes	No	Ulcers	Yes	No	Condition Requiring Cortisone Medicine
Yes	No	Pulmonary Disease	Yes	No	Glaucoma
Yes	No	Chronic Cough or Bronchitis	Yes	No	Dental Implants

(14) Please list any disease, condition, or problem you have that is not listed above? _____

Please turn over and complete other side

		Circle One	Notes
(15)	Are you having oral pain or dental discomfort at this time?	Yes No	
(16)	Are you nervous about having dental treatment?	Yes No	
(17)	Have you had a bad experience in the dental office?	Yes No	
(18)	When did you last have dental work? _____	Teeth Cleaned. _____	
(19)	How many times a day do you brush your teeth?_____	How often do you floss? _____	
(20)	What type of toothbrush do you use? Soft ____ Medium ____ Hard____	Other hygiene aids? _____	
(21)	What type of toothpaste do you use? _____		
(22)	What is your chief dental complaint?_____		

"I Attest To The Fact That The Information Given Above Is Correct And I Consent To Receive Clinical Services."

_____ What is the best time to contact you by phone? _____

(Parent or Guardian must sign for patient under age 18.) E-mail address: _____

Date	Change in Medical History	Patient Signature	DH Student Signature	Instructor Signature

Thank you for completing this form.

This section for office use only:

Date	Rx/Herbal	Dosage	Reason	Contraindications	Side effects with implications for dental treatment

Additional comments:

Date: _____

APPENDIX Q:

Demographic Questionnaire

IWH Wellness & Sport Evaluation Program
Demographic Questionnaire

Name: _____ **Date:** ____/____/20____
(Last) (First) (Middle)

Phone: (____) _____ **Email:** _____

Address: _____ **City** _____ **ST** _____ **Zip** _____

How would you prefer we contact you? ❑ Phone ❑ Email ❑ Mail

Date of Birth: ____ / ____ / _____ **Gender:** ❑ Male ❑ Female

Ethnicity: (Check all that apply)
❑ African American
❑ American Indian
❑ Asian/Pacific Islander
❑ Caucasian (non-Hispanic)
❑ Hispanic
❑ Scandinavian
❑ Other: _____

What is the highest level of education you have attained? (Please mark only one)
❑ Less than a high school diploma
❑ High school graduate
❑ Some college or technical training
❑ Associate's degree or equivalent
❑ Bachelor's degree
❑ Graduate degree

What is your present work situation? (Check all that apply)
❑ Employed full-time
❑ Employed part-time
❑ Semi-retired
❑ Fully-retired
❑ Self-employed
❑ Unemployed
❑ Homemaker
❑ Student
❑ On disability
❑ Other: _____

Are or were you a student at Texas Woman's University?
❑ Yes, I am a current student
❑ Yes, but I am not currently enrolled in any courses
❑ Yes, I am a TWU alumnus
❑ No

Are you a current employee of Texas Woman's University?
❑ Yes ❑ No

Please provide the name of a close relative or friend that we may contact, if necessary.

Name: _____ **Phone:** (____) _____

Please provide the name of your physician.

Name: _____ **Phone:** (____) _____

For office use:
Is physician clearance required?
❑ Yes ❑ No
Proof of physician clearance provided:
❑ Yes ❑ No
Approved by: _____

Proof of TWU employee/student status provided:
❑ Yes ❑ No
Approved by: _____

APPENDIX R:

Thyroid Stimulating Hormone ELISA Procedure, ALPCO Handout

ALPCO™
IMMUNOASSAYS

TSH (Thyroid Stimulating Hormone) ELISA

For the quantitative determination of TSH in human serum.

For "*In Vitro* Diagnostic" use within the United States of America.
This product is for "Research Use Only" outside of the United States of America.

Catalog Number:	25-TSHHU-E01
Size:	96 wells
Version:	030104 - ALPCO September 16, 2011

ALPCO Diagnostics

[redacted]

INTENDED USE

For the quantitative determination of thyroid stimulating hormone (TSH) concentration in human serum. The assay is useful in the diagnosis of thyroid or pituitary disorders.

INTRODUCTION

The determination of serum or plasma levels of thyroid stimulating hormone (TSH or thyrotropin) is recognized as a sensitive method in the diagnosis of primary and secondary hypothyroidism.[1] TSH is secreted by the anterior lobe of the pituitary gland and induces the production and release of thyroxine (T4) and triiodothyronine (T3) from the thyroid gland.[2] It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta.[3]

Although the concentration of TSH in the blood is extremely low, it is essential for the maintenance of normal thyroid function. The release of TSH is regulated by a TSH-releasing hormone (TRH) produced by the hypothalamus. The levels of TSH and TRH are inversely related to the level of thyroid hormone. When there is a high level of thyroid hormone in the blood, less TRH is released by the hypothalamus, so less TSH is secreted by the pituitary. The opposite action will occur when there is decreased thyroid hormone in the blood. This process is known as a negative feedback mechanism and is responsible for maintaining the proper blood levels of these hormones.[4,5]

TSH and the pituitary glycoproteins: luteinizing hormone (LH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG), have identical alpha chains.[2] The beta chains are distinct but do contain regions with identical amino acid sequences. These regions of homology can cause considerable cross-reactivity with some polyclonal TSH antisera. The use of a monoclonal antibody in this TSH ELISA test eliminates this interference, which could result in falsely elevated TSH values in either menopausal or pregnant females, a population whose evaluation of thyroid status is clinically significant.[6,7,8]

PRINCIPLE OF THE ASSAY

This TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.[9,10] The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase immobilization (microtiter wells), and goat anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60-minute or overnight incubation at room temperature, the solid phase is washed with water to remove unbound labeled antibodies. A solution of 3,3',5,5'-Tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of TSH is directly proportional to the color intensity of the test sample.

REAGENTS AND MATERIALS PROVIDED

1. *Antibody-Coated Wells (1 plate, 96 wells)*
   Microtiter wells coated with mouse monoclonal anti-TSH.
2. *Enzyme Conjugate Reagent (1 dropper bottle, 13 mL)*
   Contains goat anti-TSH conjugated to horseradish peroxidase.
3. *Reference Standard Set (1 mL/vial)*
   Contains 0, 0.5, 2.0, 5.0, 10.0 and 25.0 μIU/mL (WHO, 2nd IRP, 80/558) TSH in equine serum with preservatives. Lyophilized. See instructions for reconstitution under Reagent Preparation.
4. *TMB Reagent (1 bottle, 11 mL)*
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
5. *Stop Solution (1N HCl) (1 bottle, 11 mL)*
   Contains diluted hydrochloric acid.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes: 50 µL, 100 µL, 200 µL, and 1 mL
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450nm.
5. Orbital motion microtiter well shaker, capable of shaking at a speed of 175±25 RPM
6. Absorbent paper
7. Graph paper (semi-log, etc.)
8. Quality control material

WARNINGS AND PRECAUTIONS

1. **CAUTION:** This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[21]
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For in vitro diagnostic use.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10nm or less and an optical density range of 0 to 2 OD org reater at 450 nm wavelength is acceptable for absorbance measurement.

SPECIMEN COLLECTION AND PREPARATION

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. Avoid grossly hemolytic, lipemic, or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Reconstitute each lyophilized standard with 1.0mL dH_20. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2-8°C, and are stable at 2-8°C for at least 30 days.
4. Specimens expected to have a TSH concentration greater thatn 25µIU/mL should be diluted 1:10 with TSH-free serum (Zero calibrator).

PROCEDURAL NOTES

1. Manual Pipetting: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100µl of standards, specimens, and controls (not included in kit) into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing.
5. Incubate at room temperature (18-25°C) for 60 minutes (1 hour).
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled or dionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature, for 20 minutes.
11. Stop the reaction by adding 100µl of Stop Solution to each well.
12. Gently mix for 30 seconds. ***Ensure that all of the blue color changes completely to yellow.***
13. Read absorbance at 450nm with a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

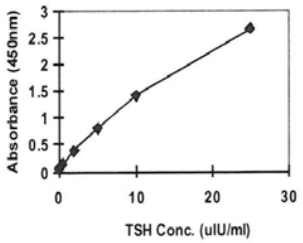
1. Calculate the mean absorbance value (OD_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in µIU/mL, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of TSH in µIU/ml from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run of the assay are shown below. The standard curve is for illustration only, and should not be used to calculate unknowns. The standard curve covers a dynamic range from 0 to 25 μIU/mL.

The absorbance (450nm) value can be varied due to incubation at different room temperatures in different laboratories.

TSH (μIU/mL)	Absorbance (450nm)
0	0.063
0.5	0.157
2.0	0.398
5.0	0.818
10.0	1.415
25.0	2.645



EXPECTED VALUES

Each laboratory should establish its own normal range based on patient population. Differences in assay technique and the use of various standards may affect results. The results provided below are based on 38 normal and 77 hyperthyroid blood specimens. The ranges were determined from the mean ± 2SD (μIU/mL TSH). These values may differ from other published data.

	Normal	Hyperthyroid
N	38	77
Mean TSH (μIU/mL)	1.44	< 0.2
Range	0.3 - 8.1	< 0.2

NORMAL REFERENCE RANGE

The reference ranges provided below are based on Tietz.[12]

	TSH (μU/mL)
Adults	
21-54 years	0.4-4.2
55-87 years	0.5-8.9
Pregnancy	
1st Trimester	0.3-4.5
2nd Trimester	0.5-4.6
3rd Trimester	0.8-5.2

PERFORMANCE CHARACTERISTICS

1. **Accuracy**
   This TSH ELISA has been compared to a comparable commercially available TSH test. The methods are similar in that they are both used for the quantitative determination of TSH in human serum, and both use TSH calibrated and labeled in µIU/ml standardized to the WHO hTSH (2nd IRP, 80/558).

   A study was conducted using 39 euthyroid and 20 hypothyroid patient samples (as determined by hospital laboratory analysis). The range of samples tested was 0.4 µIU/mL to 63 µIU/mL. The comparison demonstrated good correlation with the other kit as shown below:

   N = 59
   Correlation coefficient = 0.994
   Slope = 1.027
   Intercept = 0.787
   25-TSHHU-E01 Mean = 8.39 µIU/mL
   Comparison Mean = 9.41 µIU/ml

   Another 77 hyperthyroid patient samples also correlated well with the comparison test kit:

	# Samples with [TSH] ≤ 0.2 µIU/mL
Comparison Kit	N = 77
25-TSHHU-E01	N = 77

2. **Sensitivity**
   At TSH concentrations of 0.1 µIU/mL and 0.2 µIU/mL, the interassay CVs were determined to be 11.4% and 7.9%, respectively.

3. **Precision**
   a. *Intra-Assay Precision*
      Within-run precision was determined by replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Serum Sample	**1**	**2**	**3**	**4**
Number of Replicates	28	26	26	26
Mean TSH (µIU/ml)	0.62	1.51	15.48	26.14
Standard Deviation	0.03	0.09	0.39	0.86
Coefficient of Variation (%)	4.6%	5.7%	2.5%	3.3%

   b. *Inter-Assay Precision*
      Between-run precision was determined by replicate measurements of four different serum samples over a series of individually calibrated assays as shown below:

Serum Sample	**1**	**2**	**3**	**4**
Number of Replicates	30	24	24	24
Mean TSH (µIU/ml)	0.64	1.46	15.38	25.26
Standard Deviation	0.05	0.10	0.87	1.75
Coefficient of Variation (%)	7.6%	7.1%	5.7%	8.9%

4. Recovery and Linearity Studies

a. Recovery

Various patient serum samples of known TSH levels were combined and assayed in duplicate. The mean recovery was 98.9%.

	Expected Conc. (µIU/ml)	Observed Conc. (µIU/ml)	% Recovery
	23.94	22.92	95.7%
	11.75	11.01	93.6%
	5.46	5.53	101.3%
	2.76	3.04	110.1%
	1.48	1.39	93.9%

b. Linearity

Two patient samples were serially diluted with zero standard to determine linearity. The mean recovery was 94.6%.

#	Dilution	Expected Conc. (µIU/ml)	Observed Conc. (µIU/ml)	% Expected
1.	Undiluted		48.39	
	1:2	24.42	22.43	91.9%
	1:4	11.21	10.45	93.2%
	1:8	5.60	5.13	91.6%
	1:16	2.80	2.87	102.5%
	1:32	1.40	1.30	92.9%
				Average = 94.4%
2.	Undiluted		36.42	
	1:2	18.21	18.10	99.4%
	1:4	9.10	8.48	93.2%
	1:8	4.55	4.28	94.1%
	1:16	2.27	2.22	97.8%
	1:32	1.13	1.01	89.4%
				Average = 94.8%

6. Specificity

The following hormones were tested for cross-reactivity:

HORMONE TESTED	CONCENTRATION	PRODUCED INTENSITY EQUIVALENT TO TSH (µIUmL)
HCG - (WHO 2nd IS 61/6)	100 mIU/mL	0
	600 mIU/mL	0
	3,500 mIU/mL	0
	10,000 mIU/mL	0
	200,000 mIU/mL	0
FSH - (WHO 2nd IRP-HMG)	20 mIU/mL	0
	100 mIU/mL	0
	200 mIU/mL	<0.2
LH - (WHO 1st IRP 68/40)	75 mIU/mL	0
	150 mIU/mL	0
	300 mIU/mL	<0.2
Prolactin - (WHO 1st IRP 75/504)	10 ng/mL	0
	50 ng/mL	0
	200 ng/mL	<0.2
hGH - (WHO 1st IRP 65/217)	10 ng/mL	0
	50 ng/mL	0
	200 ng/mL	<0.2

5. **Hook Effect**
   No hook effect is observed in this assay at TSH concentrations up to 1,000 μIU/mL.

QUALITY CONTROL

Good laboratory practice requires that low, mediumm and high quality control specimens (controls) be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

STANDARDIZATION

The TSH Reference Standards are calibrated against the World Health Organization's Second International Reference Preparation of hTSH 2nd IRP-80/558).

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. This TSH assay has not been tested on newborns, and is not for use in screening newborns.

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THYROID STIMULATING HORMONE (TSH) ELISA PROCEDURE
(ALPCO Immunoassays 25-TSHHUU-E01)

IMPORTANT NOTES:

- Mix all reagents by gentle inversion or swirling prior to use. DO NOT induce foaming
- Manual Pipetting- Use **no more than 32 wells** for each assay run
- Pipetting of all standards, samples, and controls **MUST** be completed **within 3 min**
- **DO NOT** use tap water to wash plate
- **WASHING VERY CRITICAL STEP FOR THIS ASSAY- MAKE SURE TO DECANT AND WASH PROPERLY TO AVOID FALSE ELEVATION IN ABSORBANCE VALUES**
- Read plate **within 15 min** of adding stop solution

Preparation of Reagents, Standard, and Samples

Thaw Samples:

1. Begin thawing samples
2. Bring all reagents, serum references and controls (IS0104), and samples to room temperature 18-25 ^{0}C

Reconstitute Standards (at least 20 min before adding to the plate):
(Prepared standards can be stored sealed at 2-8 ^{0}C and are stable for at least 30 days)

3. Add 1 ml **(1000 µl)** of distilled or DI water to all standards
4. Mix gently by gentle inversion or swirling
5. Allow reconstituted standards to **stand** for **at least 20 min**

Standards (Serum Reference Standards):
6. Provided in the kit (1ml each)- See above for instructions to reconstitute

Standard #	Standards (µIU/ml) Final Concentration
1	0
2	0.1
3	0.5
4	2.0
5	5.0
6	10.0

Samples:

7. Bring to room temperature
8. Aliquot samples

9. Label microcentrifuge tubes for all blank, serum reference (standards), control, and samples in duplicate according to paper TSH template

Wash:

10. Use Distilled Water or Deionized water (DO NOT use tap water)

ASSAY PROCEDURE:

First Incubation (100 µl B, S, C; 100 µl enzyme conjugate):

1. Use labeled TSH template
2. Pipette **100 µl** of appropriate blank, serum reference (standards), control, and samples into assigned wells
3. Add **100 µl** of **TSH enzyme conjugate reagent** to all wells
4. Thoroughly mix for 30 sec; very important to have complete mixing
5. Set on orbital shaker at 175±25 RPM
6. Incubate **120 min (2hrs)** at room temperature

Wash (5 times with distilled/DI water):

7. Remove incubation mixture by emptying plate contents into waste container
8. Strike against absorbent towels to remove all residual water droplets
9. Wash with distilled/DI water by using a squeeze bottle or automated plate washer
10. Repeat procedure 4 times for a total of 5 washes with distilled water

Second Incubation (100 µl TMB):

11. Add **100 µl** of **TMB reagent** to all wells
12. Gently mix for 5 sec
13. Incubate at room temperature for **20 min**

Stop (100 µl Stop Sol.):

14. Add **100 µl** of 1N HCl solution (**stop solution**) to each well
15. Gently mix for 30 sec. Make sure all blue color changes to yellow completely

Read Absorbance:

16. Read absorbance at **450 nm** within **15 min**

Total time estimation to complete assay: About **4-4.5 hours**

- Thawing/Prepping Samples- 30 min-1hr
- Prepare Standards- 20 min
- First incubation-2 hr
- Wash(5 times)- 15 min

- 2nd incubation- 20 min
- Read Plate within 15 min

Pipettes Needed:

- 100 µl
- 1000 µl (1ml)

APPENDIX S:

Free T_3 ELISA Procedure, ALPCO Handout

ALPCO™
IMMUNOASSAYS

T3 (Triiodothyronine) (Free) ELISA

For the quantitative determination of free triiodothyronine (fT3) in serum.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	25-FT3HU-E01
Size:	96 wells
Version:	050211 - ALPCO January 5, 2012

ALPCO Diagnostics

[redacted]

INTRODUCTION

L-Triiodothyronine, a thyroid hormone, circulates in blood almost completely bound (>99.5%) to carrier proteins. The main transport protein is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of triiodothyronine is believed to be responsible for the biological action. Furthermore, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In individuals with normal thyroid function, as the concentrations of the carrier proteins change, the total T3 levels change in consert so that the free thriiodothyronine (fT3) concentration remains constant. Thus, measurements of fT3 concentrations correlate more reliably with clinical status than total triiodothyronine levels.

For example, the increase in total triiodothyronine levels associated with pregnancy, oral contraceptives, and estrogen therapy result in higher total T3 levels while the fT3 concentration remains basically unchanged.

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations in a direct determination of fT3.

PRINCIPLE OF THE TEST

The fT3 test is a solid phase competitive enzyme immunoassay. Serum samples, standards, and T3-Enzyme Conjugate Working Reagent is added to wells coated with monoclonal T3 antibody. fT3 in the specimen and the T3 labeled conjugate compete for available binding sites on the antibody. After a 60 minutes incubation at room temperature, the wells are washed with water to remove unbound T3 conjugate. A solution of H_2O_2/TMB is then added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 3N HCl, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled fT3 in the sample. By reference to a series of fT3 standards assayed in the same way, the condentration of fT3 in the unknown sample is quantified.

REAGENTS

Materials provided with the kit:

- T3 Antibody-Coated Microplate, 96 wells
- T3-Enzyme Conjugate, 10.5 ml, ready to use
- Free T3 Reference Standards, 0, 0.9, 2.2, 5.0, 9.0, and 19.0 pg/ml. 1 set, 1.0 ml each, ready to use
  The exact, lot-specific concentrations are listed on the vial labels.
  For SI units: 1pg/ml x 1.536= pmol/L
- Color Reagent A, 13 ml
- Color Reagent B, 13 ml
- Stop solution (3N HCl), 10 ml

Materials required but not provided:

- Pipette capable of delivering 50 µl volumes with a precision of better than 1.5%.
- Dispenser(s) for repetitive deliveries of 0.050 ml and 0.200 ml volumes with a precision of better than 1.5%.
- Microplate Reader with 450 nm wavelength absorbance capability.
- Test tubes for dilution of enzyme conjugate and for mixing Color Reagent A with Color Reagent B.
- Absorbent paper of blotting the microplate wells.
- Timer.
- Quality control materials.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood speciment obtained by acceptable medical techniques. This kit is for use with serum sample without additives only. Serum samples may be refrigerated at 2-8°C for a maximum period of 48 hours. If the samples can not be assayed within 48 hours, they may be stored at temperatures of –20°C for up to 30 days.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Working Substrate Solution – Prepare immediately before use.

To prepare H_2O_2/TMB solution, make a 1:1 mixing of Color Reagent A with Color reagent B up to 1 hour before use. Mix gently to ensure complete mixing. The prepared H_2O_2/TMB reagent should be made at least 15 minutes before use and is stable at room temperature in the dark for up to 3 hours. Discard excess after use.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-25 °C).

1. Format the microplates' wells for each serum reference, control, and specimen to be assayed in duplicate.
2. Pipette 0.050 ml (50 µl) of the appropriate serum reference, control, and specimen into the assigned well.
3. Add 0.100 ml (100 µl) of T3-Enzyme Conjugate Solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with distilled water. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 0.200 ml (200 µl) of **Working Substrate Solution** to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.** Gently mix for 10 seconds.
8. Incubate at room temperature in the dark for 20 minutes.
9. Stop the reaction by adding 50 µl of 3N HCl to each well.
10. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
11. Read absorbance at 450 nm with a microtiter well reader within 30 minutes.

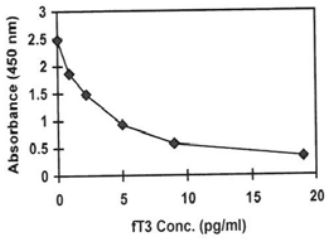
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in pg/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of fT3 in pg/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against fT3 concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

fT3 (pg/ml)	Absorbance (450 nm)
0	2.478
0.9	1.862
2.2	1.483
5.0	0.927
9.0	0.576
19.0	0.338



EXPECTED VALUES

A study of euthyroid adult population was undertaken to determine expected values for the fT3 EIA Test System. The mean (X) values, standard deviations (S.D.) and expected ranges (± 2 S.D.) are presented in the following table:

Expected Values for the Free T3 EIA Test System (in pg/ml)

	Adult (110 specimens)	Pregnancy (30 specimens)
Mean (X)	2.8	3.0
Standard Deviation	0.7	0.6
Expected Ranges (± 2 SD)	1.4 – 4.2	1.8 – 4.2

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon several factors: the specificity of the method, the population tested, and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

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FREE T3 ELISA PROCEDURE
(ALPCO Immunoassays 25-FT3HU-E01)

Preparation of Reagents, Standard, and Samples

Thaw Samples:

11. Begin thawing samples
12. Bring all reagents, serum references, and controls (IS0104) to room temperature 18-25 degree C

H2O2/ TMB Solution Reagent Preparation (Working Substrate):
(Prepared reagent stable at room temperature **in the dark** for up to **3 hours**):

13. Make a 1:1 mixing of Color reagent A with Color Reagent B up to 1 hour before use
14. Mix gently to complete mixing
15. Prepared H2O2 TMB solution must be made at least 15 min before use; store for up to **3 hours** in the **dark**
16. Cover prepared reagent with foil to keep it stable

Samples:

17. Bring to room temperature
18. Aliquot samples
19. Label microcentrifuge tubes for all blank, serum reference (standards), control, and samples in duplicate according to paper T3 template

Wash:

20. Use Distilled Water

Standards (Serum Reference Standards):
Provided in the kit (1ml each)

Standard #	Standards (pg/ml) Final Concentration
1	0
2	0.9
3	2.2
4	5.0
5	9.0
6	19.0

ASSAY PROCEDURE:

First Incubation (50 µl B, C, S; 100 µl enzyme conjugate):

17. Use labeled T3 template
18. Pipette 50 µl of appropriate blank, serum reference (standards), control, and samples into assigned wells
19. Add 100 µl of Free T3 enzyme conjugate reagent to all wells
20. Swirl microplate gently for 20-30 sec to mix
21. Cover plate
22. Incubate **60 min** at room temperature

Wash (5 times with distilled water):

23. Remove incubation mixture by emptying plate contents into waste container
24. Strike against absorbent towels to remove all residual water droplets
25. Wash with distilled water by using a squeeze bottle or automated plate washer
26. Repeat procedure 4 times for a total of 5 washes with distilled water

Second Incubation (200 µl WS):

27. Add 200 µl of H2O2 TMB working substrate to all wells
28. Gently mix for 10 sec
29. Incubate at room temperature in the dark for **20 min** (Cover with plate sealer and also use Aluminum foil to protect from light).

Stop (50 µl Stop Sol.):

30. Add 50 µl of 3N HCl solution (stop solution) to each well
31. Gently mix for 30 sec. Make sure all blue color changes to yellow completely

Read Absorbance:

32. Read absorbance at 450 nm within 30 min

Total time estimation to complete assay: About 3-3.5 hours

- Thawing/Prepping Samples- 30 min-1hr
- First incubation- 1hr
- Wash- 15 min
- 2nd incubation- 20 min
- Read Plate within 30 min

Pipettes Needed:

- 50 µl
- 100 µl
- 200 µl

APPENDIX T:

Free T_4 ELISA Procedure, ALPCO Handout

ALPCO™
IMMUNOASSAYS

T4 (Thyroxine) (Free) ELISA

For the quantitative determination of T4 (free thyroxine) in human serum.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	25-FT4HU-E01
Size:	96 wells
Version:	011311 - ALPCO December 9, 2011

ALPCO Diagnostics

[redacted]

PRINCIPLE OF THE TEST

The fT4 test is a solid phase competitive enzyme immunoassay. Serum samples, standards, and Thyroxine-Enzyme Conjugate Working Reagent are added to wells coated with monoclonal T4 antibody. fT4 in the specimen and the T4 labeled conjugate compete for available binding sites on the antibody. After a 60 minute incubation at room temperature, the wells are washed with water to remove unbound T4 conjugate. A solution of H_2O_2/TMB is then added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 3N HCl, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled fT4 in the sample. By reference to a series of fT4 standards assayed in the same way, the condentration of fT4 in the unknown sample is quantified.

REAGENTS

Materials provided with the kit:

- T4 Antibody-Coated Microplate, 96 wells
- T4-Enzyme Conjugate Reagent, ready to use, 10.5 ml
- Free T4 Reference Standards, 0, 0.3, 0.95, 2.1, 3.6, and 7.0 ng/dl*, 1 ml each
  ** Exact levels are given on the labels on a lot specific basis*
- Color Reagent A, 13 ml
- Color Reagent B, 13 ml
- Stop Solution (3N HCl), 10 ml

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Working Substrate Solution – Prepare immediately before use

To prepare H_2O_2/TMB solution, make a 1:1 mixing of Color Reagent A with Color Reagent B up to 1 hour before use. Mix gently to ensure complete mixing. The prepared H_2O_2/TMB reagent should be made at least 15 minutes before use and is stable at room temperature in the dark for up to 3 hours. Discard excess after use.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls (not included) to room temperature (18-25°C).

1. Format the microplates' wells for each serum reference, control, and sample be assayed in duplicate.
2. Pipette 0.050 ml (50 µl) of the appropriate serum reference, control and specimen into the assigned well.
3. Add 0.100 ml (100 µl) of Free T4 Enzyme Conjugate Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Incubate 60 minutes at room temperature.
6. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with distilled water. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 0.200 ml (200 µl) of Working Substrate Solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.** Gently mix for 10 seconds.
8. Incubate at room temperature in the dark for 20 minutes.
9. Stop the reaction by adding 50 µl of 3N HCl (Stop Solution) to each well.
10. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely**.
11. Read absorbance at 450 nm with a microtiter well reader within 30 minutes.

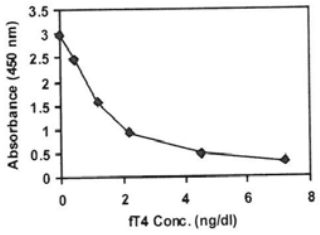
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/dl on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of fT4 in ng/dl from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against fT4 concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

fT4 (ng/dl)	Absorbance (450 nm)
0	2.496
0.3	2.292
0.95	1.903
2.1	1.295
3.6	0.819
7.0	0.410



FREE T4 ELISA PROCEDURE

(ALPCO Immunoassays 25-FT4HU-E01)

Preparation of Reagents, Standard, and Samples

Thaw Samples:

21. Begin thawing samples
22. Bring all reagents, serum references and controls (IS0104) to room temperature 18-25 ^{0} C

H2O2/ TMB Solution Reagent Preparation (Working Substrate):
(Prepared reagent stable at room temperature **in the dark** for up to **3 hours**):

23. Make a 1:1 mixing of Color reagent A with Color Reagent B up to 1 hour before use
24. Mix gently to complete mixing
25. Prepared H2O2 TMB solution must be made at least 15 min before use; store for up to **3 hours** in the **dark**
26. Cover prepared reagent with foil to keep it stable

Samples:

27. Bring to room temperature
28. Label microcentrifuge tubes for all blank, serum reference (standards), control, and samples in duplicate according to paper T4 template
29. Aliquot samples to labeled 0.5 mL microcentrifuge tubes

Wash:

30. Use Distilled Water

Standards (Serum Reference Standards):
Provided in the kit (1ml each)

Standard #	Standards (ng/dl) Final Concentration
1	0
2	0.3
3	0.95
4	2.1
5	3.6
6	7

ASSAY PROCEDURE:

First Incubation (50 µl B, C, S; 100 µl enzyme conjugate):

33. Use labeled T4 template
34. Pipette **50 µl** of appropriate blank, control, serum reference (standards), and samples into assigned wells
35. Add **100 µl** of Free T4 enzyme conjugate reagent to all wells
36. Swirl microplate gently for 20-30 sec to mix
37. Incubate **60 min** at room temperature

Wash (5 times with distilled water):

38. Remove incubation mixture by emptying plate contents into waste container
39. Strike against absorbent towels to remove all residual water droplets
40. Wash with distilled water by using a squeeze bottle or automated plate washer
41. Repeat procedure 4 times for a total of 5 washes with distilled water

Second Incubation (200 µl WS):

42. Add **200 µl** of H2O2 TMB working substrate to all wells
43. Gently mix for 10 sec
44. Incubate at room temperature in the dark for **20 min** (Cover with plate sealer and also use Aluminum foil to protect from light).

Stop (50 µl Stop Sol.):

45. Add **50 µl** of 3N HCl solution (stop solution) to each well
46. Gently mix for 30 sec. Make sure all blue color changes to yellow completely

Read Absorbance:

47. Read absorbance at **450 nm** within 30 min

Total time estimation to complete assay: About 3-3.5 hours

- Thawing/Prepping Samples- 30 min-1hr
- First incubation- 1hr
- Wash- 15 min
- 2nd incubation- 20 min
- Read Plate within 30 min

Pipettes Needed:

- 50 µl
- 100 µl
- 200 µl

APPENDIX U:

Iodoral Information Document

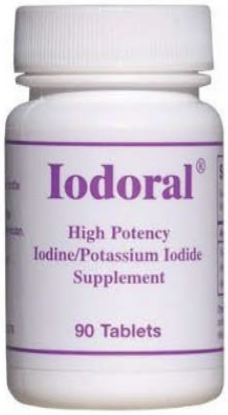
enter search term(s)

[redacted]

Optimox®
Better Health Through Nutrition*

Home | Shop | Iodoral | Iodine Research | Contact Us

[redacted]



IODORAL®

[redacted]

Iodoral® is a tablet form of Lugol solution. One 12.5 mg tablet of Iodoral® supplies an amount of total elemental iodine comparable to the average daily intake of this essential element by mainland Japanese.*

SUGGESTED USE: Take one tablet per day or as directed by a physician.

WARNING: If pregnant or nursing, consult your healthcare practitioner before use. Keep out of reach of children. Do not use if you have an iodine sensitivity, or if you are taking anti-hypertension or anti-thyroid medications. Discontinue use if you develop swollen salivary glands, stomach upset, or skin rash.

Serving Size	1 tablet
Servings per container	90 or 180
Amount per serving:	
Total Iodine/Iodide	12.5 mg
Iodine	5 mg
Iodide (as potassium salt)	7.5 mg

Other ingredients: Micosolle®, a silica-based excipient containing a non-ionic surfactant, microcrystalline cellulose, vegetable stearins and pharmaceutical glaze.

Store in a cool, dry and dark place.

More About Suggested Use

The suggested daily amount is 1-4 tablets/day as recommended by your physician. Work closely with your physician while on the Iodoral® program. Report to your physician any history of thyroid surgery and/or radiation, chronic lymphocytic thyroiditis (Hashimoto's), and any previous problem with your thyroid. Before starting Iodoral®, your physician (He/She = He) will order blood tests for hematology, blood chemistry and thyroid functions. He will also perform an ultrasound of your thyroid to measure the size and appearance of your thyroid. Depending on the results of the thyroid function tests and ultrasound, he may order some blood tests for thyroid antibodies. If you are taking thyroid hormones, let your physician know about it. Be

aware that Iodoral® enhances the response of your body to thyroid hormones.*(4,6) Subjects on Iodoral® feel warmer in cold environments and that is expected.* However, you will need to cut down the amount of thyroid hormones if you experience palpitation, anxiety, increased sweating and intolerance to heat. That is because Iodoral® decreases your need for thyroid hormones.* Your physician will guide you in titrating downward the amount of thyroid hormones. Your physician will reevaluate you one month afterward if you are on thyroid hormones, and 3 months if not. Keep a record of what you observe while on Iodoral® and show it to your physician.

To test whole body sufficiency for iodine, 4 tablets of Iodoral® are ingested, followed by 24 hr. urine collection. The more deficient a subject is in iodine, the more iodine is retained by the body and the least excreted in the urine. Sufficiency is achieved when 90% or more of the ingested amount is excreted in the urine. In most subjects tested, 3-4 tablets of Iodoral®/day were required to achieve whole body sufficiency within 3 months and the body retained approximately 1.5 gm iodine at sufficiency.*(4,8) Check with your physician for more information about the loading test.

WARNING: Ingestion of iodine and/or iodide has been associated with certain complaints. If you experience any of the following, stop ingesting Iodoral® and contact your physician:

- Acne-like skin lesions in certain areas of your body
- Headache in the frontal sinus
- Unpleasant brassy taste
- Increased salivation and sneezing
- If you experience any unusual symptom since starting on Iodoral®, contact your physician.

General Information

Iodine is an essential element. Although its main function is in the production of thyroid hormones by the thyroid gland, other organs in the body have a need for iodine in order to function normally.*(4)

Several studies have demonstrated a need for iodine intake.*(1,2) The minimum amount of iodine determined in these studies is equivalent to 0.1 mg/kg body weight/day.* For example, for a 50 kg woman, the daily amount of iodine would be 5 mg.* The thyroid gland needs iodide to function properly. The original study done 80 years ago in adolescent girls used 9 mg iodide daily.*(3)

During the early 1900's, the iodine/iodide solution called Lugol solution was used extensively, effectively and safely in medical practice, for both low activity and above normal activity of the thyroid gland.*(4) The recommended daily intake for iodine supplementation was 2 to 6 drops of Lugol solution, providing 12.5 to 37.5 mg total iodide. That amount was mentioned as lately as 1995 in a book on Pharmaceutical Sciences.(5)

Several investigators have shown convincing evidence for the need for adequate iodine intake.*(6) Japanese women living in Japan consumed a daily average of 13.8 mg total elemental iodine and some research suggests this is an important factor for their relative health.*(6) In the 1960's, one slice of bread in the USA contained the full RDA of 0.15 mg iodine. Over the last 2 decades, iodine was replaced by bromine in the bread making process. Bromine may block thyroid function and may interfere with iodine in the body.*(4),(7)

The RDA limits for vitamins and minerals were established after World War II. One of the last essential elements included in the RDA system was iodine, established in 1980 and confirmed in 1989.(8) The RDA for iodine was based on the amount of iodine/iodide needed to prevent goiter, extreme stupidity and hypothyroidism.*(9) The optimal requirement of the whole human body for iodine has never been studied. Therefore, the optimal amount of this element for physical and mental wellbeing is unknown. Based on demographic studies, the mainland Japanese consumed an average of 13.8 mg daily and they are one of the healthiest people on planet earth.*(6) Lugol solution is a time-tested preparation with a proven track record for over 150 years.* Two drops contain 12.5 mg iodine/iodide, an amount very close to the 13.8 mg average intake of mainland Japanese.*

Rationale For This Formulation

Administration of iodine/iodide in liquid solution is not very accurate, may stain clothing, has an unpleasant taste and can cause gastric irritation. Iodoral® is a precisely quantified tablet form containing 5 mg iodine and 7.5 mg iodide as the potassium salt. To prevent gastric irritation, the iodine/iodide preparation is absorbed into a colloidal silica excipient, and to eliminate the unpleasant taste of iodine, the tablets are coated with a thin film of pharmaceutical glaze.*(10)

For more information on Iodine Research: ***Click Here***

References:

1. Ghent, W., et al, Can. J. Surg., 36:453-460,1993.

2. Eskin, B., et al, Biological Trace Element Research, 49:9-19, 1995.
3. Marine, D., Atl. Med. J., 26:437-442, 1923.
4. Abraham,G.E., The Original Internist, 11:17-36, 2004.
5. Gennaro A.R., Remington: 19th Edition, 1995, Mack Publishing Co, 1267.
6. Abraham, G.E., Flechas, J.D., Hakala, J.C., The Original Internist, 9:30-41, 2002.
7. Epstein, S.S., et al, Prevention Program Macmillan, NY, 1998, pg5.
8. Abraham, G.E., The Original Internist, 11:(2) 29-38, 2004.
9. Abraham, G.E. Townsend Letter, 245:100-101, 2003.
10. Abraham, G.E., Flechas, J.D., Hakala, J.C., The Original Internist, 9:5-20, 2002.

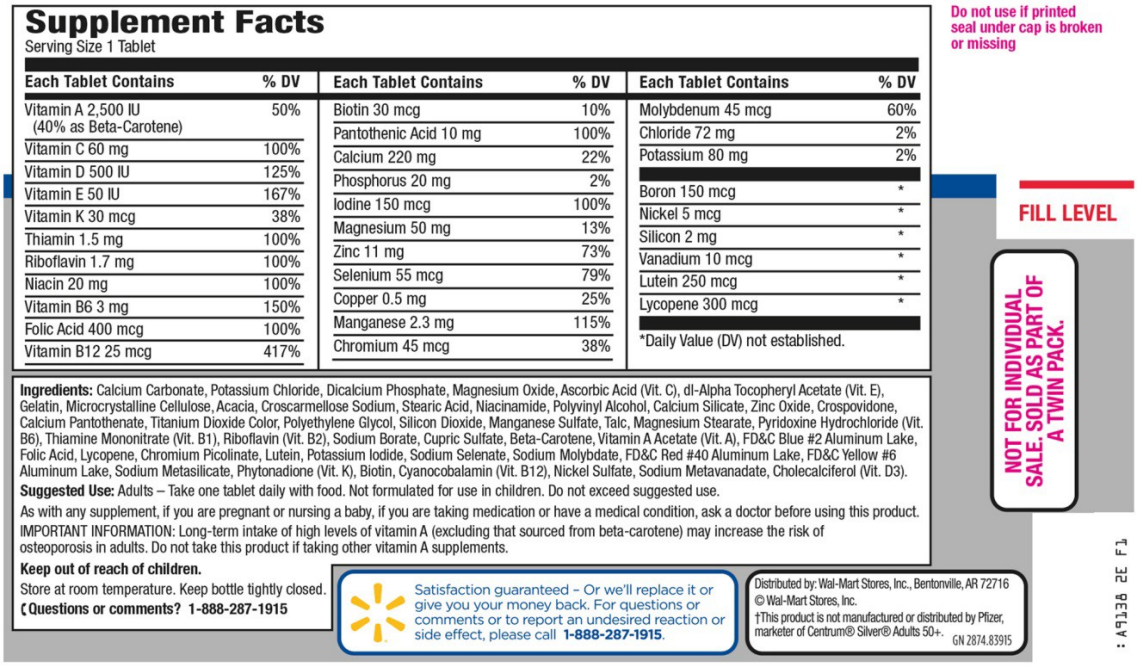
APPENDIX V:

Glucose Tablets Information Document


Directions: Chew desired amount of glucose. Please consult with your healthcare professional to determine the best serving size for you.
DISTRIBUTED BY:
Wal-Mart Stores, Inc.
Bentonville, AR 72716
DO NOT USE IF
SEAL IS BROKEN
Store at room
Keep tightly closed
from moisture.
Nutrition Facts
Serving Size 1 tablet (4.5g/0.2oz)
Amount Per Serving
Calories 20
% Daily Value *
Total Fat 0g 0%
Sodium 0mg 0%
Total Carbohydrate 4g 1%
Sugars 4g
Protein 0g
Vitamin C 10%
Not a significant source of calories from fat, saturated fat, trans fat, cholesterol, dietary fiber, vitamin A, calcium and iron.
* Percent Daily Values are based on a 2,000 calorie diet.
Ingredients: Dextrose (D-Glucose), Potassium Citrate, Microcrystalline Cellulose, Magnesium Stearate, Citric Acid, Hydrogenated Vegetable Oil (Cottonseed)†, Ascorbic Acid, Malic Acid, FD&C Red #40 Aluminum Lake, Natural and Artificial Flavor.
†Adds a trivial amount of fat
164152

APPENDIX W:

Equate Multivitamin Supplement Information Document



Supplement Facts

Serving Size 1 Tablet

Each Tablet Contains	% DV
Vitamin A 2,500 IU (40% as Beta-Carotene)	50%
Vitamin C 60 mg	100%
Vitamin D 500 IU	125%
Vitamin E 50 IU	167%
Vitamin K 30 mcg	38%
Thiamin 1.5 mg	100%
Riboflavin 1.7 mg	100%
Niacin 20 mg	100%
Vitamin B6 3 mg	150%
Folic Acid 400 mcg	100%
Vitamin B12 25 mcg	417%

Each Tablet Contains	% DV
Biotin 30 mcg	10%
Pantothenic Acid 10 mg	100%
Calcium 220 mg	22%
Phosphorus 20 mg	2%
Iodine 150 mcg	100%
Magnesium 50 mg	13%
Zinc 11 mg	73%
Selenium 55 mcg	79%
Copper 0.5 mg	25%
Manganese 2.3 mg	115%
Chromium 45 mcg	38%

Each Tablet Contains	% DV
Molybdenum 45 mcg	60%
Chloride 72 mg	2%
Potassium 80 mg	2%
Boron 150 mcg	*
Nickel 5 mcg	*
Silicon 2 mg	*
Vanadium 10 mcg	*
Lutein 250 mcg	*
Lycopene 300 mcg	*

*Daily Value (DV) not established.

Ingredients: Calcium Carbonate, Potassium Chloride, Dicalcium Phosphate, Magnesium Oxide, Ascorbic Acid (Vit. C), dl-Alpha Tocopheryl Acetate (Vit. E), Gelatin, Microcrystalline Cellulose, Acacia, Croscarmellose Sodium, Stearic Acid, Niacinamide, Polyvinyl Alcohol, Calcium Silicate, Zinc Oxide, Crospovidone, Calcium Pantothenate, Titanium Dioxide Color, Polyethylene Glycol, Silicon Dioxide, Manganese Sulfate, Talc, Magnesium Stearate, Pyridoxine Hydrochloride (Vit. B6), Thiamine Mononitrate (Vit. B1), Riboflavin (Vit. B2), Sodium Borate, Cupric Sulfate, Beta-Carotene, Vitamin A Acetate (Vit. A), FD&C Blue #2 Aluminum Lake, Folic Acid, Lycopene, Chromium Picolinate, Lutein, Potassium Iodide, Sodium Selenate, Sodium Molybdate, FD&C Red #40 Aluminum Lake, FD&C Yellow #6 Aluminum Lake, Sodium Metasilicate, Phytonadione (Vit. K), Biotin, Cyanocobalamin (Vit. B12), Nickel Sulfate, Sodium Metavanadate, Cholecalciferol (Vit. D3).

Suggested Use: Adults – Take one tablet daily with food. Not formulated for use in children. Do not exceed suggested use.

As with any supplement, if you are pregnant or nursing a baby, if you are taking medication or have a medical condition, ask a doctor before using this product.

IMPORTANT INFORMATION: Long-term intake of high levels of vitamin A (excluding that sourced from beta-carotene) may increase the risk of osteoporosis in adults. Do not take this product if taking other vitamin A supplements.

Keep out of reach of children.

Store at room temperature. Keep bottle tightly closed.

Questions or comments? 1-888-287-1915

Satisfaction guaranteed – Or we'll replace it or give you your money back. For questions or comments or to report an undesired reaction or side effect, please call **1-888-287-1915**.

Distributed by: Wal-Mart Stores, Inc., Bentonville, AR 72716
© Wal-Mart Stores, Inc.
†This product is not manufactured or distributed by Pfizer, marketer of Centrum® Silver® Adults 50+.

GN 2874.83915

Do not use if printed seal under cap is broken or missing

FILL LEVEL

NOT FOR INDIVIDUAL SALE. SOLD AS PART OF A TWIN PACK.

: A9138 2E F1

APPENDIX X:

Iodine Study Completion Consent Form

Iodine Supplementation Study Completion Consent Form

I have successfully completed the Iodine Supplementation Study being conducted at the Institute for Women's Health, Texas Woman's University.

I have received a $25.00 WalMart gift card as remuneration for my participation in the study.

Name: _____

ID#: _____

Date: _____

Signature: _____

APPENDIX Y:

Study Timeline Handout

IODINE STUDY: TIME LINE

Effect of iodine supplementation on biomarkers of thyroid function, body composition and resting metabolic rate in women, 18-45 years.

Schedule for clinic visits

Month One, Visit One:

- Informed Consent Questionnaire
- Health History Questionnaire
- Demographic Questionnaire
- Three-Day Dietary Intake Survey
- Physical Activity Questionnaire
- Pick up Iodine Loading kit

15 minutes

Month One, Visit Two:

- Iodine Loading Test
- Blood Draw –within 24 hrs of completing Iodine Loading Test
- Measurement of Resting Metabolic Rate
- Measurement of Bone Density (DXA)
- Measurement of Body Composition
- Measurement of Body Mass Index (BMI)
- Return of the Questionnaires
- Receive Supplements

1.5 hours

Months 2-6: Visits Three-Seven:
Receive Refill of Supplements, Iodine / Placebo

15 minutes

Month Six, Visit Eight:

- Measurement of Resting Metabolic Rate
- Measurement of Bone Density (DXA)
- Measurement of Body Composition
- Measurement of Body Mass Index (BMI)
- Health history questionnaire
- Demographic questionnaire
- Blood Draw – within 24 hours of completing Iodine Loading Test
- Iodine Loading Test

1.5 hours