# Visceral Adiposity is Inversely Related to Dietary Intake of Fiber

By

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A Thesis

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CERTIFICATE OF APPROVAL

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**List of Abbreviations**

| | |
|---|---|
| BMI | Body Mass Index |
| CBC | Complete Blood Count |
| CT | Computed Tomography |
| CTRC | Clinical and Translational Research Center |
| DEXA | Dual Energy X-Ray Absorptiometry |
| FFA | Free Fatty Acids |
| FFQ | Food Frequency Questionnaire |
| GI | Glycemic Index |
| GL | Glycemic Load |
| GLUT2 | Glucose Transporter 2 |
| GLUT4 | Glucose Transporter 4 |
| GLUT5 | Glucose Transporter 5 |
| HDL | High Density Lipoprotein |
| HIPAA | Health Insurance Portability and Accountability Act |
| IRB | Institutional Review Board |
| LDL | Low Density Lipoprotein |
| LT4 | Levothyroxine |
| MONW | Metabolically Obese Normal Weight |
| MMSE | Mini-Mental State Examination |
| MRI | Magnetic Resonance Imaging |
| NDSR | Nutrition Data System for Researchers |
| NHANES | National Health and Nutrition Examination Survey |
| OHSU | Oregon Health and Science University |
| OCTRI | Oregon Clinical and Translational Research Institute |
| PVAT | Percent Visceral Adipose Tissue |

| | |
|---|---|
| ROI | Region of Interest |
| SAT | Subcutaneous Adipose Tissue |
| SSB | Sugar Sweetened Beverage |
| SSF | Sugar Sweetened Food |
| TSH | Thyroid Stimulating Hormone |
| VAT | Visceral Adipose Tissue |
| WAIS-R | Wechsler Adult Intelligence Scale - Revised |

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**Abstract**

Visceral adipose tissue deposition is associated with increased risk of chronic diseases including type 2 diabetes, cardiovascular disease and accompanying metabolic abnormalities. Determining the components of diet associated with increased or decreased visceral adipose tissue deposition is critical to decreasing the risk of chronic disease in at-risk populations.

The aim of this research analysis was to determine the role of dietary carbohydrate in visceral adipose tissue deposition in participants from the "Neurocognitive and metabolic effects of mild Hypothyroidism" study conducted between 2008 to 2013. The first aim was to determine the relationship between dietary intake of simple and complex carbohydrates and the relative amount of visceral adipose tissue. The second aim was to determine the relationship between dietary intake of specific types of monosaccharides, the major contributors of added sugar in the diet and the relative amount of visceral adipose tissue. The Nutrition Data System for Research involving the multi-pass method was used to collect 3 days of dietary intake data from all participants. Visceral adipose tissue was measured by reanalysis of DEXA scans using Horizon DXA System software and the main outcome variable used for analysis was visceral adipose tissue as a percent of total body fat (PVAT).

Total fiber intake was inversely related to visceral adipose tissue ($p=0.046$) and every 5-gram increase in total fiber intake was associated with a 3% decrease in median PVAT. Age and BMI were also significantly associated with PVAT ($p<0.001$, $p<0.001$) such that each 10-year increase in age was associated with 19.6% increase in median PVAT and each 5 $kg/m^2$ increase in BMI was associated with a 21.6% increase in median PVAT. No other dietary or non-dietary variables analyzed showed a significant association with PVAT. The findings from this analysis provide further support for an influence of dietary fiber intake on visceral adipose tissue deposition.

**Chapter 1: Introduction and Specific Aims**

Body fat has several purposes including heat regulation, as a source of energy, endocrine functions and as a component of vital body structures. However, a growing body of evidence supports the idea that different types and locations of body fat have distinct metabolic properties.  For example, two types of fat in the abdomen, subcutaneous and visceral fat, have very different properties and effects on health. Subcutaneous fat is the layer of fat directly underneath the skin. Visceral fat is found inside the abdominal cavity around the intestines, pancreas, and liver. Visceral fat has been shown to play a role in development and severity of chronic illnesses including type 2 diabetes and elevated insulin resistance, cardiovascular disease and high blood pressure. In contrast, subcutaneous fat in the abdomen does not seem to be as harmful.

Long-term consumption of a hyper-caloric diet increases overall fat storage in the body. However, consumption of a typical Western-style diet, high in saturated fat and refined carbohydrates, even if not exceeding energy needs, is associated with the deposition of more visceral fat in several animal and human studies. While many studies demonstrate associations between various dietary factors and visceral fat deposition, the exact dietary components that contribute to visceral fat deposition are not well understood.

The role of dietary carbohydrate in visceral fat deposition is unclear as it has been shown to have both positive and negative associations with deposition and distribution of visceral fat. Evidence suggests that the complexity of dietary carbohydrate, measured by fiber intake, glycemic index and glycemic load; the types of monosaccharides that comprise dietary carbohydrate, as well as added sugar may prevent or promote the accumulation of visceral body fat.  To enhance our understanding of this relationship, we conducted a cross-sectional study to investigate the association between consumption of specific forms of dietary carbohydrate and the amount of visceral fat in a group of healthy women with a wide range of Body Max Index (BMI).

The project is a secondary analysis of data obtained as part of the "Neurocognitive and metabolic effects of mild hypothyroidism" study conducted by Dr. Mary Samuels. A cohort of women with normal thyroid function, demonstrated by baseline blood thyroid stimulating hormone, TSH, concentrations within normal limits, had their body composition and usual dietary intake measured prior to their involvement in the intervention arm of the original study.  This baseline data was used for the analysis and included, but was not limited to, assessment of usual dietary intake based on three unannounced 24-hr recalls and measurement of body composition including visceral adipose tissue by dual x-ray absorptiometry. The aims of this study were to:

**Aim 1:** Determine the relationship between dietary intake of simple and complex carbohydrates and the relative amount of visceral adipose tissue located in the abdomen in a sample of healthy adult women.

**Hypothesis 1:** Abdominal visceral adipose tissue, as a percent of total body fat, will be higher in participants with a higher consumption of simple carbohydrate (assessed by combined monosaccharide and disaccharide intake as a percent of total energy intake), a higher dietary glycemic index and glycemic load, and a lower consumption of complex carbohydrate assessed by fiber intake.

**Aim 2:** Determine the relationship between dietary intake of specific types of monosaccharides and major dietary contributors of added sugar and the relative amount of visceral adipose tissue located in the abdomen in a sample of healthy adult women.

**Hypothesis 2:** Abdominal visceral adipose tissue, as a percent of total body fat, will be higher in participants with higher consumption of fructose, as a percent of total carbohydrate, and a greater intake of added sugars from sweetened foods and beverages.

## Chapter 2: Background

### Visceral Fat and Chronic Diseases

The number of individuals who are overweight or obese in the United States is continuing to rise (1). Excess weight is related to many health conditions including hypertension, type 2 diabetes and high blood cholesterol (2) and the location and type of body fat associated with excess weight increases the risk of chronic disease further. This section will explain the different types of body fat and associations with chronic disease; it will describe carbohydrates, how they are digested and how they are utilized in the body; and it will discuss the evidence for the connection between dietary carbohydrate intake and visceral adipose tissue (VAT).

Several different types of body fat exist. Subcutaneous adipose tissue (SAT) accumulates immediately under the skin. Ectopic fat accumulates in unusual locations, for example, intramuscular fat, intrahepatic fat, and epicardial fat (3). VAT is a type of ectopic fat that accumulates between and around internal organs including the liver, pancreas, and intestines.

All excess fat is associated with adverse health risks; however, VAT is considered to be more deleterious to health than SAT. VAT is independently associated with risk factors of chronic disease including hyperlipidemia, hypertension and hyperinsulimia and VAT more than SAT is associated with development of cardiovascular disease and Type 2 diabetes (3). The association between VAT and metabolic derangement is evidenced in the metabolically obese, normal weight (MONW) individual. This condition is defined as a normal weight for height along with diagnosed hyperinsulinemia, insulin-resistance, a predisposition for type 2 diabetes, elevated triglycerides, and premature coronary heart disease (4). Obesity is often considered the driver of metabolic derangement and chronic disease progression. However, other factors may also play a role in chronic disease as is demonstrated in the MONW individual. Many factors play a role in the complicated case of the MONW individual however, increased central adiposity and VAT have been shown to play a role in the development of metabolic derangements in these normal-weight subjects (4, 5).

The influence of central and visceral fat on metabolic disease risk is further supported by research from Matasuzawa on Sumo wrestlers in Japan (6). Sumo wrestlers consume a high energy diet to maintain an obese body weight that is primarily SAT and very little VAT. This fat pattern is associated with normal glucose and lipid levels in the Sumo wrestlers. However, once the wrestlers retired, VAT increased along with insulin resistance.

Several theories on the connection between VAT and insulin resistance, hypertension, and cardiovascular disease have been examined. The portal hypothesis describes one proposed mechanism by which VAT may lead to insulin resistance. It is hypothesized that free fatty acids (FFA) delivered to the liver from VAT lead to liver insulin resistance. Kabir, et al induced the deposition of VAT in dogs through diet and saw that VAT increased lipolytic activity and the delivery of free fatty acids (FFA) to the liver leading to insulin resistance (7). This occurred via a significant increase in activity of hormone sensitive lipase and lipoprotein lipase in the VAT compared to SAT. The VAT also released more FFA which was transported to the liver via the portal vein. The researchers found that the triglyceride content in the liver of the dogs with excess VAT was significantly higher compared to the normal dogs. The dogs with excess VAT also had reduced insulin sensitivity and hepatic insulin clearance as evidenced by a 50% decrease in liver insulin receptor binding. This study strongly supports changes at the molecular level that are consistent with the portal hypothesis.

VAT is also associated with hypertension. Evidence suggests that this is due to the physical pressure of the VAT on the kidneys themselves (8,9). Excess VAT compresses the kidneys, which in turn puts additional pressure on the renal veins, lymph vessels, ureters, and the renal parenchyma. The renal compression and increased intrarenal pressure causes more sodium reabsorption and thus contributes to renal vasodilation and reduced macula densa feedback, leading to reductions in arteriolar resistance, glomerular hyper-filtration and increased renin secretion (8). The upregulation of renin leads to increased plasma renin activity as well as secretions of angiotensinogen from adipocytes and release of aldosterone, normally inhibited by NaCl retention. This culminates in activation of the renin-angiotensin aldosterone system, resulting in resorption of sodium and water, blood volume expansion, and hypertension (9).

Finally, dyslipidemia is associated with increased VAT as demonstrated in a study on young and middle aged women by Pascot et al. (10). VAT area was associated with altered lipoprotein-lipid and glucose and insulin concentrations in all age groups. Specifically, VAT area was significantly positively correlated with LDL-cholesterol, cholesterol to HDL cholesterol ratio, plasma glucose, plasma insulin, triglycerides and C-peptide concentrations. HDL cholesterol concentration was negatively correlated with VAT area. Further evidence for this association was reported by Rothney et al. in a cross-sectional analysis of 939 participants (11). VAT volume was measured by DEXA and fasting serum, triglycerides, HDL cholesterol, and total cholesterol concentrations were measured. VAT volume was positively associated with triglyceride concentration and negatively associated with HDL cholesterol concentration after the analysis controlled for sex, BMI, and waist circumference.

Chronic diseases including type two diabetes and cardiovascular disease have been associated with VAT. Evidence for this association includes studies of the portal hypothesis, dyslipidemia, and hypertension. Thus, accurately measuring VAT is advantageous for determining chronic disease risk.

## Measurement of Body Fat

### *Non-Imaging Methods for Estimating Body Fat*

Assessment of body fat is useful as an indicator of adverse health risks. SAT can be easily evaluated in clinical practice with skinfold calipers. After multiple sites have been measured equations can be used to estimate total body fat. This method's degree of accuracy depends on correct technique and selection of appropriate equations to estimate total body fat percent. Total body fat and body fat percentage can also be determined by bioelectrical impedance analysis (BIA) or by total body plethysmography. In BIA, the machine sends an electrical current through either the lower extremities or one side of the body depending on the type of machine used. This method measures the resistance to the electrical current which is input into standardized equations to estimate body fat. The plethysmography method measures the volume of the body by the displacement of water or air depending on the method used. Along with total body weight, body density can be calculated by plethysmography and used with predictive equations to estimate total body fat percent. While each of these methods estimate total body fat, none of them are able to measure total body fat or VAT directly.

Waist circumference is measured to determine abdominal adiposity and cut-points associated with adverse health risks have been created for men and women. A waist circumference measurement that is above the cut point suggests excessive fat deposition in the abdomen. However, a larger waist circumference could be due to additional VAT or SAT or both as waist circumference is an indicator of abdominal adiposity and does not differentiate between VAT and SAT.

*Methods to Measure Body Fat using Imaging Technology*

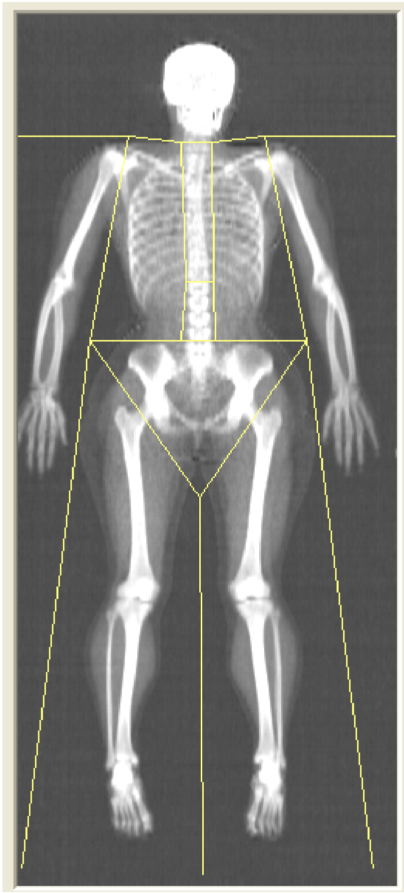


Figure 1 – DEXA scan
The different shades of gray and white are related to the density of the tissue.

Advancement in imaging technologies with X-rays enables the measurement of adipose tissue distribution with computed tomography (CT) scans and magnetic resonance imaging (MRI). This technology provides a detailed analysis of fat location including volume and area of VAT and SAT in the abdomen. However, these scans are costly and expose the individual to significant radiation. Dual energy x-ray absorptiometry (DEXA) scans, which are less expensive and require less radiation, are able to effectively measure VAT through the use of low-energy X-rays. DEXA scans are traditionally used to measure bone mineral density, but recently have been adapted to measure VAT in the abdomen. Through the imaging process, the volume and area of VAT are determined by imaging software. Several studies involving different, large populations have demonstrated that DEXA scans are as accurate at measuring VAT as CT scans (12,13). A study completed at OHSU by Kaul et al. found that DEXA was highly correlated with the CT scan in 124 males and females across a wide range of weight classes (12). Micklesfield et al. found strong agreement between DEXA and CT (13). No cut-off points associated with adverse health risks for VAT volume or area have been created, partly due to the variety of techniques used in VAT imaging (3). For this reason, a ratio of VAT to SAT is used in some studies as it takes into account overall increased abdominal fat.

The sections above described VAT location, the effect on health, and options for estimating or measuring VAT. Determining the dietary factors that play a role in VAT deposition will be key in helping patients make correct behavior changes to reduce this type of fat in the body.

Carbohydrates Briefly Defined

Recent evidence implicates carbohydrates as a contributing factor in VAT deposition. This section will describe the types of carbohydrate in the diet and how they are digested and utilized in the body.

Carbohydrates comprise a large part of the Western diet, and recommendations for carbohydrate intake range from 45-65% of total calories (14). A carbohydrate is made up of a single molecule or a combination of molecules of glucose, galactose or fructose molecules. Monosaccharides are a single molecule of glucose, fructose, or galactose that is absorbed into the body in the small intestines. Disaccharides are combinations of monosaccharide molecules; two molecules of glucose form maltose, one molecule of glucose with one molecule of fructose forms sucrose, and one molecule of galactose with one molecule of glucose forms lactose.

The most common disaccharide in the diet is sucrose which may be added to food products during processing. This is called “added sugar” and is usually in the form of sucrose or high fructose corn syrup, which are both a disaccharide of glucose and fructose. High fructose corn syrup, generated by the addition of glucose isomerase to corn syrup, contains 55% fructose and 42% glucose, sucrose contains 50% fructose, 50% glucose (15). Glucose isomerase converts some of the glucose in corn syrup to fructose creating a sweeter product. Sugar can also be added to foods and beverages at the time of consumption in the form of honey, agave, coconut sugar, raw sugar, and rice syrup. These sugars are less common in processed foods but all contain fructose and/or glucose as monosaccharides and/or sucrose. Naturally-occurring sugar is the difference between total sugars and added sugars. Naturally-occurring sugars can include the disaccharides; lactose and maltose, as well as monosaccharides. The key is that naturally-occurring sugars are found in foods before processing blends in added sugars.

Polysaccharides are long chains of glucose units, and fiber is an indigestible polysaccharide. Several types of fiber include cellulose, pectic polysaccharides, and other associated polysaccharides. Although fiber is not broken down during digestion, it provides additional benefits to the body. It slows the release of food from the stomach to the small intestine, decreases the absorption of cholesterol, and is beneficial to the microbiome in the large intestine (16). A complex carbohydrate includes both fiber and polysaccharides.

*Digestion of Carbohydrates*

The type of carbohydrate consumed determines how it will be digested in the body. The digestion of all carbohydrates starts in the mouth with the enzyme salivary amylase, which begins the breakdown of carbohydrates by cleaving the bond between molecules in the chain of carbohydrates. Carbohydrate digestion is temporarily paused in the stomach due to the acidic environment. In the small intestine, carbohydrates that are ingested as monosaccharides are rapidly moved into the cells of the small intestine via the glucose transporter 2 (GLUT2), for glucose transport, or by GLUT5, for fructose transport (17).

Digestion of complex carbohydrates starts in the oral cavity with salivary amylase and continues in the small intestine. Pancreatic amylase continues the breakdown of carbohydrate in the small intestine and allows other enzymes, sucrase, maltase, and lactase, to break apart each matching disaccharide to release free glucose, galactose and fructose. Free monosaccharides are transported into intestinal cells via glucose transporters, GLUT2 and GLUT5. The free monosaccharides diffuse through the cell and are released into the hepatic portal vein by GLUT2 on the basolateral membrane (18). In the liver, fructose is rapidly phosphorylated and accumulates as fructose-1-phosphate which ultimately forms two glyceraldehyde-3-phosphate molecules. Glyceraldehyde-3-phosphate, from fructose, can be converted to pyruvate and used for ATP synthesis by the cell; it can serve as the glycerol backbone for the synthesis of triglycerides and phospholipids, or through a stage of conversions is converted to glucose or glycogen (18).

The metabolism of fructose to glyceraldehyde-3-phosphate is unregulated. The metabolism of glucose in the liver is regulated by phosphofructokinase and fructose-1,6-diphosphatase, the metabolism of fructose does not require phosphofructokinase and in return is less regulated and readily metabolized (18). This difference in fructose metabolism is hypothesized to be a cause for increased concentrations of plasma triglycerides and increased synthesis and secretion of triglycerides by the liver with high fructose intake (19).

Glucose in the liver can be used to produce energy, stored as glycogen or released into the blood stream to supply other tissues. The liver is the primary organ responsible for maintaining a steady flow of glucose into the blood stream. However, digestion also plays a role in blood glucose concentrations. Simple carbohydrates with low or no fiber move through digestion quickly and raise blood glucose levels rapidly (20). The speed that carbohydrates raise blood glucose levels is an important factor in glucose homeostasis.

*Glycemic Response to Carbohydrates*

As carbohydrates are broken down and enter the blood stream the response on blood glucose concentrations can be measured. The glycemic index ranks carbohydrate containing foods on a scale of 1-100 based on the post-prandial effect of the food on blood glucose concentration. This is a continuous ranking system from complex carbohydrate, with low glycemic index scores, to simple carbohydrate, with higher glycemic index scores (21). A simple carbohydrate is considered a high glycemic food because it is rapidly digested, absorbed and released into the blood stream causing a rapid rise in blood glucose concentrations. Glycemic load takes the portion into account by multiplying the available carbohydrate in the food consumed by the glycemic index for that food.

Equation for Glycemic Load (22):

$$\frac{\text{Available carbohydrate in a particular food (g)} \times \text{Glycemic index for that particular food}}{100}$$

Dietary Intake Assessment Methods

Several ways to determine the dietary carbohydrate intake of an individual have been created and validated. These include laboratory-based dietary studies, diet records, food frequency questionnaires (FFQ) and diet recalls. This section will briefly describe each method and justify the use of the 24-hour recall as the dietary assessment tool used in this research study.

*Feeding Studies*

A research kitchen is used to prepare all meals and snacks for participants enrolled in a feeding study. This ensures that the exact energy and nutrient composition is known for all foods consumed. The exact amount of food consumed by participants is determined by the amount of food left uneaten. This method is exceptionally accurate (23); however, it is expensive and time consuming. It also forces the participant to consume a set diet that may be very different from the type of diet normally consumed.

*Diet Records*

Diet records require the participant to write down and/or photograph everything they eat for a certain number of days. What is included in a record can vary between studies and participants; however, they often include at least food type and frequency of consumption. Usually these records are for periods of 1-7 days. Weighed records require that the participant weigh their food before cooking, when it is prepared, or the left-over food. Dietary records can place a large burden on participants and can vary widely in accuracy. Also, participants may alter dietary intake due to the process of recording all food eaten. Diet records are not a gold-standard method for gathering dietary intake data; however, it is a validated method for determining dietary intake in participants (24).

*Food Frequency Questionnaires*

FFQs are surveys that quantify how often certain foods are consumed during a specific period of time. Participants report how often they consume a food: daily, 3-4 times per week, 1-2 times per week, 1-2 times per month, <1 time per month, or never. Questionnaires can range from a few items to 200 items. This is an inexpensive and relatively quick way to assess past dietary intake. However, the accuracy of this method is low (25).

*Dietary Recall*

The multiple day 24-hour recall, which was used in this analysis, requires participants to describe everything they consumed during the last 24 hours. Often this recall is repeated 3-7 times to collect information on different days for each participant. This process will be described further in the method section. It is considered a gold standard as it allows for analysis of the dietary intake of free living participants in a controlled, defined manner (26).

Visceral Adipose Tissue Deposition and Carbohydrate Intake

This section will describe evidence for the relationship between VAT and dietary carbohydrate.

Ongoing research into dietary factors that contribute to the risk for chronic disease provide strong evidence for an association between change in VAT and several dietary variables including, but not limited to, carbohydrate intake. The European Prospective Investigation into Cancer and Nutrition (EPIC) (27) was a five-year prospective study conducted in Europe that analyzed data from 48,631 Europeans from Italy, the United Kingdom, the Netherlands, Germany, and Denmark. Country specific FFQs were used to determine baseline dietary intake and every year for five years participants were weighed and provided waist circumference measurements. Waist circumference adjusted for BMI was used as a proxy measure for VAT. The authors examined the associations between the change in waist circumference adjusted for BMI with glycemic index, glycemic load, energy density, fiber, alcohol, and macronutrient intake by percent of total energy.

The findings from the EPIC study indicate that consumption of an energy-dense diet and high glycemic index foods were independently associated with significant increases in waist-circumference adjusted for BMI. One calorie per gram greater energy density predicted an annual increase in waist circumference adjusted for BMI of 0.09 cm in men and 0.15 cm in women. Higher glycemic load, as well as low fiber intake, predicted a higher annual change in waist circumference adjusted for BMI in women. This study supports the hypothesis that diets high in simple carbohydrate increase VAT accumulation. While waist circumference adjusted for BMI was considered a good proxy measure of VAT for this study, it is not as accurate of a measure of VAT as DEXA (12) and further research into these associations should use a more direct measure of VAT.

The EPIC study population consisted of only adults; however, similar results were seen in a recent cross-sectional study on 74 overweight adolescents 13-19 years of age (28). The cross-sectional study by Mollard et al. compared the consumption of total carbohydrates, fried foods, sugars, and fiber to VAT and to the VAT-to-SAT ratio. Dietary intake was recorded at baseline using the Harvard Youth Food Frequency Questionnaire. VAT was measured by MRI and used to divide the participants between low or high VAT. The cut-off point that divided participants and the researchers determined to be associated with adverse health risks was 80 $cm^2$.

Total daily sugar intake and soda intake were significantly higher in adolescents with large VAT areas compared to those with small VAT areas. After adjustment for confounders, total carbohydrate intake was positively associated with a higher VAT-to-SAT ratio. Fried food intake was positively associated with hepatic steatosis before adjustment for confounders but was not associated with an increased risk for elevated VAT-to-SAT ratio. In contrast, increased fiber intake was associated with an 18% reduced odds risk of high VAT. Daily soda intake, but not sugar-sweetened beverage intake, was associated with higher odds of an increased VAT-to-SAT ratio and VAT >80 $cm^2$. This research supports the hypothesis that sugar and soda consumption and decreased fiber intake are associated with high VAT in adolescents. However, these results were found in a small group of adolescents in which dietary intake was determined by FFQ.

While the studies listed above are helpful for generating hypotheses they do not demonstrate cause and effect relationships. These studies provide a direction for future research due to the observational design; however, some of the methods such as waist circumference adjusted for BMI and FFQ are not the gold standards. The use of stronger methods for measuring body composition and dietary intake, such as DEXA scans and multiple 24-hour recalls, in this thesis will help progress this field of research.

Visceral Adipose Tissue and Reduction in Carbohydrate Intake

Significant correlations between carbohydrate intake and VAT were found in the above mentioned observational studies and additional evidence for a causative relationship between carbohydrate intake and VAT is described in a recent clinical trial. Sasakabe et al. (29) determined that decreasing overall carbohydrate consumption resulted in a decrease in VAT. This study, completed in Japan, recruited 45 men and 31 women diagnosed with type 2 diabetes. Three dietary recalls were collected at baseline and after a three-month intervention period. The intervention, performed by a Registered Dietitian, taught dietary carbohydrate reduction through removal of one carbohydrate source at breakfast and dinner. Following the intervention, a significant decrease was seen in total carbohydrate intake, VAT, and BMI. Carbohydrate intake decreased from 51% of total calories to 41% in men and from 54% to 42% in women. Total energy intake also decreased, men consumed 400 calories per day and women consumed 200 fewer calories per day less after the 3-month intervention. In men, the change in carbohydrate intake was significantly and positively correlated, a Spearman correlation of 0.398, with a change in VAT area and the percentage of VAT but not to a change in the SAT area. In women, however, the change in carbohydrate intake was not significantly correlated with change in VAT area and VAT percentage although it did trend down over the three months with a Spearman correlation of 0.27.

This study suggests that in Japanese men, a decrease in total carbohydrate consumption can cause a reduction in VAT without a change in SAT. The lack of association seen in women in this study suggests that the relationship may be more complex and supports the need for further examination of the variables that contribute to excess VAT in women. A more detailed examination of the relationship was described by Goss et al. (30). This study was a randomized, clinical trial to test if a low glycemic load diet would reduce total fat and VAT under eucaloric and hypocaloric conditions.

A cohort of 69 healthy, overweight men and women participants were divided into low glycemic load or high glycemic load diet groups for two phases. The first phase was a eucaloric feeding for eight weeks, followed by eight weeks of hypocaloric feeding. Participants were blinded to the diet assigned and received all their food from the research center during the study. The low glycemic load diet was comprised of 43% of total calories from carbohydrate and 39% of total calories from fat and the high glycemic load diet was comprised of 59% of total calories from carbohydrate and 27% of total calories from fat. The percent of total calories from protein was the same in both diets. The low glycemic index diet was created by reducing the percent of total calories from carbohydrates and including low glycemic index foods. Both diets included foods commonly found in the American diet including a mix of whole grain and simple carbohydrate sources. Body weight was monitored throughout the study and energy intake was adjusted as needed for maintenance in the eucaloric phase and decreased by 1,000 calories during the hypocaloric phase. All participants underwent DEXA scans to measure total body fat mass and lean mass, and VAT and SAT area were measured by CT scan.

Following eight weeks on the eucaloric diet the women on the low glycemic load diet, lost 15% of VAT on average compared with only 1% in the women on the high glycemic load diet, while body weight remained the same for both groups. Furthermore, the men consuming the low glycemic load diet lost 4.3% of VAT compared to the men on the high glycemic load diet who gained 4.2% VAT, despite no alteration in total body weight.  All groups during the eucaloric phase, except women on the low glycemic load diet, lost some SAT as well. The lack of change in SAT in women on the low glycemic load diet provides evidence for a transitioning from fat stored as VAT to storage as SAT. After the eight-week hypocaloric phase all groups lost total fat mass, however there was no significant difference between the groups for the percent change in VAT or SAT.

These results suggest that consumption of a low glycemic load diet may influence energy partitioning in the body resulting in less fat stored in the VAT over SAT. The precise factors and mechanisms to explain this enhanced loss of fat by the mobilization of fatty acids from adipose tissue within the abdominal cavity are unclear and require further examination. Our analysis will clarify one contributing dietary factor in a group of free living woman and allow for mechanistic experiments to be designed in the future.

## Added Sugars

While glycemic index and glycemic load are often used to assess the impact that dietary carbohydrates have on blood glucose levels, many factors influence the rate of their digestion and absorption. A more direct measure of simple carbohydrate in the diet is by estimating the intake of the monosaccharides and disaccharides from foods and beverages. The primary contributor to dietary simple sugars is sucrose, either found naturally in fruits and some vegetables, or from added sucrose or high fructose corn syrup in soft drinks, sweets, and other processed foods. Recent evidence suggests that it is the addition of sucrose to foods and beverages that has caused the increase in simple carbohydrate intake over the last few decades and may be the driving force for the rise in obesity rates (31). It is unclear if the high intake of added sugar in the diet contributes to the selective partitioning of fat storage to the VAT compared to SAT in the abdomen.

Evidence for the association between intake of added sugar and VAT was analyzed in 2,596 middle-aged adults from the Framingham Heart Study Offspring study (32). VAT volume was measured with an 8-slice CT scan and sugar-sweetened beverage and diet soda intake was collected by FFQs for the cross-sectional analysis. Of all the participants, 17% were non-consumers and the remaining 83% were regular or daily consumers of sugar-sweetened beverages. The sugar-sweetened beverages with the highest consumption among participants included cola, non-carbonated fruit drinks, carbonated non-cola beverages, and caffeine free cola. The analysis revealed that a greater VAT volume was associated with sugar-sweetened beverage intake after adjustment for SAT. Daily consumers of sugar-sweetened beverages had a 10% higher absolute VAT volume, adjusted for SAT volume, and 15% higher VAT-to-SAT ratio compared to non-consumers; however, neither value was adjusted for total fat mass or BMI. Sugar-sweetened beverage consumption was negatively associated with SAT. Thus, added sugar was correlated with VAT but not SAT, suggesting that added sugar may be related to altered fat partitioning.

Similarly, a cross-sectional analysis by Odegaard et al. (33) of 791 healthy, non-Hispanic adults employed the Willett semi-quantitative FFQ to compare intake of sugar-sweetened beverages with percent VAT. Overall adiposity was measured by DEXA and VAT was measured by MRI. The results of the MRI were used to calculate the variable percent VAT of abdominal fat, defined as: (%VAT = (VAT/ (VAT + SAT)) x100). Sugar-sweetened beverage intake was calculated to be the sum intake of carbonated soft drinks, carbonated sweetened drinks, and non-carbonated sweetened fruit or punch drinks. The frequency of sugar-sweetened beverage intake was defined as never or none, 1-2 per week, 3-6 per week, or ≥ 1 per day.

Increased frequency of sugar-sweetened beverage consumption was associated with percent VAT calculated from the MRI scan. Participants that consumed at least one sugar-sweetened beverage per day had a significantly higher percent VAT compared to participants that consumed less than one sugar-sweetened beverage per day. Sex stratified trends were similar to the main results. In men, percent VAT increased from a mean of 40.9% in those that did not consume sugar-sweetened beverages to a mean of 44.4% in men that consumed at least one sugar-sweetened beverage per day. This association in men was not significant; however in women the difference was significant. The mean percent VAT increased from 22.1% in women with no sugar-sweetened beverage consumption to a mean percent VAT of 24.5% in women who consumed at least one sugar-sweetened beverage per day.

*Increased Sugar-sweetened Beverage Intake and Visceral Adipose Tissue*

Further support for the association between sugar-sweetened beverage consumption and VAT was demonstrated in a randomized, intervention study on 47 healthy, overweight adults (34). The participants were divided into four groups and required to drink 1 liter of sucrose-sweetened cola, milk, diet cola, or water every day for six months. The cola and milk were iso-caloric, water and diet cola provided no calories. Slightly more men were randomly assigned to the regular cola group; beyond this, the four groups were not significantly different at baseline. VAT volume and SAT volume were measured by MRI and DEXA was used to measure total fat mass, lean body mass, and bone mass.

The effect of daily, sucrose-sweetened cola consumption was a greater increase in volume of VAT from baseline compared to all other beverage categories.  The VAT-to-SAT ratio was also significantly increased with cola consumption compared to milk consumption. Liver and muscle fat was elevated from baseline in the regular cola group compared with the three other beverages. No significant differences in VAT accumulation after 6 months were found between the participants that consumed milk, diet cola, or water. Both of the energy-containing drinks, milk and cola, led to an increase in SAT after 6 months whereas water and diet cola caused a reduction in SAT. These changes occurred without significant differences in body weight or total fat mass between any of the beverage groups after the intervention. Thus, the sucrose-sweetened beverage, even compared to iso-caloric milk, significantly increased VAT without changing total body fat mass suggesting sucrose consumption causes increased VAT deposition.

The three studies described above support the thesis hypothesis and continued research on the association between added sugar and VAT. The clinical intervention study on sugar-sweetened cola, milk, diet cola and water provide strong results; however, continued research is needed as the number of subjects was small and the study was un-blinded. The FFQs used in the cross-sectional studies allowed for collection of diet data on a large population; however, 24-hour recalls are considered gold-standard measurements of diet. The output from the Nutrition Data System for Researchers used in the thesis provides accurate information on sucrose intake by measuring disaccharide intake from all foods whereas the study above only analyzed sugar-sweetened beverage intake.

### Dietary Glucose versus Fructose

The research on disaccharides has shown that increased consumption of sucrose and high fructose corn syrup is associated with VAT. However, added sugar can also be consumed in the form of a monosaccharide, a single unit of glucose or fructose, and the type of monosaccharide consumed may influence the accumulation of VAT.

Much of the research on specific monosaccharide intake focuses on consumption of fructose. A study published in 2009 by Stanhope et al. found that fructose intake increased VAT in overweight and obese adults (35). This double-blind, randomized trial included 32 subjects, 40-72 years old, who were overweight without any chronic diseases. The study consisted of three phases: a two-week inpatient baseline period with an energy balanced diet, an eight-week outpatient intervention period with the fructose or glucose sweetened beverage providing 25% of daily energy needs along with usual *ad libitum* diet, and a two-week inpatient intervention period with fructose or glucose sweetened beverages providing 25% of daily energy requirements in an energy-balanced diet. The two two-week inpatient diets were very similar except for the adjustment for 25% of daily energy from the sweetened beverage. The purpose of the two-week inpatient period was to allow for comparison of high fructose or glucose diets under well-controlled conditions. The eight-week outpatient period allowed for the possibility of weight fluctuation, most likely weight gain that is common with consumption of sugar-sweetened beverages.

After the intervention, subjects consuming glucose did not have significantly changed total fat or VAT area from baseline as measured by CT scans. Both total abdominal fat and VAT area increased significantly from baseline in participants consuming the fructose beverage. By week ten, those consuming the fructose beverage had 14% greater VAT and 8.6% greater total abdominal fat from baseline. Both groups had comparable total body weight gain. This could be due to the unregulated metabolism of fructose compared to the tightly regulated metabolism of glucose.

This trial demonstrated that the consumption of a fructose-sweetened beverage caused an increase in VAT. Thus, in the thesis project, it will be imperative to consider the type of monosaccharide consumed by participants and analyze for the relationship between VAT and fructose consumption. This thesis analysis is relevant as the women are free-living and able to consume fructose in commonly found forms rather than in a solely fructose-sweetened beverage.

## Dietary Fiber

Dietary complex carbohydrates include a category of non-digestible polysaccharides that together are referred to as fiber. Dietary fiber has many known health benefits and several prospective and cross-sectional studies associate fiber intake with decreased VAT deposition. In adolescents, fiber intake was associated with an 18% reduced odds ratio of high VAT (28) and the EPIC study found that low fiber intake predicted a higher annual change in waist circumference adjusted for BMI in women (27).

Two studies examining VAT and dietary fiber intake found negative correlations between fiber intake and VAT. A cross-sectional study by Parikh et al. of 559 adolescents (36) compared dietary fiber intake, as assessed by four to seven 24-hour recalls, with measurements of total and central adiposity by MRI. Multiple linear regression was used to adjust for age, race, tanner stage, fat-free soft tissue mass, energy intake, and physical activity. Both fat mass and VAT volume were negatively associated with the intake of dietary fiber in girls and boys. All correlations were statistically significant with correlation coefficients of -0.102 in boys and -0.344 in girls.

Finally, a five-year prospective study by Hairston et al. examined types of dietary fiber and their association with fat localization in minority populations in the US (37). 1,114 African-American or Hispanic-American participants between 18 and 81 years old were included and abdominal CT scans were taken at baseline and at follow-up. Physical activity was documented by self-report and FFQs were used to determine dietary patterns. Increased physical activity and soluble fiber intake was associated with decreased VAT in participants, independent of changes in BMI. Every 10 grams of soluble fiber intake was associated with a 3.73% decreased accumulation of VAT.

This research provides evidence for the role of fiber, and possibly soluble fiber specifically, in VAT accumulation and supports continued research in this area. It is important for future research to use more accurate dietary intake assessment methods and include additional populations to better understand the benefit that dietary fiber provides in the protection against VAT accumulation.

Other Contributing Factors to Visceral Adipose Tissue

The primary aim of this study was to determine associations between dietary components and VAT; however, additional factors, including age, race, sex, menopausal status, BMI, activity level, and education status have also been shown to be associated with VAT accumulation.

A cross-sectional analysis by Katzmarzyk et al., measured total body fat, SAT, and VAT in Caucasian and African-American adults (38). Within the 1,967 participants, VAT accumulation was significantly higher in Caucasian compared to African-American men and women. The significance remained after adjustment for the covariates age and total body fat mass in men and women and after adjustment for age, total body fat mass, smoking and menopausal status in women. Caucasian women had significantly lower SAT than African-American women. Thus, it will be important to control for race in future research evaluating VAT.

Several studies have provided evidence for age-related changes in fat deposition, particularly increases in VAT with age. In a large cross-sectional analysis of 52,952 women, as age increased fat was more likely to be stored in the abdominal area rather than the gluteofemoral area (39). This may also be due to menopausal status since post-menopausal women accumulate more VAT than premenopausal women and VAT deposition is associated with reduced estrogen levels after menopause (40). However, when young women were compared with middle-aged premenopausal women, the middle aged women carried significantly more VAT even before menopause (39). This suggests that even before menopause, increased age is associated with increased deposition of VAT.

The deposition of VAT seems to be more pronounced in men than in women (41). A study of 164 men and women found that men had significantly higher VAT volume after correction for total body fat mass. However these differences were less pronounced when men were compared to post-menopausal women.

Physical activity is associated with VAT as revealed in a study of 1,114 people (37). While physical activity was documented by self-report only, the researchers found that increased levels of reported physical activity correlated with a decrease in VAT independent of change in BMI after 5 years. Finally, while no studies have shown that education status is related specifically to VAT, an analysis of a large prospective study in Europe found that higher educational status was associated with lower BMI (42). The differences between BMI groups in early adulthood became more pronounced with differences in education achievement over time. All of these factors, including sex, race, menopausal status, age, physical activity and education are important covariates in the analysis of VAT.

Conclusion

VAT influences the occurrence of chronic disease as demonstrated in research by Kabir (7), Despres (3), and Matasuzawa (6) as well as other researchers (9,10). Increased VAT accumulation is associated with insulin resistance, Type 2 diabetes, and hypertension. Multiple subtypes of carbohydrate, including simple sugars and fiber, have been shown to be both positively and negatively associated with VAT amounts. Furthermore, the monosaccharide fructose may play a greater role in VAT accumulation compared to glucose. Finally, studies have shown that non-dietary characteristics such as race, educational status, sex, age and physical activity level may affect VAT accumulation.

However, gaps exist in the literature in regards to study population and methods. Although studies on a variety of populations exist, few have looked specifically at free-living women. This thesis helps to fill the gap in the literature by focusing on a population of free-living women. Assessment of diet by multiple 24-hour recalls, used in this thesis, is also lacking in the literature. No studies have looked at total intake of monosaccharides and disaccharides. This is a critical gap that will be addressed in specific aim 1. The second aim also addresses a gap in the literature as few studies have determined if a relationship exists between fructose intake and VAT in a cohort of free-living women.

The analyses in this thesis test whether there is a significant association between the intake of types of carbohydrates and VAT, and if so, how large of an influence diet has in a free-living population of adult women.

# Chapter 3: Methods

## General Study Design

This study was a cross-sectional analysis of data obtained as part of the "Neurocognitive Effects of Hypothyroidism" study, conducted between 2008 and 2013. The subjects, described in detail below, included healthy control women and women with a history of primary hypothyroidism. Women diagnosed with hypothyroidism were all treated with levothyroxine (LT4) and had blood TSH concentrations within the normal reference range for at least 3 months at the time of data collection. Body composition, dietary intake, and physical activity information was gathered from each participant within three-weeks prior to initiating the study intervention, control subjects were not followed after this data was collected. Subjects were "free living" and were instructed not to make major alterations in their exercise and diet regimens.

All procedures took place at the Oregon Health & Science University (OHSU) Marquam Hill Campus in the Clinical Translational & Research Center (CTRC) of the Oregon Clinical & Translational Research Institute (OCTRI). The study was reviewed and approved by the OHSU Institutional Review Board (IRB) and all participants signed informed consent and Health Insurance Portability and Accountability Act (HIPAA) forms.

## Study Participants

This was a baseline, cross-sectional study of 104 free-living women, 20-75 years old, living in the Portland, OR metropolitan area. The participants met the inclusion criteria in Table 1 and included 16 healthy controls and 88 participants with primary hypothyroidism. All participants included in this secondary analysis were euthyroid for at least 3 months as confirmed by blood TSH concentrations within the laboratory reference range.

**Table 1: Inclusion and Exclusion Criteria**

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| • Age: 20 – 75 years<br>• Primary hypothyroidism on stable dose of LT4 for 3 months<br>• Euthryroid determined by normal blood TSH concentration for 3 months<br>• No acute or chronic medical or psychiatric illnesses<br>• Weight stable for 3 months<br>• Normal score on screening Mini-Mental State Examination (MMSE)<br>• Normal vision by screening examination<br>• Normal hearing by screening examination<br>• Non-smokers | • Failure to meet any of the inclusion criteria<br>• Inability to speak and comprehend English<br>• Significant abnormalities on screening electrocardiogram<br>• Pregnancy or intent to become pregnant during study (screening pregnancy tests routinely done before DEXA scans)<br>• Intentional interventions for weight modification<br>• Present or recent use of medications that affect thyroid hormone levels or interfere with thyroid hormone effects; beta blockers, lithium, glucocorticoids (non-systemic glucocorticoid use allowed), or iodine containing agents<br>• Current use of illegal drugs<br>• History of coronary artery disease<br>• Screening hemoglobin <10 g/dL<br>• Screening white blood cell count > 10,000 cells/mcL<br>• Clinically significant abnormalities on screening metabolic set<br>• Screening low density lipoprotein cholesterol >160 mg/dL<br>• Screening triglycerides > 300 mg/dL |

<u>Participant Recruitment</u>

Potential study participants were identified through the endocrinology clinic at OHSU. The participants were first screened over the phone and, if they initially qualified, they were asked to fill out an "Authorization to Use and Disclose Protected Health Information Release Form" to obtain their medical records to confirm that they met all eligibility criteria. The study was explained to interested participants, and the consent form was reviewed prior to enrollment.

Additional participants were recruited from other OHSU clinics by flyers and advertisements posted on the OHSU campus. Interested participants were directed to call the study staff who described the study, screened for potential eligibility and invited participants to attend a screening visit.

Participants were also identified from prior thyroid research studies and from a query of the Research Data Warehouse at OHSU (IRB protocol # e4076). These potential participants were sent a letter inviting them to participate in the study and informing them of a follow-up phone call. A query of the electronic health record at The Vancouver Clinic, Vancouver, Washington was also done. The study coordinators contacted the primary care providers or endocrine specialist of potential participants for permission to contact their patients.

Each participant attended a screening visit to check for general health, thyroid status, and mood or cognitive disorders. The participants came in after an overnight fast and if relevant, were asked to delay taking LT4 until after the visit. Participants were screened for alcohol or drug abuse by standard questioning and for cognitive disorders by the Mini-Mental State Examination, a thirty question test that measures cognitive impairment. The following fasting blood measures were drawn for the study: hemoglobin, TSH (if not done within the prior 6 weeks), glucose, free T4, CBC and chemistry battery, LDL cholesterol and triglyceride concentrations. An electrocardiogram was performed on all participants and a urine pregnancy test was conducted on women of child bearing age. The participants' degree of schooling was recorded, and they completed the vocabulary subtest of the WAIS-R. The WAIS-R Vocabulary subtest is a standardized measure of general intellectual functioning. The following measurements were made within a three-week period following the screening visit:

Dietary Assessment

Dietary intake data was collected using 24-hour recalls conducted by registered dietitians trained in the use of the Nutrition Data System for Research (NDSR) software. Three recalls, one weekend day and two weekdays, were collected over the telephone (22) from all participants. Recalls were unannounced and participants were asked about their food intake during the previous 24 hours using the multi-pass method (22). The passes are outlined below:

1. Collect a list of all foods and beverages consumed over the last 24 hours
2. Review the list of foods probing for missing items
3. Determine the details of the food or beverage, including type or brand, any added items, the amount consumed and the method of preparation
4. Probe for commonly forgotten foods
5. Review all the information collected with the participant.

The multi-pass method is a validated method for collecting dietary information and is considered one of the most accurate dietary collection tools (25). The amount of specific nutrients and the number of food group servings consumed daily was averaged for the analysis.

*Nutrition Data System for Research*

The NDSR database includes over 18,000 foods with complete nutrient information (43). The nutrient output from NDSR that was used for this analysis included total energy (kcals/day), total carbohydrate (grams/day), fiber (grams/day), added sugar (grams/day), monosaccharides and disaccharides (grams/day), average glycemic index and glycemic load, and the number of servings of sugar-sweetened foods and beverages (servings/day).

### Anthropometric Measurements

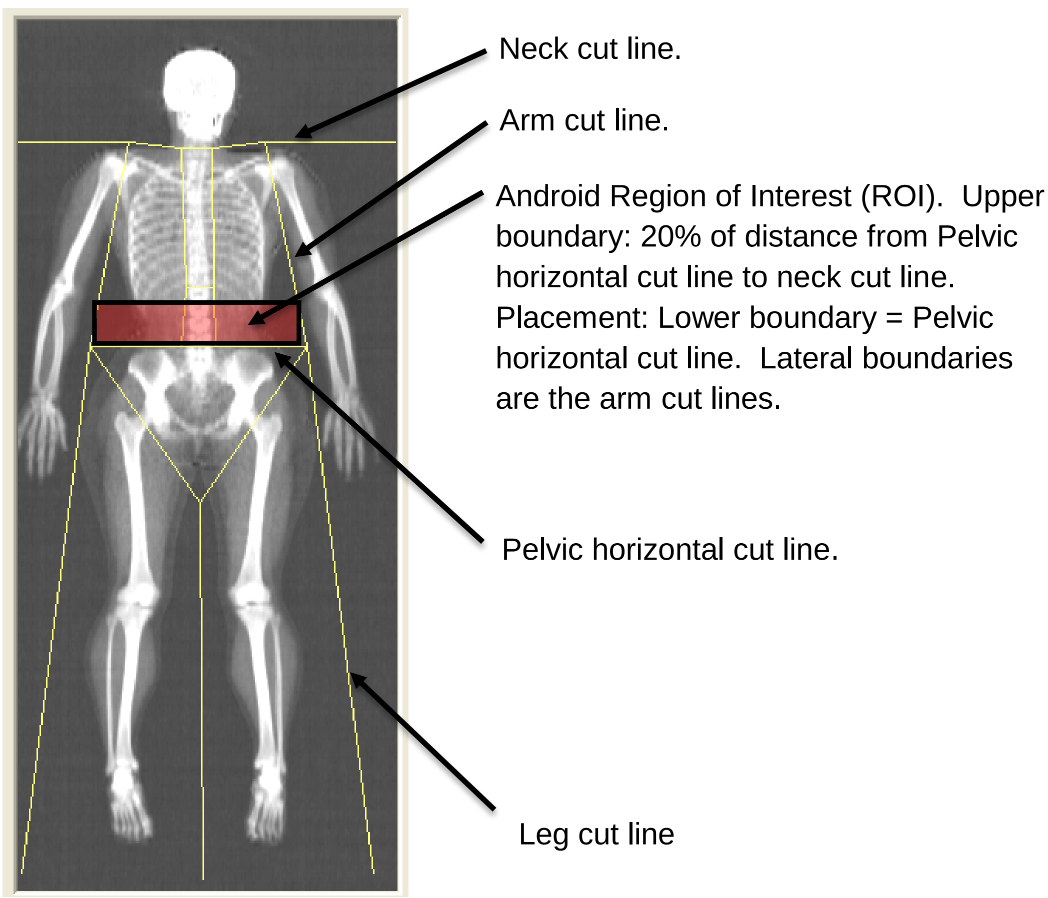
Anthropometric measures were taken at the first study visit following the consent/screening visit. Body weight was measured and recorded with a digital scale (Scale-Tronix, Model 5002, Wheaton, IL) to the nearest 0.01 kilogram while the participant was dressed in a hospital gown and after an overnight fast. Height was measured without shoes using a wall-mounted stadiometer (Harpenden Stadiometer, Holtain Ltd, Crymych, UK) to the nearest 0.1 cm.

### Body Composition Measurements

Total body composition (total lean and fat mass) and body fat distribution were measured using whole body Dual Energy X-ray Absorptiometry (DEXA) scans. After a negative pregnancy test was verified, the licensed DEXA technician positioned the subject within the quadrants of the scanning bed. Measurements were made using a Hologic QDR Discovery A Densitometer (Hologic, Inc., Bedford, MA.)

The whole-body DEXA scans were initially analyzed by the licensed DEXA technician for total and regional lean mass and fat distribution. These same DEXA scans were reanalyzed using Hologic's new Horizon DXA System software to measure visceral adipose tissue (VAT) by a trained, single operator. The region of interest for VAT assessment was defined as 20% of the distance from the pelvic horizontal cutline to the neck cut line. The pelvic horizontal cut line served as the lower boundary and one-fifth of the distance from the pelvic cut line to the neck cut line was the upper boundary, see Figure 2 below. These boundaries were identified by automated software algorithms and confirmed visually by the operator. The automated software then identified the subcutaneous fat ring, the inner abdominal muscle wall, and the visceral cavity at the level of the fourth lumbar vertebrae. The software calculated the area ($cm^2$) and volume ($cm^3$) of VAT by subtracting subcutaneous adipose tissue (SAT) from total abdominal fat. This is considered an accurate location and measurement of VAT and SAT in the body (44). These measures were used to determine VAT as a percent of total body fat (PVAT). DEXA scans have been shown to be as accurate at calculating VAT as computed tomography (CT) and magnetic resonance imaging (MRI) scans (12, 13).

**Figure 2: DEXA Image**



Activity monitoring

The Actical® activity-monitoring device (Philips-Respironics, Bend, OR) utilizes a multidirectional accelerometer to monitor the occurrence and intensity of motion, or epoch. The Actical® device measures three cm by three cm, weighs 17.0 grams, and is securely attached to a waistband and placed around the waist. The device was worn and data was collected for four to seven days. Data was downloaded from the accelerometer using an ActiReader and converted into total activity counts, average activity (counts per minute), time interval duration (minutes), activity ranges during sedentary, light, moderate, and vigorous activity, and accumulated time within each activity range (minutes). Using software provided by Philip-Respironics, average minutes spent in moderate plus vigorous physical activity was calculated. This variable was included in the analysis as a possible covariate.

Descriptive Statistics

The statistical analyses were run on all subjects combined into one group to test the primary study hypotheses. Data representing descriptive variables were averaged and expressed as the mean ± SD (or median [IQR]) for each continuous variable. Descriptive variables included age, weight (kg), BMI ($kg/m^2$), total body fat percent, VAT mass (g), area ($cm^2$) and volume ($cm^3$), and VAT as a percentage of total body fat (PVAT). The statistical analysis software used was STATA Data Analysis and Statistical Software for Windows (version 14; College Station, Texas).

Statistical Analysis Plan

Multivariable linear regression was used to identify and describe significant ($p<0.05$) associations between the outcome of interest (PVAT) and key dietary variables while adjusting for possible confounding variables age, BMI, years of education, minutes spent in moderate plus vigorous physical activity, ethnicity (defined as white and other), and menopausal status (defined as premenopausal, premenopausal on hormone therapy, post-menopausal, or post-menopausal on hormone therapy). Only those covariates which significantly changed the model were included in further analyses.

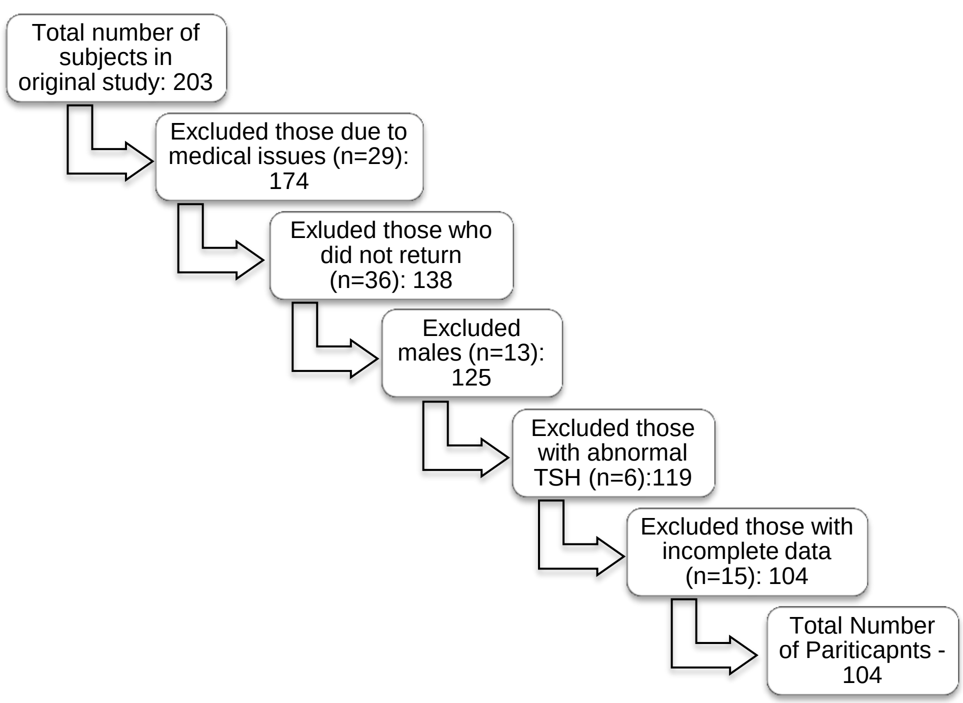
Models were separately built for each specific aim by first including (and retaining) all variables intended for purposes of adjustment, and then supplementing this baseline multivariable model with those key dietary variables of interest that significantly improved model performance. To address the first specific aim, PVAT was tested for relationships with total simple sugar intake as a percent of total energy intake, glycemic index, glycemic load, and fiber intake. To address the second specific aim, correlations between PVAT and fructose consumption as a percent of total carbohydrate intake, added sugar intake, and servings of sugar-sweetened foods and beverages were determined.

## Chapter 4: Results

A subset of the baseline data from the Neurocognitive and Metabolic Effects of Mild Hypothyroidism study was used to examine the relationship between diet and body composition. All measures including questionnaires, DEXA scans, activity monitoring, and 24-hour recalls, were collected prior to the initiation of any study intervention. These measures were collected when participants were euthyroid based on measured serum TSH concentrations within the normal reference range. Original DEXA scans were reanalyzed for VAT after the study was concluded.

Data from 104 participants were included in the analysis from the original group of 203 study participants. The selection process for inclusion of these participants is shown in Figure 3. During the screening process, twenty-nine people were excluded for medical issues and thirty-six did not return for the baseline visit. Thirteen males and 6 people with abnormal serum TSH concentrations were removed. Finally, fifteen participants had incomplete data and were excluded.

**Figure 3 – Inclusion Process**



Participant Characteristics

The group of participants included in this analysis ranged in age from 21 - 68 years with a mean age of 46 ± 11 years. Educational level was recorded in total years and the participants had a mean educational level of 16 ± 3.1 years which ranged from 9-26 years. Duration of moderate plus vigorous physical activity was measured in 99 of the 104 participants and the average time spent in moderate plus vigorous physical activity was 144.8 ± 77.8 minutes per day with a range of 40-481 minutes per day. The majority (88.5%) of participants identified as white.

Menopausal and hormone therapy status is described in Table 2. Slightly over half of the participants were premenopausal, 60.6%, and 49 (77.8%) of those women were pre-menopausal and not on hormone therapy. Forty-one women (39.4%) were post-menopausal and the majority, 90.2%, of those participants were not receiving any hormone therapy. Overall, 86 (82.7%) women were not on hormone therapy.

**Table 2 – Menopausal Status of Participants**

| Menopausal Status | No Hormone Therapy | Hormone Therapy | Total |
|---|---|---|---|
| Pre-menopausal | 49 (77.8%) | 14 (22.2%) | 63 (60.6%) |
| Post-menopausal | 37 (90.2%) | 4 (9.8%) | 41 (39.4%) |
| Total | 86 (82.7%) | 18 (17.3%) | 104 |

Physical Characteristics of Participants

Body composition results are shown in Table 3. Overall, the majority of women were overweight with a mean body weight of 74.3 ± 17.1 kg and a mean BMI of 27.5 ± 6.1 kg/m$^2$. The average overweight status was confirmed by the average total body fat percent of 38.0 ± 7.4%. The amount of abdominal VAT was assessed within a region above the pelvis (VAT ROI) and the average area, volume and mass are shown in Table

3. To examine the amount of VAT relative to total body fat, VAT ROI mass is shown as a percent of total fat mass (PVAT). Which is the main outcome variable used for further analyses.

**Table 3 – Physical Measures and Body Composition of Participants**

| Variables | Mean ± SD | Minimum | Maximum |
|---|---|---|---|
| Weight (kg) | 74.3 ± 17.1 | 47.4 | 127.4 |
| BMI (kg/m$^2$) | 27.5 ± 6.1 | 18.7 | 46.0 |
| Total Body Fat (kg) | 29.2 ± 12.1 | 12.4 | 68.9 |
| Body Fat Percent (%) | 38.0 ± 7.4 | 24.2 | 54.6 |
| VAT ROI area* (cm$^2$) | 94.3 ± 62.5 | 8.3 | 252.0 |
| VAT ROI Volume* (cm$^3$) | 491.4 ± 326.1 | 43.5 | 1316.0 |
| VAT ROI Mass* (g) | 454.5 ± 301.6 | 40.2 | 1217.0 |
| PVAT (%) | 1.4 ± 0.58 | 0.28 | 3.26 |

*VAT measured at ROI (region of interest); VAT – visceral adipose tissue, PVAT – percent visceral adipose tissue mass of total body fat mass

Dietary Intake

The 3-day average intake of individual nutrients for the group is shown in Table 4. The mean energy intake was 1,957 ± 525 kilocalories (kcals/day) with 47.7 ± 7.8% of the total calories provided by carbohydrates. The average simple sugar intake accounted for 43.4% of the total carbohydrates consumed, while fiber was 9.3%. Within the total simple sugars, the monosaccharides, glucose and fructose, accounted for a similar portion of the sugars, 18.6% and 18.8%, respectively, while the disaccharide, sucrose, made up 43.9% of simple sugar intake. The mean intake of added sugars was 54.4 ± 32.8 grams and accounted for 22.7% of total carbohydrate intake and 10.8% of total energy intake. Naturally-occurring sugars were calculated by subtracting added sugars from total sugars and accounted for 10.2% of total energy. The mean intake of sugar-sweetened beverages (SSB) was less than half of an 8 fluid ounce serving per day (0.4 ± 0.7 servings) and the mean servings of sugar sweetened foods (SSF) was 3.2 ± 2.6 servings per day. Finally, the average daily glycemic index (GI) for this group was 58 ± 4.7 with an average glycemic load of 127.2 ± 46.0.

**Table 4 – Dietary Intake of the Study Participants**

| Nutrient Variables | Mean ± SD | Minimum | Maximum |
|---|---|---|---|
| Total Energy Intake (kcal/day) | 1957 ± 525 | 872 | 3285 |
| Total Fat (g) | 77.2 ± 27.6 | 23.6 | 133.9 |
| % of Total Energy | 34 ± 6.5% | 8.5% | 49.0% |
| Total Protein (g) | 73.6 ± 18.8 | 36.7 | 129.4 |
| % of Total Energy | 16.0 ± 3.7% | 9.4% | 31.9% |
| Total Carbohydrate (g) | 240.0 ± 76.8 | 99.3 | 488.1 |
| % of Total Energy | 47.7 ± 7.8% | 21.4% | 68.7% |
| Total Simple Sugars (g) | 104.2 ± 45.8 | 23.4 | 225.2 |
| % of Total Energy | 21.1 ± 6.5 | 6.6 | 40.0 |
| Monosaccharides (g) | 39.1 ± 23.5 | 7.3 | 133.6 |
| Fructose (g) | 19.5 ± 11.9 | 3.3 | 59.7 |
| % of Total Carbohydrate | 8.0 ± 3.8 | 1.5 | 20.2 |
| Glucose (g) | 19.3 ± 11.8 | 3.8 | 72.9 |
| Disaccharide (g) | 65.1 ± 31.6 | 9.5 | 152.2 |
| Sucrose (g) | 45.7 ± 25.3 | 3.9 | 119.5 |
| Glycemic Index (Index: 0-100) | 58.0 ± 4.7 | 41.3 | 67.7 |
| Glycemic Load | 127.2 ± 46.1 | 34.6 | 271.3 |
| Total Fiber (g) | 22.2 ± 9.1 | 7.5 | 58.8 |
| Naturally-occurring Sugar (g) | 50.1 ± 24.9 | 7.9 | 148.9 |
| Added Sugar (g) | 54.4 ± 32.8 | 1.7 | 143.2 |
| % of Total Energy | 10.8 ± 5.1 | 0.5 | 24.0 |
| Sugar-Sweetened Beverages (servings) | 0.4 ± 0.7 | 0 | 2.9 |
| Sugar-Sweetened Foods (servings) | 3.2 ± 2.6 | 0 | 13.5 |

In order to control for differences in total energy intake, the number of grams or servings for each nutrient were calculated per 1,000 kcal and the values for the key nutrient are shown in Table 5. Daily intake of fructose and glucose per 1,000 kcal were similar (10.0 vs. 9.8 grams, respectively) as was the consumption of added sugars compared to naturally-occurring sugars (26.9 vs.26.0 grams, respectively.)

**Table 5 – Nutrient Density**

| Nutrient Variables | Mean ± SD | Minimum | Maximum |
|---|---|---|---|
| Fructose (g/1,000 kcal) | 10.0 ± 5.4 | 1.6 | 27.0 |
| Glucose (g/1,000 kcal) | 9.8 ± 5.0 | 2.1 | 32.4 |
| Sucrose (g/1,000 kcal) | 22.8 ± 9.4 | 2.1 | 51.1 |
| Naturally-occurring Sugar (g/1,000 kcal) | 26.0 ± 11.2 | 6.6 | 56.1 |
| Added sugar (g/1,000 kcal) | 26.9 ± 12.6 | 1.2 | 60.0 |
| Fiber (g/1,000 kcal) | 11.6 ± 4.2 | 4.2 | 24.6 |
| Sugar-Sweetened Beverages (Servings/1,000 kcal) | 0.20 ± 0.34 | 0 | 1.6 |
| Sugar-Sweetened Foods (Servings/1,000 kcal) | 1.6 ± 1.1 | 0 | 5.26 |

kcal – kilocalories

<u>The Relationship between Menopausal Status, Age and Percent Visceral Adipose Tissue</u>

Participants were divided into subcategories by menopausal status (Table 6). An F-test was run to determine significant differences between all groups. VAT ROI mass (p=0.025) and PVAT (p=0.0064) were significantly different among all four groups. Physical activity (moderate + vigorous) was not significantly different among all the groups (p-value = 0.22). When the Scheffe post-hoc, pair-wise comparison was performed VAT mass was not significantly different. PVAT was significantly different (p<0.01) between the post-menopausal group without hormone therapy and the premenopausal group with hormone therapy.

**Table 6 – Visceral Adipose Tissue between Menopausal Groups**

| Category Mean ± SD | Premenopausal | Premenopausal on hormone therapy | Post-menopausal | Post-menopausal on hormone therapy | p-value across all groups |
|---|---|---|---|---|---|
| Number of subjects (% of total) | 49 (47.1%) | 14 (13.5%) | 37 (35.5%) | 4 (3.85%) | |
| VAT ROI mass (g) | 457 ± 273 | 270 ± 216 | 535 ± 342 | 262 ± 121 | 0.045* |
| PVAT (%) | 1.4 ± 0.47 | 1.1 ± 0.54[a] | 1.6 ± 0.65[a] | 1.2 ± 0.38 | 0.0024* |
| Moderate + Vigorous PA (minutes/day) | 160 ± 84.6 | 148 ± 66.3 | 127 ± 72.5 | 109 ± 43.8 | 0.22 |

Mean ± SD for F-test; VAT measured at ROI (region of interest); VAT – visceral adipose tissue, PVAT – percent visceral adipose tissue mass of total body fat mass; PA – physical activity
*p-value ≤ 0.05 is considered statistically significant
Scheffe post-hoc analysis for difference between pairs; [a]p-value < 0.01

The participants were divided into three groups based on age: less than 40 years old, between 40 and 60 years old and older than 60 years. An F-test was run to determine differences between all three groups. Table 7 shows the mean ± standard deviation for VAT ROI mass, PVAT, and moderate plus vigorous physical activity by age. VAT ROI mass and PVAT were significantly different (p-value 0.0014 and <0.001) among all three groups. However, moderate plus vigorous physical activity in minutes per day was not significantly different among the groups (p-value = 0.0561). Scheffe post-hoc analysis of pairs demonstrated that for VAT mass the oldest and youngest groups were significantly different. The post-hoc analysis for PVAT showed that the oldest group compared to both the youngest group and the middle group had significantly higher PVAT (%).

**Table 7 – Visceral Adipose Tissue between Age Groups**

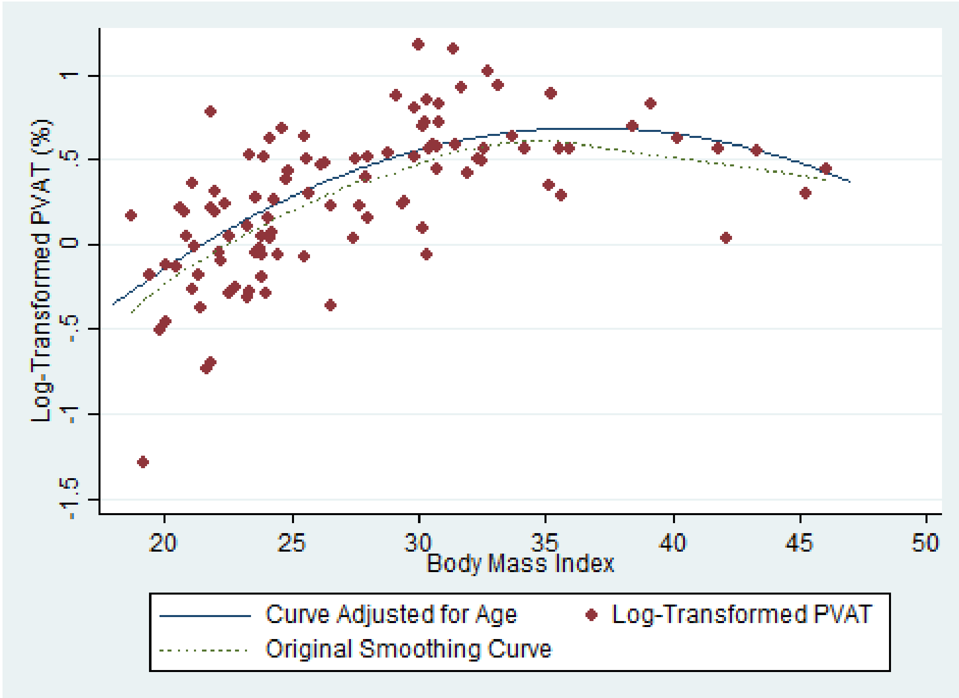
| Category | < 40 years | 40-60 years | > 60 years | p-value |
|---|---|---|---|---|
| Number of subjects (%) | 32 (30.8%) | 61 (58.7%) | 11 (10.5%) | |
| VAT ROI mass (g) | 333 ± 260[a] | 474 ± 281 | 698 ± 372[a] | 0.0014* |
| PVAT (%) | 1.1 ± 0.4[bc] | 1.5 ± 0.54[b] | 1.9 ± 0.66[c] | <0.001* |
| Moderate + Vigorous PA (minutes/day) | 160 ± 81.0 | 146 ± 78.8 | 91.9 ± 30.1 | 0.0561 |

Mean ± SD for F-test; VAT measured at ROI (region of interest); VAT – visceral adipose tissue, PVAT – percent visceral adipose tissue mass of total body fat mass; PA – physical activity
*p-value ≤ 0.05 is considered statistically significant;
Scheffe post-hoc pairwise comparison; [a] p=0.002; [b] p<0.001; [c]p<0.001

Relationship between Body Mass Index and Percent Visceral Adipose Tissue

BMI showed a curvilinear relationship with log-transformed PVAT as shown in Figure 4. The maximum median PVAT was estimated to occur when BMI was 35.8 (95% CI: 34.0—39.1) kg/m$^2$. At this point, the median PVAT was 1.94% (95% CI: 1.77—2.13%). The relationship between BMI and PVAT was positive when BMI was below 35.8 kg/m$^2$ and negative once BMI exceeded 35.8 kg/m$^2$. For example, within the healthy BMI category the median PVAT of women with a BMI of 22 kg/m$^2$ was 24% higher than those with a BMI of 20 kg/m$^2$. The association was negative above a BMI of 35.8 kg/m$^2$ as evidenced by the negative slope. For example, in obese category III, those with a BMI of 46 kg/m$^2$ had 13% lower median PVAT than women with a BMI of 44 kg/m$^2$. In Figure 4 the dotted line shows the unadjusted relationship between BMI and PVAT and the solid line demonstrates the relationship between BMI and PVAT when controlling for age.

**Figure 4 – Correlation between Body Mass Index and Percent Visceral Adipose Tissue with Fitted Line for Age**




Linear Regression Models

Linear regression analysis was performed for each dietary variable and log-transformed PVAT. Each correlation coefficient was then multiplied by a factor that allowed for clinically relevant values to be determined from the linear regression, the columns noted multiplicative effect in Table 8. Column 2 of Table 8 shows the simple linear regression analysis without any covariates included for each variable. The significant findings include age and BMI. Each 10-year increase in age was associated with a 19.6% increase in median PVAT (95% CI: 11.9-27.8% increase, p-value <0.001). For BMI, each 5 kg/m$^2$ increase was associated with a 21.6% (95% CI: 14.9-28.7%) increase in median PVAT (p<0.001). Only total carbohydrate, total fiber, naturally-occurring sugars, and SSBs were significantly associated with PVAT before inclusion of the covariates, age and BMI (0.952, 0.947, 0.942 and -.035, respectively). The columns on the far right of Table 8 show the results of the analysis when age and BMI were included in the regression analysis model. Only total fiber was still significantly associated with PVAT, such that it was estimated to decrease median PVAT by 3% (95% CI: 0.1-5.9% decrease) with each 5 grams of additional fiber (p-value = 0.046).

Linear regression analysis was performed with dietary variables converted to grams or servings per 1,000 kcals shown in Table 9. Before age and BMI were included in the analysis SSF was significantly associated with log-transformed PVAT (p-value=0.01). However, including BMI and age resulted in no significant associations between dietary variables per 1,000 kcals and log-transformed PVAT.

**Table 8 – Regression Analysis of Dietary Variables with Percent Visceral Adipose Tissue**

| Variable | Simple linear regression | | | Linear Regression[a] | | |
|---|---|---|---|---|---|---|
| | Multiplicative effect | p-value | Confidence Intervals | Multiplicative effect | p-value | Confidence Intervals |
| Age<br>per 10 years | 1.196 | <0.001 | 1.121-1.275 | | | |
| BMI<br>per 5 units | 1.216 | <0.001 | 1.149-1.287 | | | |
| Hormone Supplementation | 0.678 | 0.053 | 0.351-1.004 | 0.991 | 0.935 | 0.781-1.202 |
| Menopause | 1.154 | 0.069 | 0.988-1.320 | 0.963 | 0.686 | 0.781-1.145 |
| Ethnicity | 1.026 | 0.843 | 0.770-1.281 | 1.128 | 0.163 | 0.947-1.308 |
| Education<br>per 5 years | 0.878 | 0.075 | 0.761-1.101 | 0.925 | 0.096 | 0.843-1.014 |
| Moderate plus Vigorous Physical Activity<br>per 60 minutes | 0.942 | 0.062 | 0.884-1.003 | 0.993 | 0.796 | 0.941-1.048 |
| Total Energy<br>per 500 kcal | 0.943 | 0.125 | 0.874-1.017 | 0.990 | 0.709 | 0.937-1.045 |
| Total Carb<br>per 50 g | 0.952 | 0.039 | 0.908-0.997 | 1.002 | 0.925 | 0.962-1.044 |
| Total Simple Sugars<br>per 40 g | 0.950 | 0.147 | 0.885-1.019 | 1.017 | 0.545 | 0.963-1.073 |
| Monosaccharides + Disaccharides (% points of total energy)<br>per 5% | 0.989 | 0.719 | 0.928-1.053 | 1.025 | 0.282 | 0.980-1.072 |
| Monosaccharide (g)<br>per 20 g | 0.973 | 0.509 | 0.897-1.056 | 1.026 | 0.362 | 0.971-1.084 |

**Table 8 (continued) – Regression Analysis of Dietary Variables with Percent Visceral Adipose Tissue**

| Variable | Simple linear regression | | | Linear Regression[a] | | |
|---|---|---|---|---|---|---|
| | Multiplicative effect | p-value | Confidence Intervals | Multiplicative effect | p-value | Confidence Intervals |
| Fructose (g) per 10 g | 0.979 | 0.602 | 0.904-1.060 | 1.027 | 0.354 | 0.970-1.088 |
| Fructose (% points of Total Carbohydrate) per 1% | 1.011 | 0.362 | 0.987-1.035 | 1.014 | 0.076 | 0.999-1.029 |
| Galactose (g) Per 1 g | 0.834 | 0.202 | 0.630-1.104 | 1.005 | 0.962 | 0.831-1.215 |
| Glucose (g) per 10 g | 0.969 | 0.461 | 0.892-1.054 | 1.023 | 0.383 | 0.971-1.079 |
| Disaccharides (g) per 10 g | 0.981 | 0.104 | 0.958-1.004 | 1.002 | 0.869 | 0.983-1.02 |
| Maltose (g) per 2 g | 0.980 | 0.551 | 0.916-1.048 | 1.007 | 0.784 | 0.960-1.056 |
| Sucrose (g) per 10 g | 0.981 | 0.160 | 0.954-1.008 | 1.003 | 0.779 | 0.984-1.002 |
| Lactose (g) per 10 g | 0.954 | 0.304 | 0.871-1.045 | 0.996 | 0.913 | 0.933-1.064 |
| Glycemic Index per 5 | 1.074 | 0.083 | 0.991-1.166 | 1.030 | 0.286 | 0.975-1.087 |
| Glycemic Load per 25 | 0.973 | 0.162 | 0.936-1.011 | 1.009 | 0.562 | 0.978-1.042 |
| Total Fiber (g) per 5 grams | 0.947 | 0.005 | 0.913-0.983 | 0.970 | 0.046* | 0.941-0.999 |
| Added Sugar (g) per 10 g | 0.997 | 0.807 | 0.975-1.020 | 1.010 | 0.169 | 0.996-1.025 |
| Naturally-occurring Sugars (g) per 10 g | 0.962 | 0.037 | 0.927-0.998 | 0.997 | 0.985 | 0.962-1.032 |

**Table 8 (continued) – Regression Analysis of Dietary Variables with Percent Visceral Adipose Tissue**

| Variable | Simple linear regression | | | Linear Regression[a] | | |
|---|---|---|---|---|---|---|
| | Multiplicative effect | p-value | Confidence Intervals | Multiplicative effect | p-value | Confidence Intervals |
| Added Sugar (% points of Total Energy) per 5% | 1.020 | 0.586 | 0.948-1.098 | 1.041 | 0.096 | 0.993-1.091 |
| SSB Per 1 serving | 1.025 | 0.666 | 0.915-1.149 | 1.084 | 0.096 | 0.986-1.191 |
| SSF per 1 serving | 0.966 | 0.002 | 0.945-0.987 | 0.993 | 0.537 | 0.972-1.015 |

Linear Regression analysis
[a] Linear regression including covariates BMI and age
*p-value ≤ 0.05 is considered statistically significant
VAT measured at ROI (region of interest); VAT – visceral adipose tissue, SSF – Sugar-Sweetened Foods; SSB – Sugar-Sweetened Beverages

**Table 9 – Regression Analysis of Nutrient Density with Percent Visceral Adipose Tissue**

| Variable | Simple linear regression | | | Linear Regression[a] | | |
|---|---|---|---|---|---|---|
| | Multiplicative effect | p-value | Confidence Intervals | Multiplicative effect | p-value | Confidence Intervals |
| Total Sugars (g/1,000 kcal) | 0.999 | 0.713 | 0.994-1.004 | 1.002 | 0.264 | 0.998-1.006 |
| Naturally-occurring Sugars (g/1,000 kcal) | 0.996 | 0.317 | 0.988-1.004 | 1.00 | 0.950 | 0.994-1.007 |
| Fructose (g/1,000 kcal) | 1.005 | 0.544 | 0.988-1.022 | 1.009 | 0.118 | 0.998-1.02 |
| Glucose (g/1,000 kcal) | 1.001 | 0.910 | 0.984-1.018 | 1.008 | 0.163 | 0.997-1.019 |
| Sucrose (g/1,000 kcal) | 0.998 | 0.538 | 0.990-1.005 | 1.001 | 0.772 | 0.996-1.006 |
| Fiber (g/1,000 kcal) | 0.987 | 0.105 | 0.971-1.003 | 0.989 | 0.071 | 0.976-1.001 |
| SSB (servings/1,000 kcal) | 1.072 | 0.550 | 0.851-1.352 | 1.184 | 0.055 | 0.996-1.406 |
| SSF (servings/1,000 kcal) | 0.932 | 0.01 | 0.883-0.983 | 0.984 | 0.493 | 0.938-1.032 |

Linear Regression analysis
[a]Linear regression including covariates BMI and age
VAT measured at ROI (region of interest); VAT – visceral adipose tissue, SSF – Sugar-Sweetened Foods; SSB – Sugar-Sweetened Beverages

Multi-Variable Regression Models

A multi-variable model was created to determine if specific nutrients grouped together demonstrated significant associations with PVAT. These models controlled for only age and BMI as no other non-dietary variables were significantly associated with PVAT. The grouping of nutrients in Table 10 is related to the first specific aim and demonstrates that total fiber in grams was significantly correlated with PVAT (p-value = 0.033). This is an inverse correlation as the correlation coefficient multiplied by the multiplicative effect is less than 1. No other dietary variables included in the model were significantly related to PVAT.

Several other models were also created including combinations of variables related to the specific aims and groupings of nutrients based on how they could be consumed in a typical food or meal. For example, each monosaccharide per 1,000 kcals was combined in one model.  Other models included analysis of nutrients such as added sugars, naturally-occurring sugars and fiber per 1,000 kcals. However, none of these models demonstrated any significant correlations between dietary variables and PVAT.

**Table 10 – Association of Dietary Variables with Percent Visceral Adipose Tissue**

| Variables | Multiplicative Correlation | p-value |
|---|---|---|
| Total Simple Sugars (1% of Total Energy) | 1.002 | 0.74 |
| Glycemic Index Per 5 | 1.000 | 0.99 |
| Glycemic Load Per 25 | 1.023 | 0.42 |
| Total Fiber (g) Per 5 grams | 0.961 | 0.033* |

Multivariable linear regression model included covariates age and BMI;
*p-value ≤ 0.05 is considered statistically significant

In conclusion, the only significant finding of this secondary analysis was that total fiber intake was inversely associated with PVAT.  This association remained significant with inclusion of covariates and in the multi-variable model.  No other dietary factors were significantly associated with PVAT. Age and BMI were associated with PVAT as predicted, although menopausal status, ethnicity, years of education and physical activity were not.

## Chapter 5: Discussion

This study examined the relationship between specific dietary components and VAT. The significant finding from this study was that increased intake of total fiber was associated with decreased VAT as a percent of total body fat at the ROI (PVAT). The other hypotheses were rejected. PVAT was not associated with intake of monosaccharides, disaccharides, glycemic index, glycemic load, fructose, added sugar, or servings of sugar-sweetened beverages (SSB) or sugar-sweetened foods (SSF).

### Participant Demographics

The participants for this analysis were drawn from the Neurocognitive and Metabolic Effects of Mild Hypothyroidism study. These women were highly educated, middle-aged, overweight, and mostly white in comparison to the general U.S. population. The median age of participants was 7.5 years older than the median age of all women in the United States (45) and the average education level was slightly more than a Bachelor's degree. The mean BMI was 27.5 kg/m$^2$ and the range was wide; however, 56% of women in the study were overweight. This is similar to national data which estimate the average BMI for adult women in the US is 28.7 kg/m$^2$ (46). The women were recruited from Portland area clinics, limiting the racial and ethnic diversity of the participants. As a result, this group of participants is only representative of women in the Northwest area and the findings from this study are only generalizable to similar individuals and groups.

Given the intense debate around added sugar consumption and the increasing rates of obesity the goal of this analysis was to determine the influence of dietary carbohydrate intake on VAT. We found that the participants in the current analysis consumed a slightly healthier diet than the average American adult. Participant consumption of total energy and distribution of macronutrients were within the recommendations of Dietary Guidelines for Americans (47). Compared to U.S. adults studied by the National Health and Nutrition Examination Survey (NHANES), a nationally representative survey that examines the nutritional status of adults and children every year (48), the current participants consumed more fiber, less added sugar and fewer SSBs. NHANES data from 2005-2010 found that American adults consumed an average of 13% of total energy as added sugar. Women aged 20-39 years consumed 14.5% of total energy as added sugar and women 40-59 years old consumed 12.9% of total energy as added sugar (49). Participants in the current analysis consumed 10.8% of total energy as added sugar, less than both groups of women and the average intake of all U.S. adults. The study participants also consumed fewer servings of SSBs -- less than half of an 8-ounce beverage per day on average. An analysis of NHANES data from 2009-2010 found that 50.6% of men and women consumed one or more SSB per day and 25% consumed two or more SSBs per day (50). Average consumption of total fiber in the study population had a wide range and was close to the recommendation of 21-25 grams per day for adult females (47). U.S. adults have an average intake of 16.1 grams of total fiber per day (51), 6.1 grams on average less than the current group of participants. The lower intake of SSBs and added sugar as well as increased fiber intake suggest that this study's participant population was consuming a healthier and a diet more similar to the Dietary Guidelines for Americans than the average US adult. This may have limited the opportunity for finding a significant association between dietary variables and VAT.

### Visceral Adipose Tissue and Demographic Characteristics

Age, menopausal status, BMI and physical activity were included in the current analysis as possible covariates to VAT. PVAT and VAT mass at the ROI were significantly different between age groups, and as age increased PVAT increased in the current study. This result supports other research on VAT and age. In a large cross-sectional study of African-American and European-American women, VAT was positively associated with age (52). Lanaska et al. (39) also found a positive association between age and VAT deposition in women before menopause, suggesting that increasing age may contribute to an increase in VAT in women, regardless of change in menopausal status. Increased VAT deposition in men is also associated with age. Men, compared to women, have more VAT stores and preferentially store fat in the trunk and upper body and have continued deposition with increasing age (53).

The sex hormones estrogen and testosterone facilitate adipose deposition and function in men and women. Low levels of testosterone in men are associated with increased VAT and hormone therapy, which returns testosterone levels to normal, causes a decrease in VAT accumulation (53). In pre-menopausal women, estrogen released from the ovaries supports accumulation of fat in the subcutaneous layer. During and after menopause, circulating estrogen is reduced and estrogen production from fat stores becomes important (53). The reduction in circulating estrogen with menopause is hypothesized to be the mechanism for increased VAT in post-menopausal women (54, 55). Palmer et al. (56) hypothesized that the increase in conversion of subcutaneous fat to VAT during menopause serves as a source of estrogen production. Hormone replacement therapy is associated with a slower rate of VAT accumulation supporting the role of estrogen in VAT deposition (40). In the current analysis, VAT ROI mass and PVAT were significantly different between groups of women based on menopausal status and hormone replacement therapy. However, once age and BMI were included in the analysis, menopausal status was no longer significantly associated with PVAT. This suggests that age and BMI have a greater effect on VAT accumulation than menopausal status. Nevertheless, the current study might not have been large enough to differentiate between the effect of age, menopause and hormone therapy on VAT because most of the participants fell within the pre-menopausal category and a much smaller number of participants were postmenopausal or receiving hormone replacement therapy.

Overall adiposity is also associated with VAT. This relationship is often accounted for in other research studies (11, 28) by including either SAT volume or BMI in the analysis. In the current analysis, BMI was associated with PVAT, however the association was curvilinear. Below a BMI of 35.8 kg/m$^2$ the relationship was positive; a change in BMI from 20-22 kg/m$^2$ was associated with a 24% increase in median PVAT. The relationship between BMI and PVAT became negative with BMI above 35.8 kg/m$^2$. For example, median PVAT was calculated to decrease by 13% with a change in BMI from 44 kg/m$^2$ to 46 kg/m$^2$. This association has not been seen in other research on VAT and may be due to the small number of participants in the current study and the small percentage of participants with BMIs greater than 35.8 kg/m$^2$. In other populations, data shows a steady increase in VAT with BMI (57).

An active lifestyle involving regular moderate and vigorous physical activity is associated with reduced VAT (8, 37). Murabito et al. (57) found an inverse relationship between time spent in moderate and vigorous physical activity and VAT volume in women. Physical activity was measured by Actical® accelerometer for 5-7 days in 1,249 participants. Every 30 minutes per day increase in moderate plus vigorous physical activity was significantly associated with an average decrease in VAT even after controlling for SAT and BMI. However, physical activity was not significantly associated with PVAT in the current analysis. This is surprising considering the level of accuracy used to monitor physical activity and the high number of minutes spent in moderate and vigorous activity. The reason for the lack of association may be due to the small amount of time spent in vigorous activity. The majority of activity by participants was moderate intensity which includes activities such as walking, climbing stairs, and housework. Thus, a large amount of moderate activity may have less effect on VAT.

Percent Visceral Adipose Tissue and Nutrient Intake

To determine any significant associations between dietary intake variables and PVAT, linear regression models, first excluding and then including age and BMI as covariates, were created. Before inclusion of BMI and age in the models, several dietary variables were significantly associated with PVAT, including total carbohydrate, total fiber, naturally-occurring sugar, and servings of SSBs. However, once variation due to age and BMI was taken into account, only total fiber remained significantly associated with PVAT. This agrees with other research on total fiber and VAT in adults (27, 37). However, when total fiber was calculated as grams per 1,000 kcals in the current analysis it was no longer significantly associated with PVAT. This does not completely discount the significance of the original finding as total energy was never significantly associated with PVAT, thus controlling for it may have been unnecessary.

In a study by Hairston et al., decreased VAT accumulation was associated with increased total fiber intake in adult African-American and Hispanic-Americans (36). In this prospective study, after five years, each 10-gram increase in daily soluble fiber intake was associated with a 3.73% decrease in VAT accumulation rate. An inverse relationship between total fiber intake and VAT has also been found in overweight youth (28, 36, 58, 59). The mechanism for the protective effect that fiber has on VAT deposition may be related to fiber's high satiety level when compared to other carbohydrates such as refined carbohydrates and added sugar (60). The high satiety of fiber can result in meals with lower energy density and to reduced weight gain, however total energy intake was not significantly associated with VAT in this analysis.

Another putative mechanism that may cause the inverse relationship between fiber and VAT is the blunting effect of fiber on blood sugar and insulin levels (61). Intake of a low glycemic load diet, and thus high fiber, is associated with a reduced glucose and thus reduced insulin response (21). Lower insulin levels are hypothesized to cause increased fatty acid mobilization from abdominal cavities and specifically VAT (30). In addition, VAT is more responsive to postprandial insulin. VAT compared to SAT has two times the insulin-stimulated glucose uptake rate likely due to greater expression of GLUT4, a glucose transporter in adipose tissue, in the presence of insulin (62). Thus, increased insulin release with a high glycemic load diet will support increased VAT deposition. However, in the current analysis a lower average glycemic index or load was not associated with reduced VAT.

While the current analysis did not find any significant associations between VAT and other dietary variables, several studies have found significant associations between VAT and glycemic index and load (30) and SSB consumption (32). In a 6-year longitudinal study from the third generation cohort of the Framingham Heart Study, increased SSB intake was positively associated with change in VAT volume (63). Greater than one serving per day of SSB was associated with a significant increase in VAT volume. However, the association between SSB and PVAT was not significant in the current study. This could be due to the study design, small sample size or non-representative population.

The lack of any statistically significant associations between dietary variables, besides fiber, and VAT is not unique to this study. In the same studies on adolescents that found an inverse relationship between fiber and VAT, added sugar intake was not positively associated with VAT (58, 59). A study of 1,089 older adults and their dietary patterns did not find any significant differences in the VAT of women when they consumed a "healthier foods" diet compared to women who consumed a diet defined by sweets and desserts, refined grains, and breakfast cereals (64). The lack of an observable association may be due to a cross-sectional design compared to more precise and sensitive designs such as intervention and longitudinal studies which have found significant associations between diet and VAT.

The association between fructose intake and VAT was of specific interest in the current analysis due to a randomized trial by Stanhope et al. (35). Stanhope found that feeding participants a fructose sweetened beverage caused the participants to significantly gain VAT compared to those that consumed a glucose sweetened beverage. However, the positive relationship between fructose intake and VAT in the current analysis was not statistically significant. Another recent publication also found no effect of fructose intake on VAT accumulation. In opposition to the results of the Stanhope study, a randomized, controlled trial by Silbernagel et al. did not find a significant increase in VAT when participants were fed a fructose compared to glucose sweetened beverage (65). The two studies have several differences that may have led to the opposing results. Participants in the Silbernagel study consumed 150 grams of fructose or glucose per day for four weeks in addition to a eucaloric diet. In the Stanhope study, participants consumed the fructose or glucose sweetened beverage as 25% of daily energy needs for two weeks as part of a eucaloric diet followed by 2 weeks of beverage supplementation in addition to a eucaloric diet. The slight differences in how the sweetened beverage was provided to participants and the amount given could have caused the difference in findings, although neither study protocol caused significant differences in weight gain between groups. The populations in each study were also different: Stanhope included 32 matched, overweight or obese, middle-aged subjects without chronic disease and Silbernagal studied 20 young to middle-aged, normal weight to overweight subjects. It is possible that older, overweight and obese adults are at increased risk for VAT accumulation with increased intake of fructose compared to younger, healthy adults. The current analysis included both young and middle-aged, as well as normal and overweight, adult women which could diminish any effect of fructose on VAT in middle-aged and/or overweight adults.

Multi-Variable Models

In order to determine the relative contribution of individual variables to PVAT in our study, multivariable models were developed. This type of analysis is similar to other studies which created models or controlled for several dietary variables in one model (27, 28, 32). In the current analysis, when total fiber, total simple sugars, glycemic index and glycemic load were included in one model, total fiber was still significantly associated with PVAT.

A diet quality score allows for inclusion of many dietary variables in a way that is similar to a model; however, a model allows for determination of associations between specific dietary components. In other studies, higher diet quality scores that reflect greater similarity to the 2005 Dietary Guidelines or the Mediterranean diet, have been shown to be inversely associated with VAT. For example, in a group of multi-ethnic participants, Shah et al. (66) found that a higher diet quality score was associated with lower VAT. The group with the highest diet score had 12.8% less VAT area than the group with the lowest diet score. A study published in 2009 (67) found an inverse association between higher diet quality and VAT in men and women. VAT was significantly lower in women with a higher diet score, however, the higher diet score was associated with improved SAT and VAT equally. These conclusions provide further support for the benefit of a healthy diet on body composition, however they are not able to elucidate which aspects of the diet are protective against VAT deposition. The multivariable model in the current analysis was able to show that fiber, even when analyzed with other dietary variables, was significantly associated with reduced VAT.

Strengths

The strengths of this study include the use of three unannounced 24-hour recalls to gather dietary data on all participants. This method has been shown to be one of the most accurate methods for collecting diet information (26) and surpasses the FFQ in accuracy, a method often used in cross-sectional and longitudinal study designs (24). Physical activity was monitored over 4-7 days by accelerometer, which provides more accurate information on physical activity than questionnaires (68). The use of DEXA scans to measure VAT is also a strength of the current study. DEXA scans provide a measurement of VAT at the ROI which is associated with better accuracy than proxy measures such as waist circumference or waist-to-hip ratio (12). Micklesfield et al. compared VAT measured by CT and DEXA and found that the DEXA measurement of VAT was strongly correlated with VAT measured by CT (13). This finding supports the use of DEXA scans in research studies evaluating VAT. Finally, the wide range in participant age, BMI, intake of total fiber and added sugar are strengths of the study.

Limitations

The limitations of this study include the small sample size of 104 participants and limited generalizability. The participants were mostly middle-aged, over-weight, white women with increased years of education, suggesting a higher socioeconomic status. This population type may be consuming a healthier and more nutrient dense diet than the average U.S. population. This limits the opportunity for a significant association between a diet high in added sugar, refined carbohydrates and VAT. Generalizability of the results will also be limited to adult females with similar demographics as the study participants. The cross-sectional nature of the study is a weakness as the results only demonstrate association and do not provide evidence for any cause and effect relationships.

The original study was designed to test the metabolic and cognitive effects of thyroid status. As a sub-sample from this larger study, a majority of the participants in the current analysis had a diagnosis of thyroid disease. While all subjects were within the normal range for TSH and weight stable for at least three months prior to data collection, it is unclear if prior imbalances in thyroid levels could have caused long term changes to body composition. Therefore, it is possible that our sample is not representative of a healthy adult population.

Weaknesses in the method used to collect dietary information include the assumption that an average of three days reflects the usual diet of the participant. The variability within each participant and between participants can affect the results of the dietary analysis. Depending on the within-subject variability, more dietary recalls may be needed to collect accurate information on true intake. Evidence suggests that for some nutrients up to eight recall days need to be analyzed to capture usual intake (69). Nelson et al. found that to reach a correlation of 0.9 between observed and true nutrient intake, the least number of collected days needed for total sugars was three to eight. In the same study, accurate intake of energy, protein, fat and carbohydrates required greater than seven recall days to reach a correlation of 0.9. Another study in 140 women found that seven, 24-hour recalls were needed to reach a correlation of 0.9 between observed and true intake of total energy (70) and eight recall days were needed for total carbohydrate intake to reach a correlation of 0.9. Micronutrients often require even more recalls due to increased intra-person variation. For example, Vitamin A required 19 days in the same group of women to reach a correlation of 0.9. Thus, it may take more than three 24-hour dietary recalls to gather accurate information on a participant’s diet.

Furthermore, 24-hour recalls have been criticized for bias towards underreporting, even in normal weight subjects (71). For example, the participant may consciously refuse to list all foods consumed or they may forget to include some foods consumed. One large study in adults found an average underreporting of 12% when three 24-hour recalls were averaged (72) and Thompson et al. found underreporting to range up to 34% (73).

The measurement of VAT by DEXA scan, while providing some benefits over MRI and CT analysis, does not fully match the accuracy that the more expensive and hazardous procedures can provide (74). The current analysis would have been significantly improved if the DEXA analysis software had been able to provide information on the amount of SAT contained in the ROI. Other studies routinely assess the VAT-to-SAT ratio. We examined VAT as a percent of total body fat, which includes both SAT and VAT, and this might weaken any associations that are present. Furthermore, calculating the VAT/SAT ratio would have allowed our results to be more directly compared to other research on the dietary contributors to VAT.

Finally, with an increasing number of statistical analyses performed on a dataset, comes an increasing risk of type I error. This category of error means that a positive result is found when none actually exists (75). However, this error is less likely in this analysis considering the consistency of the confidence intervals throughout the analysis.

## Conclusions

In conclusion, we partially reject the first specific aim that VAT will be increased with increased consumption of individual monosaccharides and disaccharides or a diet with a higher glycemic index and load. We accept the second part of the first specific aim that VAT will be increased with decreased fiber intake. The second specific aim is rejected as VAT was not significantly associated with fructose, SSF, and SSB consumption.

## Future Directions

The role of estrogen on VAT deposition through the menopausal transition is unclear. Several studies point to decreased levels of estrogen causing increased VAT in post-menopausal women (54, 55). However, a few studies have found that age and not menopausal status was a better predictor of VAT. Further studies designed to distinguish the effects of age versus hormone status on VAT deposition are needed.

Future research on VAT deposition and diet are needed. Our analysis was limited to carbohydrates and sugar intake. Additional dietary variables were not included in our original hypotheses. Another study found a positive association of dietary fat consumption with VAT (76) and in a study of middle-aged men, alcohol intake was positively associated with VAT deposition (77). It is possible that these dietary components may play a greater role in VAT deposition than simple carbohydrate intake.

The role of fructose on VAT has yet to be fully elucidated; the two randomized, controlled feeding studies on fructose present conflicting results that another trial may resolve. Furthermore, it is unclear if one can attribute the positive association that we saw with fiber to fiber intake alone. Fiber is a nutrient that accompanies a variety of healthy foods. Foods high in fiber, such as fruits and vegetables, also contain phytoestrogens. A study including 939 Framingham Heart post-menopausal women found that increased intake of phytoestrogens, specifically lignans, were inversely associated with central adiposity measured by waist-to-hip ratio, a surrogate for VAT (78). In this group, phytoestrogens were more likely to have biological effects due to low endogenous estrogen. Since estrogen therapy has shown an inverse relationship with VAT deposition, lignans, which can act in a similar way to estrogen, may be protective against deposition of VAT in the same way. The current analysis did not analyze intake of phytoestrogens and this would be an interesting area of future study.

Fiber is also found in whole grains. A recent cross-sectional study by McKewon et al. (79) found that whole-grain intake was inversely associated with VAT and refined-grain intake was positively associated with VAT. In this study, total dietary fiber and glycemic index were included in the analyses suggesting that the consumption of whole grains, and not fiber, may be protective against VAT. Thus, our analysis might be detecting the association of fiber that accompanies a diet rich in whole grains. The mechanisms for how fiber or whole grain intake reduces VAT deposition are poorly understood. Intervention trials to determine the mechanism by which fiber or whole grains decrease VAT deposition are needed.

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**Appendix A**

**Evidence Table**

| **Authors** | **Citation** | **Study Design and Methods** | **Type of diet** | **Participant Description** | **Conclusions** |
|---|---|---|---|---|---|
| | | | | | |
| **VAT and Chronic Disease** | | | | | |
| Jean-Pierre Despre´s | Després J-. Body fat distribution and risk of cardiovascular disease: An update. Circulation 2012;126:1301-13. | History of VAT. VAT is a consistent marker of CVD risk as well as other conditions including: hyperinsulinemia, glucose intolerance, type 2 DM, inflammation, VLDL, altered cytokine. | NA | NA | Excess VAT may be due to hypothalamic-pituitary-adrenal axis leading to increased control of carb and lipid metabolism by glucocorticoids more of which are in VAT leading to preferential fat deposition in VAT. Sex hormones may also play a role. Substantial differences in proportion of VAT between black and white adults; Caucasian adults had more. VAT could be a marker of inability of SAT to expand. |
| Fried SK, Russell CD, Grauso NL, Brolin RE | Fried SK, Russell CD, Grauso NL, Brolin RE. Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men. J Clin Invest 1993;92:2191-8. | Human adipose tissue was collected. Samples were tested for LPL activity and expression of LPL mRNA in visceral verses subcutaneous adipose tissue. Response to insulin on LPL expression was also tested. | NA | 44 women, 18 men undergoing gastrointestinal surgery for morbid obesity. | Insulin increases LPL expression/activity; LPL in SAT is more responsive to insulin than LPL in VAT suggesting that glucose sweetened beverages could cause greater LPL activity in SAT and increased uptake of TG by SAT due to higher insulin levels. Fructose does not raise insulin, suggesting decreased insulin-mediated LPL activity in SAT, causing a greater uptake of TG by VAT. |

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| Ruderman N, Chisholm D, Pi-Sunyer X, Schneider S. | Ruderman N, Chisholm D, Pi-Sunyer X, Schneider S. The metabolically obese, normal-weight individual revisited. Diabetes 1998;47:699-713. | Metabolically obese normal weight individuals with hyperinsulinemic insulin-resistance are predisposed to type 2 diabetes, hypertriglyceridemia, and are at risk for premature coronary heart disease. Specific factors predispose individuals including - central fat distribution, inactivity, and low VO2 max. | NA | NA | Argument to identify these individuals early on and treat before conditions worsen. These individuals should be younger and more able to change habits and behaviors and perform activities to improve health. It is possible as demonstrated by two large studies over 5 years in which diabetes was diminished in this population however, more research is needed. |
| Lim S, Meigs JB | Lim S, Meigs JB. Links between ectopic fat and vascular disease in humans. Arterioscler Thromb Vasc Biol 2014;34:1820-6. | Review of different types of ectopic fat and potential mechanisms for associated CVD risk. VAT increased FFA, cytokines, reactive oxygen species production, mechanical stress leading to insulin resistance, inflammation, oxidative stress, renin angiotension system activation, decreased cardiac output. | NA | NA | VAT alters the redox system, resulting in increased reactive oxygen species production which may be involved in insulin resistance in obesity. In obese with high VAT, systemic renin-angiotensin system (RAS) is impaired. Increased activity of VAT may play a key role in hypertension through secretion of angiotensinogen. Interaction between hyperinsulinemia by increased VAT and influence of adipocytokines released from VAT on RAS system. |

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| Tchernof A, Després JP | Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. Physiol Rev 2013;93:359-404. | Review article of history, risks, etiology, mechanisms of VAT. | NA | NA | Visceral adipose tissue must be considered as it is an important risk factor for diabetes and CVD and has it's own metabolic properties. Etiology of VAT is related to age, gender, sex hormones, genetics, and ethnicity. Portal theory - increased VAT predicted to contribute to nearly 50% of portal vein non-esterified fatty acid release, compared to 5-10% in lean individuals. |
| Matsuzawa Y, Shimomura I, Nakamura T, Keno Y, Kotani K, Tokunaga K | Matsuzawa Y, Shimomura I, Nakamura T, Keno Y, Kotani K, Tokunaga K. Pathophysiology and pathogenesis of visceral fat obesity. Obes Res 1995;3 Suppl 2:187S-94S. | VAT has been shown to have high lipogenesis and lipolysis and its accumulation can induce high levels of free fatty acids in portal circulation and into the liver. Increased free fatty acids may lead to up regulation of lipid synthesis and gluconeogenesis leading to insulin resistance and resulting in hyperlipidemia, glucose intolerance and atherosclerosis. | NA | NA | Visceral fat syndrome disease proposed, which may increase susceptibility to atherosclerosis due to multiple risk factors induced by visceral fat accumulation. |

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| Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. | Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms. Circ Res 2015;116:991-1006. | Review of metabolic properties of VAT which lead to chronic disease | NA | NA | Elevated visceral fat increases BP by physically compressing the kidneys, increasing sodium reabsorption, loop of Henle reabsorption, and renin secretion. |
| Kabir M, Catalano KJ, Ananthnarayan S, Kim SP, Van Citters GW, Dea MK, Bergman RN. | Kabir M, Catalano KJ, Ananthnarayan S, Kim SP, Van Citters GW, Dea MK, Bergman RN. Molecular evidence supporting the portal theory: A causative link between visceral adiposity and hepatic insulin resistance. American Journal of Physiology - Endocrinology and Metabolism 2005;288:E454-61. | Randomized clinical trial, that induced visceral fat deposition in dogs and measured markers of liver insulin resistance and lipolytic activity in VAT. | 12 weeks of either normal diet for control dogs or moderate fat diet for test dogs | 12 dogs; 6 control, 6 test dogs | After the intervention period the test dogs had a higher ratio of visceral to subcutaneous for HSL, LPL, PPARy. In the livers of test dogs, FABP tended to be greater and sREBP-1 was significantly greater compared to control dogs. Liver triglycerides were increased by 45% in test dogs and test dogs demonstrated reduced insulin receptors and insulin clearance compared to control dogs. |

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| Rydén M, Andersson DP, Bergström IB, Arner P. | Rydén M, Andersson DP, Bergström IB, Arner P. Adipose tissue and metabolic alterations: regional differences in fat cell size and number matter, but differently: a cross-sectional study. J Clin Endocrinol Metab. 2014;99:E1870-1876. | Cross-sectional study on obese patients scheduled for gastric bypass surgery. Abdominal SAT was obtained prior to surgery and VAT cells were gathered at the beginning of surgery. Participants also underwent a hyperinsulinemic-euglycemic clamp to calculate glucose disposal rates. | NA | 204 patients preparing for Roux-en-Y surgery for obesity. Participants were 18-60 years old, BMI >30 with complications or BMI >35 without complications. | In VAT cell volume correlated significantly with insulin sensitivity, plasma levels of insulin, triglycerides, and HDL-cholesterol. |

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| Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, Vasan RS, JM, Meigs JB, Cupples LA, D'Agostino RB, O'Donnell CJ. | Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu C-, Vasan RS, Murabito JM, Meigs JB, Cupples LA, et al. Abdominal visceral and subcutaneous adipose tissue compartments: Association with metabolic risk factors in the Framingham heart study. Circulation 2007;116:39-48. | Cross sectional study on Framingham Heart Third Generation Study participants. CT measure of VAT and SAT, risk factors and covariates measured during examination. Covariates determined to be age, smoking, physical activity, alcohol use, menopausal status, and hormone replacement therapy. | NA | 3001 Offspring and Third Generation Study participants, men > 35, women >40, free of CVD | VAT correlated with age in both sexes, all risk factors were correlated with both SAT and VAT (except cholesterol and SAT in men and physical activity and VAT in men). VAT was highly correlated with fasting plasma glucose, log triglycerides and HDL cholesterol. The results for men were similar although the VAT was more strongly associated with metabolic risk factors in women than men. Heritability for SAT was 57% and VAT was 36%. |

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| Rothney, M P, Catapano, AL., Xia J, Wacker W K, Tidone C, Grigore L., Xia Y and Ergun DL. | Rothney MP, Catapano AL, Xia J, Wacker WK, Tidone C, Grigore L, Xia Y, and Ergun D L. Abdominal visceral fat measurement using dual-energy X-ray: Association with cardiometabolic risk factors. Obesity, 2013 21: 1798–1802. | Cross-sectional analysis of cardiometabolic risk factors and VAT volume. VAT was measured by DEXA and fasting blood sample was provided to test triglycerides, HDL cholesterol, total cholesterol, fasting plasma glucose, impaired fasting glucose, systolic and diastolic blood pressure. | NA | 939 subjects (541 females and 398 males) average age 56 and average BMI 26. | In both men and women VAT was associated with age, triglycerides, HDL cholesterol, systolic and diastolic blood pressure, and fasting plasma glucose. After additional analysis VAT volume was associated with increased odds for type 2 diabetes in men and metabolic syndrome in men and women. |
| **DEXA measurement of VAT verses CT and MRI measurement of VAT** | | | | | |
| Kaul S, Rothney MP, Peters DM, Wacker WK, Davis CE, Shapiro MD, Ergun DL. | Kaul S, Rothney MP, Peters DM, Wacker WK, Davis CE, Shapiro MD, Ergun DL. Dual-energy X-ray absorptiometry for quantification of visceral fat. Obesity 2012;20:1313-8 | All subjects were scanned to measure visceral fat in the abdomen with DEXA and CT scans to determine the accuracy of DEXA scans. | NA | 109 adult men and women (100 white, 5 Hispanic, 4 Asian) with a range of BMIs | Strong correlation was found with the measurement of VAT by DEXA and CT. When the group was divided by gender and BMI the correlation remained strong in all groups. |

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| Lisa K. Micklesfield, Julia H. Goedecke, Mark Punyanitya, Kevin E. Wilson and Thomas L. Kelly | Micklesfield LK, Goedecke JH, Punyanitya M, Wilson KE, Kelly TL. Dual-energy X-ray performs as well as clinical computed tomography for the measurement of visceral fat. Obesity 2012;20:1109-14. | Cross sectional study. Measurement of VAT by CT, DXA, anthropometric and demographic models all analyzed and compared | NA | 272 black and white South African women | DXA-VAT performed as well as a clinical read of VAT from a CT scan and better than anthropomorphic and demographic models |
| Xia Y, Erqun DL, Wacker WK,Wang X, Davis CE, Kaul S. | Xia Y, Erqun DL, Wacker WK,Wang X, Davis CE, Kaul S. Relationship between dual-energy x-ray absorptiometry volumetric assessment and x-ray computed tomography-derived single-slice measurement of visceral fat. J Clin Densitom 2014;17:78-83. | Statistical analysis of DEXA compared to CT scans to determine algorithm to compute single-slice area values at L2/3 and L4/5. | NA | 55 males, 21-77 years old, BMI 21.1-37.9. | Correlation coefficients between DEXA and CT were 0.94 for L2/3 and 0.96 for L4/5. The average difference between CT and DEXA at L2/3 was 5 $cm^2$ and 3.8 $cm^2$ at L4/5. |

| Reduction in Dietary Carbohydrate and VAT | | | | | |
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| Sasakabe T, Haimoto H, Umegaki H, Wakai K. | Sasakabe T, Haimoto H, Umegaki H, Wakai K. Association of decrease in carbohydrate intake with reduction in abdominal fat during 3-month moderate low-carbohydrate diet among non-obese Japanese patients with type 2 diabetes. Metabolism: Clinical and Experimental 2015;64:618-25. | Randomized, control trial to test reduced carbohydrate diet on visceral fat deposition over 3 months. Measures taken every month; body weight, blood pressure, HgbA1c, fasting blood glucose, fasting serum insulin, serum triglycerides, serum LDL and HDL cholesterol, VAT area and total adipose tissue area. | Medium-low carbohydrate diet for 3 months. Participants with an HbA1c < 9% were taught to remove one carbohydrate from the dinner meal. Participants with HbA1c >= 9% were taught to remove one carbohydrate source at breakfast and dinner. | 45 men, 31 women with T2DM | After 3 months, carbohydrate intake, BMI, HbA1c, absolute value of VAT and SAT, was significantly reduced from baseline in men and women. In men reduction in carbohydrate intake was significantly related to reduction in VAT and not SAT, In women the change in carbohydrate intake was associated with change in VAT but was not significant. |

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| Gower BA, Goss AM | Gower BA, Goss AM. A lower-carbohydrate, higher-fat diet reduces abdominal and intermuscular fat and increases insulin sensitivity in adults at risk of type 2 diabetes. J Nutr 2015;145:177S-83S. | Randomized, controlled trial on low-fat verses low carbohydrate diet and visceral fat deposition. Serum concentrations were assess after 4 weeks on eucaloric diet. Main outcomes were fasting glucose, fasting insulin, insulin sensitivity, beta cell responsiveness, body composition and fat distribution. | 8 week eucaloric, 8 week hypocaloric on low-fat or low-carbohydrate diet. | 69 overweight or obese men and women. | In eucaloric phase loss of intra-abdominal fat was significantly greater in low-carbohydrate diet (11%) compared to the low-fat diet. No difference between groups in the hypocaloric phase. |
| Gower BA, Goss AM | Gower BA, Goss AM. A lower-carbohydrate, higher-fat diet reduces abdominal and intermuscular fat and increases insulin sensitivity in adults at risk of type 2 diabetes. J Nutr 2015;145:177S-83S. | Randomized, controlled trial on low fat verses low carbohydrate diet. Main outcomes were fasting glucose, fasting insulin, insulin sensitivity, beta cell responsiveness, body composition and fat distribution. | 8 week eucaloric low-fat or low-carbohydrate diet. | 30 women with PCOS | Women with PCOS - low-carbohydrate diet women lost both intra-abdominal and intra-mesenteric fat. Low-fat women lost lean mass. No change in intra-abdominal fat in low-fat diet. |

| **Glycemic Index and Glycemic Load and VAT** | | | | | |
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| Romaguera D, Ängquist L, Du H, Jakobsen MU, Forouhi NG, Halkjær J, Feskens EJM, Daphne LA, Masala G, Steffen A, et al. | Romaguera D, Ängquist L, Du H, Jakobsen MU, Forouhi NG, Halkjær J, Feskens EJM, Daphne LA, Masala G, Steffen A, et al. Dietary determinants of changes in Waist circumference adjusted for body mass index - a proxy measure of Visceral adiposity. PLoS ONE 2010;5: | Five year prospective, observational study in Europe (EPIC). Compared energy density, macronutrients, alcohol, fiber, GI, GL, with annual change in waist circumference adjusted for BMI. This measure considered to be a proxy measure for visceral fat. | NA | 48,631 Europeans w/out chronic disease or pregnant, 18-60 years old. | Higher energy density and glycemic index diets associated with significant increase in waist circumference adjusted for BMI. Alcohol intake was associated with increased waist circumference adjusted for BMI in women, and fiber intake was negatively associated with waist circumference adjusted for BMI in women. |

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| Goss AM, Goree LL, Ellis AC, Chandler-Laney PC, Casazza K, Lockhart ME, Gower BA. | Goss AM, Goree LL, Ellis AC, Chandler-Laney PC, Casazza K, Lockhart ME, Gower BA. Effects of diet macronutrient composition on body composition and fat distribution during weight maintenance and weight loss. Obesity (Silver Spring, Md.) 2013;21:1139-42. | Randomized, clinical trial. Two phases, 8 weeks eucaloric, followed by 8 weeks hypocaloric. Participants divided into low glycemic load (GL) or high GL diets for both phases. | 8 weeks eucaloric, 8 weeks hypocaloric. Two groups, low GL or high GL. | 69 healthy, overweight men and women. | After eucaloric phase low GL had 11% less IAAT than high GL. After weight loss those on low GL had 4.4% less total fat mass than those on high GL. Low GL diet may affect energy partitioning reducing IAAT without weight change and fat loss relative to lean mass during weight loss. |
| **VAT and Added Sugars** | | | | | |
| Ma J, McKeown NM, Hwang SJ, Hoffmann U, Jacques PF, Fox CS. | Ma J, McKeown NM, Hwang SJ, Hoffmann U, Jacques PF, Fox CS. Sugar-Sweetened Beverage Consumption Is Associated With Change of Visceral Adipose Tissue Over 6 Years of Follow-Up. Circulation 2016;133:370-7. | Prospective study that assessed patients at baseline and 6 years later. SSB and diet soda intake was assessed by FFQ, VAT and SAT measured by CT. | NA | 1,003 participants in the Framingham's Third generation cohort. | Higher SSB intake was associated with greater change in VAT volume after adjustment for body weight, sex, age, smoking status, physical activity, alcohol intake, daily energy intake, saturated fatty acids, multivitamin use, intakes of individual foods (whole grains, vegetables, coffee, nuts, fish). Diet soda consumption not associated with change in VAT. |

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| Silbernagel G, Machann J, Unmuth S, Schick F, Stefan N, Haring HU, Fritsche A. | Silbernagel G, Machann J, Unmuth S, Schick F, Stefan N, Haring HU, Fritsche A. Effects of 4-week very-high-fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. Br J Nutr 2011;106:79-86. | Randomized, controlled trial, participants consumed fructose or glucose sweetened beverage. VAT and SAT measured by MRI and analyzed for difference in VAT, liver fat, intramyocellular lipids. | 4 weeks of 150 grams of fructose or glucose sweetened beverage in addition to eucaloric diet. | 20 healthy, young-middle aged adults (mean age 30.5). | Insulin sensitivity decreased in both groups and TAG increased in fructose group. No treatment effects were seen with liver fat, VAT, SAT, or intramyocellular lipids. |
| Mollard RC, Sénéchal M, MacIntosh AC, Hay J, Wicklow BA, Wittmeier KD, Sellers EA, Dean HJ, Ryner L, Berard L, McGavock JM. | Mollard RC, Senechal M, MacIntosh AC, Hay J, Wicklow BA, Wittmeier KD, Sellers EA, Dean HJ, Ryner L, Berard L, et al. Dietary determinants of hepatic steatosis and visceral adiposity in overweight and obese youth at risk of type 2 diabetes. Am J Clin Nutr 2014;99:804-12. | Cross-sectional study on consumption of fat, fried foods, sugar and fiber in youth. Measurement of hepatic steatosis, VAT, SAT by CT and dietary intake by FFQ during baseline testing. | Ad libitum | 74 overweight adolescents, physically inactive | Total daily sugar intake higher in adolescents with VAT obesity. Available carbohydrate intake positively associated with VAT to SAT ratio. VAT to SAT ratio negatively associated with fiber intake and positively associated with total sugar intake - not significant after adjustment for confounders. Fiber intake associated with 18% decreased odds of VAT, daily soda associated with significantly higher odds of VAT. |

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| Maersk M, Belza A, Stødkilde-Jørgensen H, Ringgaard S, Chabanova E, Thomsen H, Pedersen SB, Astrup A, Richelsen B. | Maersk M, Belza A, Stødkilde-Jørgensen H, Ringgaard S, Chabanova E, Thomsen H, Pedersen SB, Astrup A, Richelsen B. Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: A 6-mo randomized intervention study. Am J Clin Nutr 2012;95:283-9. | 6 month randomized, clinical, intervention study on visceral fat deposition in adults with increased intake of sugar-sweetened beverage. | 1 L of either regular cola, milk, diet cola, water per day for six months | 47 Healthy, overweight adults | Consumption of sucrose sweetened cola was associated with significantly higher liver fat, skeletal muscle fat, VAT, blood triglycerides, and total cholesterol. Total fat mass was not different between all four groups. No significant difference in SAT between groups although both energy containing beverages lead to increased SAT and non-caloric containing beverages resulted in reduction in SAT. |

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| Ma J, Sloan M, Fox CS, Hoffmann U, Smith CE, Saltzman E, Rogers GT, Jacques PF, McKeown NM | Ma J, Sloan M, Fox CS, Hoffmann U, Smith CE, Saltzman E, Rogers GT, Jacques PF, McKeown NM. Sugar-sweetened beverage consumption is associated with abdominal fat partitioning in healthy adults. J Nutr 2014;144:1283-90. | Cross-sectional analysis of sugar-sweetened beverage and diet soda consumption determined by FFQ visceral and subcutaneous fat. | Ad libitum | 2,596 middle aged adults from the Framingham Heart Study Offspring and 3rd generation cohort. | Inverse association between SSB and SAT, higher SSB positively associated with VAT/SAT ratio, daily consumers of SSB had 10% higher absolute VAT and 15% greater VAT/SAT ratio. |
| Odegaard AO, Choh AC, Czerwinski SA, Towne B, Demerath EW | Odegaard AO, Choh AC, Czerwinski SA, Towne B, Demerath EW. Sugar-sweetened and diet beverages in relation to visceral adipose tissue. Obesity (Silver Spring) 2012;20:689-91. | Cross-sectional analysis of beverage consumption (from FFQ) analyzed for correlation with VAT, SAT, and total body fat. | Ad libitum | 791 healthy non-Hispanic participants, 18-70 years old. | In adjusted model, significant increase in waist circumference and VAT with increase in SSB frequency. No association with total body fat percentage or BMI. |

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| Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, Hatcher B, Cox CL, Dyachenko A, Zhang W, et al. | Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, Hatcher B, Cox CL, Dyachenko A, Zhang W, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 2009;119:1322-34. | Double-blinded parallel arm study with matched subjects and 3 phases: (a) 2 week inpatient baseline period, subjects consumed energy-balanced diet (b) 8 week outpatient intervention, subjects consumed either fructose or glucose sweetened beverages providing 25% of daily energy along with usual diet (c) a 2 week inpatient period, subjects consumed fructose or glucose sweetened beverages providing 25% of daily energy needs with energy-balanced diet. | 2 weeks energy balanced, high-complex carbohydrate diet, then 8 week outpatient intervention and fructose or glucose sweetened beverages at 25% of energy needs (3 servings a day of sweetened beverage), then 2 weeks in patient where subjects consumed 25% energy from fructose or glucose sweetened beverages. | 39 subjects, 40-72 years, BMI 25-35. | Dietary fructose specifically promotes dyslipidemia, decreases insulin sensitivity, increased deposition of visceral fat. Total fat and VAT volumes were not significantly changed in subjects consuming glucose, SAT volume was significantly increased. Both total fat and VAT volume significantly increased in subjects consuming fructose. Men consuming fructose had greater increase in VAT than women despite comparable weight and fat gain. |

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| Pollock NK, Bundy V, Kanto W, Davis CL, Bernard PJ, Zhu H, Gutin B, Dong Y | Pollock NK, Bundy V, Kanto W, Davis CL, Bernard PJ, Zhu H, Gutin B, Dong Y. Greater fructose consumption is associated with cardio metabolic risk and visceral adiposity in adolescents. J Nutr 2012;142:251-7 | Cross-sectional study on association between total fructose intake (free fructose plus one-half the intake of free sucrose) with type of adiposity. Dietary intake determined by 4 to 7 24-hour recalls. | Ad libitum | 559 adolescents from the Augusta, Georgia area, 14-18 years old, Caucasian or African American | Total fructose associated with systolic blood pressure, fasting serum glucose, HOMA-IR, plasma TG, plasma HDL-cholesterol, plasma LDL-cholesterol, plasma CRP, and VAT. Only VAT associated with total fructose intake after controlling for co-variates. Intakes of free fructose, free sucrose, total sugar, added sugar, 100% juice, sugar-sweetened food and beverages were associated with increased VAT. |
| **VAT and Fiber Consumption** | | | | | |
| Ventura E, Davis J, Byrd-Williams C, Alexander K, McClain A, Lane CJ, Spruijt-Metz D, Weigensberg M, Goran M. | Ventura E, Davis J, Byrd-Williams C, Alexander K, McClain A, Lane CJ, Spruijt-Metz D, Weigensberg M, Goran M. Reduction in risk factors for type 2 diabetes mellitus in response to a low-sugar, high-fiber dietary intervention in overweight Latino adolescents. Arch Pediatr Adolesc Med 2009;163:320-7. | Randomized, controlled trial to determine reduction in risk factors for T2DM (VAT) after intervention with diet changes and increased activity. Participants divided into three groups – no intervention, only diet intervention, diet + strength training. | Ad libitum – intervention to increase intake of fiber and decrease added sugar consumption. | Latino high school students from the Los Angeles County with a BMI in the $85^{th}$ percentile or higher but not already diagnosed with any chronic diseases. | 55% of participants decreased added sugar intake, 59% increased fiber intake. No change was seen in VAT with decreased added sugar but increased fiber was associated with VAT (-10%). |

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| Davis JN, Alexander KE, Ventura EE, Toledo-Corral CM, Goran MI. | Davis JN, Alexander KE, Ventura EE, Toledo-Corral CM, Goran MI. Inverse relation between dietary fiber intake and visceral adiposity in overweight Latino youth. Am J Clin Nutr 2009;90:1160-6. | Longitudinal (2 years) study on change in diet and metabolic risk factors – VAT area. Dietary intake was assessed from two 24-hour recalls at each annual visit. | NA | 85 overweight Latino youth aged 11-17 years old from Southern California. | Increased total fiber and soluble fiber intake (g/1,000 kcal) was associated with decreases in VAT area independent of change in SAT. Increased added sugar was not associated with increases in VAT. |
| Parikh S, Pollock NK, Bhagatwala J, Guo D-, Gutin B, Zhu H, Dong Y. | Parikh S, Pollock NK, Bhagatwala J, Guo D-, Gutin B, Zhu H, Dong Y. Adolescent fiber consumption is associated with visceral fat and inflammatory markers. J Clin Endocrinol Metab 2012;97:E1451-7. | Cross sectional study to compare dietary fiber intake with inflammatory biomarkers, and total and central adiposity (VAT). Dietary intake determined by 4 to 7 24-hour recalls. | Ad libitum | 559 adolescents from the Augusta, Georgia area, 14-18 years old, Caucasian or African American. | Dietary fiber intake was negatively associated with visceral adipose tissue. All correlations were statistically significant with correlation coefficients of -0.102 in boys and -0.344 in girls. |

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| Hairston KG, Vitolins MZ, Norris JM, Anderson AM, Hanley AJ, Wagenknecht LE | Hairston KG, Vitolins MZ, Norris JM, Anderson AM, Hanley AJ, Wagenknecht LE. Lifestyle factors and 5-year abdominal fat accumulation in a minority cohort: The IRAS family study. Obesity 2012;20:421-7. | Prospective study on lifestyle factors and VAT, SAT. Documented VAT, SAT, physical activity, dietary intake with FFQ, smoking and compared with 5 year change in VAT and SAT. | Ad libitum | 1114 people, 18-81 years old, African American and Hispanic American. | Every 10 g of soluble fiber decreased the rate of VAT accumulation by 3.73%. Significant association between percent calories from sweets and protein and SAT. Increased physical activity associated with decreased VAT. |
| **VAT and Dietary Fat Intake** | | | | | |
| Bruce W. Bailey, Debra K. Sullivan, Erik P. Kirk, and Joseph E. Donnelly | Bailey BW, Sullivan DK, Kirk EP, Donnelly JE. Dietary predictors of visceral adiposity in overweight young adults. Br J Nutr 2010;103:1702-5. | Cross-sectional study using baseline data from the Midwest Exercise Trial. All meals eaten in café (measured using observer-recorded and weighed plate waste) and 24 hour recall used to measure food eaten outside of café. | Ad libitum - all intakes for 14 days at university cafeteria. Participants could choose between 8-10 entrees per meal. | 109 young adults (17-35), sedentary, BMI 25-35, healthy, in energy balance | Total dietary fat was best predictor of VAT and SAT in women. Saturated fat associated with VAT and SAT in women, total energy intake predicted SAT. Carbohydrate intake was not significantly associated with VAT |

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| Larson DE, Hunter GR, Williams MJ, Kekes-Szabo T, Nyikos I, Goran M | Larson DE, Hunter GR, Williams MJ, Kekes-Szabo T, Nyikos I, Goran MI. Dietary fat in relation to body fat and intraabdominal adipose tissue: A cross-sectional analysis. Am J Clin Nutr 1996;64:677-84. | Cross-sectional study on fat intake by average of 3 day food record and VAT/SAT and physical activity. Stepwise multivariate analyses to test macronutrient intake on VAT/SAT/fat mass. | Ad-libitum | 135 men mean age 44, 214 women mean age 45, all Caucasian. | Results showed that dietary fat was not associated with VAT after adjustment for sex, age and physical activity. |

| Alcohol Consumption and VAT | | | | | |
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| Kondoh T, Takase H, Yamaguchi TF, Ochiai R, Katashima M, Katsuragi Y, Sakane N. | Kondoh T, Takase H, Yamaguchi TF, Ochiai R, Katashima M, Katsuragi Y, Sakane N. Association of dietary factors with abdominal subcutaneous and visceral adiposity in Japanese men. Obes Res Clin Pract 2014;8:e16-25. | A pooled cross-sectional analysis to evaluate the associations between total energy intake, energy intake from breakfast, lunch, supper, snacks, protein, fat, carbohydrate and alcohol consumption and subcutaneous fat area and visceral fat area. Dietary records collected for 3, 5, 8 days average of 5.8 days of collection. | Ad libitum | 301 Japanese men, 21-65 years old | Coefficient of alcohol intake was positive for visceral fat area and negative for subcutaneous fat. Alcohol intake correlated with visceral fat (p 0.009). The risk of increased visceral fat > 100 cm was 2.07 times higher in those who consumed > 3g/d alcohol. |
| Cigolini M, Targher G, Bergamo IA, Tonoli M, Agostino G, De Sandre G | Cigolini M, Targher G, Bergamo Andreis IA, Tonoli M, Agostino G, De Sandre G. Visceral fat accumulation and its relation to plasma hemostatic factors in healthy men. Arterioscler Thromb Vasc Biol 1996;16:368-74. | Cross-sectional study to examine association between VAT, plasma hemostatic factors, physical activity, alcohol intake, and smoking habit. | Ad libitum | 52, 38-year-old, healthy men. | Did not find significant differences in daily alcohol intake in either group. No significant differences were found in daily physical activity or smoking. Larger VAT area, when matched for BMI, was associated with an increased thrombogenic risk profile. |

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| Must A, Bandini L, Tybor D, Janssen I, Ross R, Dietz W | Must A, Bandini L, Tybor D, Janssen I, Ross R, Dietz W. Behavioral risk factors in relation to visceral adipose tissue deposition in adolescent females. International Journal of Pediatric Obesity 2008;3:28-36. | Prospective study in adolescent girls. VAT deposition measured before and after 4 years and compared with smoking and alcohol use. | NA | 41 females mean age 13.5 assessed at menarche, follow-up data available for 24 subjects after 4 years. | Smoking and alcohol use was associated with the change in VAT over the 4-year period, before and after adjustment for total body fat. |
| **VAT and Dietary Patterns** | | | | | |
| Shah RV, Murthy VL, Allison MA, Ding J, Budoff M, Frazier-Wood AC, Lima JA, Steffen L, Siscovick D, Tucker KL, et al. | Shah RV, Murthy VL, Allison MA, Ding J, Budoff M, Frazier-Wood AC, Lima JA, Steffen L, Siscovick D, Tucker KL, et al. Diet and adipose tissue distributions: The Multi-Ethnic Study of Atherosclerosis. Nutr Metab Cardiovasc Dis 2016;26:185-93. | Cross-sectional study, used FFQ to determine dietary patterns and create diet score and compared to VAT and SAT and pericardial fat and hepatic attenuation. | NA | 5,079 participants included in the Multi-Ethnic Study of Atherosclerosis, a subset received imaging for SAT and VAT | Higher DietQuality score was associated with lower VAT area. Greater whole grain intake and fat intake as unsaturated was associated with less VAT. Processed meat was associated with greater VAT. |

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| Anderson AL, Harris TB, Houston DK, Tylavsky FA, Lee JS, Sellmeyer DE, Sahyoun NR. | Anderson AL, Harris TB, Houston DK, Tylavsky FA, Lee JS, Sellmeyer DE, Sahyoun NR. Relationships of dietary patterns with body composition in older adults differ by gender and PPAR-gamma Pro12Ala genotype. Eur J Nutr 2010;49:385-94. | Prospective study on dietary patterns determined by FFQ. Clusters of diet patterns were created and analyzed in relation to VAT. | NA | 1,089 participants with completed dietary patterns from the Health, Aging and Body composition Study in older adults. | Dietary patterns did not influence VAT. Women who consumed a healthier dietary pattern did not have less VAT than those who consumed a diet defined by sweets, desserts, refined grains, and breakfast cereals |
| **Non-Dietary Factors and VAT** | | | | | |
| Demerath EW, Rogers NL, Reed D, Lee M, Choh AC, Siervogel RM, Chumlea WC, Towne B, Czerwinski SA | Demerath EW, Rogers NL, Reed D, Lee M, Choh AC, Siervogel RM, Chumlea WC, Towne B, Czerwinski SA. Significant associations of age, menopausal status and lifestyle factors with visceral adiposity in African-American and European-American women. Ann Hum Biol 2011;38:247-56. | Cross-sectional analysis of women to determine associations between age, menopausal status, smoking, physical activity, and VAT and SAT, by MRI. | NA | 111 African American and 617 European American, aged 18-80 years old, without type 1 or type 2 diabetes. | VAT and VAT% were higher in European Americans, age was associated with VAT deposition before menopause. |

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| Murabito JM, Pedley A, Massaro JM, Vasan RS, Esliger D, Blease SJ, Hoffman U, Fox CS | Murabito JM, Pedley A, Massaro JM, Vasan RS, Esliger D, Blease SJ, Hoffman U, Fox CS. Moderate-to-vigorous physical activity with accelerometry is associated with visceral adipose tissue in adults. J Am Heart Assoc 2015;4:e001379. | Cross-sectional analysis of moderate-vigorous physical activity (MVPA) with accelerometry and VAT volume by CT scan. | NA | 1249 Framingham Third Generation and Omni II cohorts, average 51.7 years old, 47% women. | MVPA was associated with less VAT and SAT and better fat quality. |
| Lee CG, Carr MC, Murdoch SJ, Mitchell E, Woods NF, Wener MH, Chandler WL, Boyko EJ, Brunzell JD. | Lee CG, Carr MC, Murdoch SJ, Mitchell E, Woods NF, Wener MH, Chandler WL, Boyko EJ, Brunzell JD. Adipokines, inflammation, and visceral adiposity across the menopausal transition: a prospective study. J Clin Endocrinol Metab 2009;94:1104-10. | Prospective cohort study of women through the menopausal transition. Body composition measures were assessed by CT at pre- and post-menopausal visits as well as fasting blood sample for markers of inflammation. | NA | 69 healthy women followed from pre-menopause into post-menopause status. | From baseline to follow-up the women had increases in BMI, weight, percent truncal fat, intra-abdominal fat and subcutaneous abdominal fat without changes in total body fat percent. IAF increased by 21% across the transition. |

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| Kanaley JA, Sames C, Swisher L, Swick AG, Ploutz-Snyder LL, Steppan CM, Sagendorf KS, Feiglin D, Jaynes EB, Meyer RA, et al. | Kanaley JA, Sames C, Swisher L, Swick AG, Ploutz-Snyder LL, Steppan CM, Sagendorf KS, Feiglin D, Jaynes EB, Meyer RA, et al. Abdominal fat distribution in pre- and postmenopausal women: The impact of physical activity, age, and menopausal status. Metabolism 2001;50:976-82. | Cross-sectional analysis of women to determine relationship between menopausal status and VAT deposition, percent visceral abdominal fat and VAT area, determined by MRI. The women were matched for BMI and total body fat mass. | NA | 23 premenopausal, 27 postmenopausal, and 28 postmenopausal women on estrogen therapy. | Percent visceral abdominal fat was lower in the premenopausal women than postmenopausal women. But age and menopausal status were not significant predictors of VAT or SAT. Physical activity was a predictor for VAT and percent VAT. |

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| Molenaar EA, Massaro JM, Jacques PF, Pou KM, Ellison RC, Hoffmann U, Pencina K, Shadwick SD, Vasan RS, O'Donnell CJ, et al. | Molenaar EA, Massaro JM, Jacques PF, Pou KM, Ellison RC, Hoffmann U, Pencina K, Shadwick SD, Vasan RS, O'Donnell CJ, et al. Association of lifestyle factors with abdominal subcutaneous and visceral adiposity: the Framingham Heart Study. Diabetes Care 2009;32:505-10. | Cross-sectional analysis of associations between life style factors, diet quality and SAT and VAT volumes | NA | 2,926 Framingham Heart Study participants, average 50 years old. | Diets consistent with the 2005 Dietary Guidelines (assess with FFQ, used 2005 dietary guidelines adherence index) resulted in less SAT and VAT. Closer to Dietary guidelines or higher DGAI associated with lower SAT and VAT. |
| Katzmarzyk PT, Bray GA, Greenway FL, Johnson WD, Newton RL Jr, Ravussin E, Ryan DH, Smith SR, Bouchard C. | Katzmarzyk PT, Bray GA, Greenway FL, Johnson WD, Newton RL,Jr, Ravussin E, Ryan DH, Smith SR, Bouchard C. Racial differences in abdominal depot-specific adiposity in white and African American adults. Am J Clin Nutr 2010;91:7-15. | Cross-sectional analysis VAT and SAT between white and African American adults. Measured total body fat, SAT, VAT. | Ad libitum | 1,967 adults (18-84 years old) white and African-American. | Abdominal VAT was significantly higher in white than in African American men and women, before and after adjustment for covariates. White women had significantly lower SAT than African American women. |

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| Gambacciani M, Ciaponi M, Cappagli B, Piaggesi L, De Simone L, Orlandi R, Genazzani AR. | Gambacciani M, Ciaponi M, Cappagli B, Piaggesi L, De Simone L, Orlandi R, Genazzani AR. Body weight, body fat distribution, and hormonal replacement therapy in early postmenopausal women. J Clin Endocrinol Metab 1997;82:414-7. | Randomized control trial to test effects of hormonal replacement on bone mineral density and central adiposity over 12 months. No significant differences between the groups at baseline. DEXA used to determine total body bone mineral, lean and adipose tissue weight and abdominal fat weight. | NA | Early postmenopausal women, control group (n=12) given calcium and test group (n=15) given hormonal replacement therapy for 12 months. | After the intervention, total body bone mineral was increased in the hormonal group, body weight and BMI did not show any significant modification. In the calcium group trunk fat was significantly increased after 12 months as well as arm fat. No increase in trunk fat was seen in the hormonally treated group. |

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| Lemieux S, Prud'homme D, Bouchard C, Tremblay A, Després J-. | Lemieux S, Prud'homme D, Bouchard C, Tremblay A, Després J-. Sex differences in the relation of visceral adipose tissue accumulation to total body fatness. Am J Clin Nutr 1993;58:463-7. | Cross-sectional study to determine correlations between age and gender and abdominal and visceral fat. Abdominal fat determined by CT scan and visceral fat area was measured by delineating the abdominal cavity within the muscle wall. Subcutaneous fat was determined by subtracting visceral area from the total abdominal fat area. Predictive equations were also used to calculate total visceral fat in men and women. | NA | 89 men (30-42 years old), 75 premenopausal women (23-50 years old) all free of metabolic disease. | Men presented with significantly higher visceral fat area when calculated and when measured with CT than women even though women had overall more body fat. |

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| Pascot A, Lemieux S, Lemieux I, Prud'homme D, Tremblay A, Bouchard C, Nadeau A, Couillard C, Tchernof A, Bergeron J, et al. | Pascot A, Lemieux S, Lemieux I, Prud'homme D, Tremblay A, Bouchard C, Nadeau A, Couillard C, Tchernof A, Bergeron J, et al. Age-related increase in visceral adipose tissue and body fat and the metabolic risk profile of premenopausal women. Diabetes Care 1999;22:1471-8. | Cross-sectional analysis comparing visceral fat in young and middle-aged women. CT scans were used to determine total abdominal fat area as well as visceral and subcutaneous fat area. Physical activity was recorded in journals by participants. Lipoproteins and insulin-glucose homeostasis was also measured. | NA | 122 young women, 52 middle aged premenopausal women | Middle aged women had greater body fat mass, waist circumference, total abdominal fat area and visceral fat area than younger women. For every level of body fat mass middle-aged women had higher levels of visceral fat area compared with younger women. Middle-aged women had significantly greater plasma cholesterol, LDL cholesterol, LDL, HDL, and fasting glucose compared to younger women. Overall increased levels of VAT area were associated with altered plasma lipoprotein-lipid and glucose-insulin concentrations in both age groups. Visceral fat made the largest contribution to the variance of most of the metabolic variables in young women. |

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| Rohrmann S, Steinbrecher A, Linseisen J, Hermann S, May A, Luan J, Ekelund U, Overvad K, Tjonneland A, Halkjaer J, et al. | Rohrmann S, Steinbrecher A, Linseisen J, Hermann S, May A, Luan J, Ekelund U, Overvad K, Tjonneland A, Halkjaer J, et al. The association of education with long-term weight change in the EPIC-PANACEA cohort. Eur J Clin Nutr 2012;66:957-63. | Follow-up cohort study on educational attainment and annual change in weight in follow-up time. Educational attainment was defined as the highest obtained school level. | NA | 361,467 participants of the European Prospective Investigation into Cancer and Nutrition (EPIC) study. | After follow-up all groups gained body weight however gain was lowest in men and women with a university degree; high verses low education -120 g per year for men and -70 g per year for women. |
| **Estimating Nutrient intake via Dietary Recalls and other methods** | | | | | |
| Nelson M, Black AE, Morris JA, Cole TJ. | Nelson M, Black AE, Morris JA, Cole TJ. Between- and within-subject variation in nutrient intake from infancy to old age: estimating the number of days required to rank dietary intakes with desired precision. Am J Clin Nutr 1989;50:155-167. | Determine the accuracy of dietary intake data from 6 different studies and participants to determine the number of days needed to determine intake of energy, 28 nutrients and the ratio of polyunsaturated to saturated fatty acids. | NA | 6 different studies including different populations of – toddlers, families, schoolchildren, dietitians, pregnant women, and elderly subjects | 7 days of records did not generate the kind of accuracy normally assumed. Some nutrients required many more days and some required less and could be accurately estimated by diet histories or questionnaires. |

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| Pereira RA, Araujo MC, Lopes Tde S, Yokoo EM. | Pereira RA, Araujo MC, Lopes Tde S, Yokoo EM. How many 24-hour recalls or food records are required to estimate usual energy and nutrient intake? Cad Saude Publica 2010;26:2101-11. | Analysis to determine the number of recalls or food records needed to reach a correlation of 0.9 between observed and true intake | NA | 300 adults and 169 adolescents in Rio de Janeiro Brazil. | 23 replications needed to determine energy intake was a precision of 90% and 7 to classify energy intake with a correlation coefficient of 0.9. |
| Moshfegh AJ, Rhodes DG, Baer DJ, Murayi T, Clemens JC, Rumpler WV, et al. | Moshfegh AJ, Rhodes DG, Baer DJ, Murayi T, Clemens JC, Rumpler WV, et al. The U.S. Department of Agriculture automated multiple-pass method reduces bias in collection of energy intakes. Am J Clin Nutr 2008;88:324-332. | Determine the accuracy of the automated multiple-pass method for dietary recall assessment by comparing reported energy intake with total energy expenditure by doubly labeled water. | NA | 524 adults 30-69 y/o. First recall conducted in person with 2 follow-ups over the phone. DLW dosed on first day of 2-wk study period. | Subjects underreported by average of 11% for total energy intake, normal weight subjects underreported by 3% and those with obese BMI were classified as low energy reporters. |

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| Asbeck, Mast M, Bierwong A,Westenhofer J, Acheson KJ, Muller MJ. | Asbeck, Mast M, Bierwong A,Westenhofer J, Acheson KJ, Muller MJ. Severe underreporting of energy intake in normal weight subjects: use of an appropriate standard and relation to restrained eating. Pub Health Nutr 2001;5:683-690 | Cross-sectional study to determine the influence of restrained eating and different standards on underreporting. Doubly-labelled water used to assess TEE and compared to 7-day dietary record. | NA | 83, young adults (20-38 y/o) weight-stable, non-obese, healthy subjects | Severe-underreporting was found in 37% of subjects, more frequently in women than men, and higher restraint was associated with higher degree of underreporting. |
| Thompson FE, Sugar AF. | Thompson FE, Sugar AF. Dietary assessment methodology. 3rd ed. In: Coulston AM, ed. Nutrition in the prevention and treatment of disease. San Diego, CA: Elsevier Press, 2013:5-46. | NA | NA | NA | Description of different dietary recall methods and accuracy of methods used in research. Discusses types of recalls used depending on the study design and population. |
| Rutishauser IH | Rutishauser IH. Dietary intake measurements. Public Health Nutr 2005;8:1100-7. | Review of different methods to obtain dietary intake. | NA | NA | Description of the different ways to collect dietary data. Includes strengths and weaknesses of each type. |

| Miscellaneous | | | | | |
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| Banerjee A, Chitnis UB, Jadhav SL, Bhawalkar JS, Chaudhury S. | Banerjee A, Chitnis UB, Jadhav SL, Bhawalkar JS, Chaudhury S. Hypothesis testing, type I and type II errors. Ind Psychiatry J 2009;18:127-31. | NA | NA | NA | Description of hypothesis testing process and types of errors possible. Describes statistical concepts including power, type I and type II error and p-values. |
| De Kleijn MJJ, Van Der Schouw YT, Wilson PWF, Grobbee DE, Jacques PF. | De Kleijn MJJ, Van Der Schouw YT, Wilson PWF, Grobbee DE, Jacques PF. Dietary intake of phytoestrogens is associated with a favorable metabolic cardiovascular risk profile in postmenopausal U.S. women: The framingham study. J Nutr 2002;132:276-82. | Cross-sectional study on associations between waist-hip ratio, blood pressure, lipoprotein levels and dietary phytoestrogens (isoflavones and lignans). | NA | 939 post-menopausal women from the Framingham Offspring Study | Those in the highest quartile for lignin intake had a lower waist-hip-ratio than women with the lowest lignin intake. |

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| McKeown NM, Troy LM, Jacques PF, Hoffmann U, O'Donnell CJ, Fox CS. | McKeown NM, Troy LM, Jacques PF, Hoffmann U, O'Donnell CJ, Fox CS. Whole- and refined-grain intakes are differentially associated with abdominal visceral and subcutaneous adiposity in healthy adults: the Framingham Heart Study. Am J Clin Nutr 2010;92:1165-71. | Cross-sectional analysis of intake of whole and refined-grains assessed by FFQ and SAT and VAT volumes determined by 8-slice MDCT scanner. | NA | 2,834 Framingham Heart Study participants, 49.4% women, aged 32-83 years old. | Whole-grain intake was inversely associated with SAT, VAT after adjustment for age, sex, smoking status, total energy and alcohol intake. Refined grain was associated with SAT and VAT. SAT and VAT were evaluated VAT remained associated with whole and refined grains. |
| Sallis JF, Saelens BE. | Sallis JF, Saelens BE. Assessment of physical activity by self-report: status, limitations, and future directions. Res Q Exerc Sport 2000;71 Suppl 2:1-14. | NA | NA | NA | Certain methods for determining physical activity of participants are more accurate than others such as accelerometer. |
| Ervin RB, Ogden CL. | Ervin RB, Ogden CL. Consumption of added sugars among U.S. adults, 2005-2010. NCHS Data Brief 2013;1-8. | Summary of data from NHANES on added sugar consumption in adults. | NA | Representative sample of U.S. adults. | U.S. adults on average consume 13% of total calories as added sugar, women 20-39 consume an average of 14.5% of total calories as added sugar, and women 40-59 consume 12.9% of total calories as added sugar. |

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| Kit BK, Fakhouri TH, Park S, Nielsen SJ, Ogden CL. | Kit BK, Fakhouri TH, Park S, Nielsen SJ, Ogden CL. Trends in sugar-sweetened beverage consumption among youth and adults in the United States: 1999-2010. Am J Clin Nutr 2013;98:180-8. | Analysis on NHANES data from 1999-2010 on SSB consumption by U.S. population. | NA | Representative sample of U.S. adults. | 50.6% of U.S. adults consume 1+ SSB per day, 25% consume 2+ SSB per day. |
| McGill CR, Fulgoni VL,3rd, Devareddy L. | McGill CR, Fulgoni VL,3rd, Devareddy L. Ten-year trends in fiber and whole grain intakes and food sources for the United States population: National Health and Nutrition Examination Survey 2001-2010. Nutrients 2015;7:1119-30. | Study on trends in fiber and whole grain consumption by NHANES data of US population from 2001-2010. | NA | Representative sample of U.S. adults and children. | Mean fiber intake was 16.1 grams per day in adults and did not increase significantly over the time span, except in adults 51+. |

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| Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. | Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. J Am Med Assoc 1999;282:1523-9. | Cross-sectional analysis of NHANES III data from 1988-1994. Described the relationship between weight status and health conditions including - type 2 diabetes, gallbladder disease, coronary heart disease, high blood cholesterol, high blood pressure, or osteoarthritis | NA | 16,884 men and women 25 years and older, all overweight and obese by BMI | Prevalence of adverse health conditions increased with severity of overweight and obesity for all conditions except coronary heart disease in men and high blood cholesterol in men and women. The prevalence of having 2 or more health conditions increased with weight category. |
| Gabriela Radulian, Emilia Rusu, Andreea Dragomir, and Mihaela Posea | Radulian G, Rusu E, Dragomir A, Posea M. Metabolic effects of low glycaemic index diets. Nutrition Journal 2009;8:1 | Review of literature on glycemic index and its possible metabolic effects including: metabolic syndrome, insulin resistance, unfavorable lipid profiles, raised inflammatory status, weight loss, and blood glucose levels. | NA | NA | Low GI associated with rapid weight loss, decrease in fasting glucose and insulin levels, reduction of circulating triglycerides and improvement of blood pressure. |