THE RELIABILITY OF AN ABBREVIATED FAT TOLERANCE TEST: A COMPARISON TO THE ORAL GLUCOSE TOLERANCE TEST

By

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Abstract: **Background**: Postprandial lipemia (PPL) is predictive of cardiovascular disease risk. However, the current method for assessing PPL is a burdensome process. Recently, the validity of an abbreviated fat tolerance test (AFTT) has been established. As a continuation of this research, the purpose of this study was to determine whether the AFTT is reliable and compare it to the reliability of the oral glucose tolerance test (OGTT). **Methods**: In this randomized crossover trial, twenty healthy adults (10 male and 10 female) completed two AFTTs and two OGTTs separated by a one week washout period. For the AFTT, triglycerides (TG) were measured at baseline and 4 hours after a high-fat meal, during which time participants were able to leave the lab. The OGTT involved measurement of glucose at baseline and 2-hours post-consumption of a 75-gram glucose drink, and participants remained in the lab. To determine reliability, we calculated within-subject coefficient of variation (WCV) and intraclass correlation coefficient (ICC). **Results**: The mean 4-hour TG WCV for the AFTT was 12.6%, while the mean 2-hour glucose WCV for the OGTT was 10.5%, indicating similar reliability for both tests. ICC values for 4-hour TG and TG change were >0.7 and higher than ICC values for 2-hour and glucose change, indicating good reliability. **Conclusion**: The AFTT was observed to be similarly reliable to the OGTT, supporting its potential as a standard clinical test for determining PPL. Further clinical trials are needed to establish its true utility.

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CHAPTER I

INTRODUCTION

Cardiometabolic syndrome (CMS) involves a combination of risk factors including type 2 diabetes, hypertension, visceral obesity, and hyperlipidemia. Research has shown that a strong correlation exists between CMS and risk of cardiovascular diseases (CVD) such as a coronary artery disease, myocardial infarctions, and stroke (Kelli et al., 2015). CVD is a global concern with approximately 18 million deaths per year, and diabetes and hypertension are major predisposing factors (Kelli et al., 2015). Without suitable assessment methods that can be used in a clinical setting to identify risk and detect disease in its early state, CMS and CVD will continue to be a problem.

One commonly used risk factor in the clinical setting is elevated levels of triglycerides (TG). This blood test is typically examined in a fasted state (Emelia et al., 2017). However, it is well-known that non-fasted or postprandial lipid levels are a significant risk marker for CVD (Boren et al., 2014). The current method for assessing postprandial TG is a burdensome process, in which the patient must stay in the laboratory for up to 6 or more hours with blood drawn every hour after consuming a high-fat meal (HFM) (Maraki et al., 2011). This process is clearly burdensome, which is why standardizing an abbreviated protocol is an important next step with regard to the clinical utility of postprandial triglyceride assessment.

To address this issue, we recently completed a study investigating the validity of a novel abbreviated fat tolerance test (AFTT) (Sciarrillo et al. 2019). In this test, blood draws are only

taken at baseline and the 4-hour postprandial mark, and participants can leave the laboratory between test meal consumption and the follow-up blood draw. Triglyceride results were compared between the AFTT and the standard 6-hour protocol. Our results demonstrated no significant differences between the AFTT and the standard test in which participants stayed in the laboratory, suggesting that the AFTT is valid relative to the standard postprandial protocol, while also being much more clinically feasible.

In order for a clinical test to be useful, there is a critical need for the test to yield consistent results within an individual. In this context, it is important that the reliability of the AFTT be determined. Thus, the primary purpose of the present study was to determine whether or not the AFTT is reliable. The research question was: Does a given individual consistently demonstrate a similar TG response following a HFM when tested with an AFTT? In order to evaluate the reliability of the AFTT, we compared it to the oral glucose tolerance test (OGTT), an accepted and clinically standardized metabolic test. Therefore, the AFTT may be sufficiently consistent in a clinical setting if it yields TG results that are similarly reliable (or more so) relative to the glucose results produced by an OGTT. In addition, we aimed to preliminarily determine the association between AFTT and OGTT responses with other indicators of cardiometabolic risk, in order to initially gauge which test is a stronger indicator of risk.

Aim 1. Determine the reliability of the AFTT over 1 week in a sample of young healthy individuals. We hypothesized that the AFTT would demonstrate moderate to excellent reliability as observed by within-subject coefficient of variation (WCV) of <20% and an intraclass correlation coefficient (ICC) of >7.

Aim 2. Evaluate the reliability of the AFTT by comparing it to the OGTT, a clinically utilized metabolic test. We hypothesized that the AFTT would demonstrate similar or better reliability compared to the OGTT.

Exploratory Aim 3. Preliminarily evaluate the utility of the AFTT compared to the OGTT for predicting cardiovascular risk by determining its association with vascular function. We

hypothesized that the AFTT would demonstrate a similar or better indication of risk in healthy individuals compared to the OGTT.

Upon completion of the study, we found the AFTT to demonstrate similar reliability to the OGTT, strengthening the case for potential clinical utilization of postprandial lipid testing via the AFTT. Specifically, our data provides preliminary evidence that the AFTT is reliable and clinically informative, supporting our previous findings that it is valid relative to standard postprandial assessments (Sciarrillo et al. 2019). This research is important not only for improving postprandial TG testing in a clinical setting, but in a research context as well. Researchers studying postprandial lipemia and their participants would benefit greatly from an abbreviated protocol in which there is a single post-meal blood draw. The wider implication of this study is augmenting the early detection of cardiometabolic risk. If there is an enhanced ability to detect CVD risk earlier, then lifestyle and medical interventions can be utilized at a more effective stage within the disease development continuum.

CHAPTER II

REVIEW OF LITERATURE

Cardiometabolic Disease

Prevalence and Statistics

Cardiometabolic syndrome (CMS), also known as metabolic syndrome X, refers to a cluster of diseases related to multiple risk factors (Kelli et al., 2015). These factors include hypertension, hyperlipidemia, obesity, and elevated blood glucose. All of these factors are associated with the development of atherosclerotic cardiovascular disease (CVD) and type 2 diabetes, which is a determinant of CVD itself. Because CVD continues to be a leading cause of death around the world, accounting for over 18 million deaths each year, early detection of CMS is key in preventing and reducing the risk of cardiometabolic disease (Kelli et al., 2015).

An increase in high-calorie, low-fiber fast foods and sedentary lifestyle has contributed to the prevalence of CMS, which is often paralleled with the incidence of type 2 diabetes and obesity. Patients with CMS characteristics are at a 5-fold increased risk of developing diabetes (Kelli et al., 2015). According to the American Heart Association (AHA), adults with diabetes are two to four times more likely to die from heart disease compared to adults without diabetes. Centers for Disease and Control Prevention (CDC) data published in 2017 revealed that about 30.2 million adults in the U.S. had type 2 diabetes, and CMS prevalence was 3 times as much with one third of adults affected (Saklayen et al., 2018). According to the 2017 update report from the AHA, metabolic syndrome prevalence is rising faster in women and younger individuals, and existing metabolic syndrome worsens with advancing age in over three quarters of adults. Presence of

CMS is associated with an increased risk for coronary artery disease, stroke, renal failure, cancer, and other co- morbidities (Kelli et al., 2015).

Although global data for CMS prevalence is scarce, it is estimated that one quarter of the world population has CMS (Saklayen et al., 2018) – that is over 1 billion people worldwide. Even more alarming is the increasing incidence of CMS in children and adolescents. According to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III), approximately 1 million, or 4%, of U.S. adolescents have CMS. This percentage is reported to be similar for adolescents worldwide (Kelli et al., 2015). Obesity rates are also rising around the world, with an estimated 1.1 billion adults being overweight and 312 million being obese. This is likely explained in part by Western lifestyle (i.e., consumption of high-calorie processed foods and minimal physical activity) spreading rampantly across the globe, particularly in urban areas of developing nations. It is thought that 85% of all CVD occurs in low- and middle-income countries (Kelli et. al, 2015).

Clearly, CMS is a global epidemic that has catastrophic consequences on both health and the economy (Saklayen et al., 2018). The total cost of the condition including potential economic loss and cost of health care is in the trillions of dollars. CMS has negative subsequent impacts on the cardiovascular, renal, immune, and cerebrovascular systems as well as cancer diagnoses (Kelli et al., 2015). Collectively, CMS is projected to cause tens of millions of avoidable deaths. Considering the enormity of this tragic impact, serious action needs to take place in order to combat this silent and often overlooked epidemic. Approaches to reducing CMS are focusing on both improving cardiovascular health in those with less than optimal levels of lipids, blood pressure, and glucose as well as maintaining health in those with ideal levels (Emelia et al., 2017).

Criteria for Cardiometabolic Syndrome

Clinical definitions for CMS vary among organizations. However, the International Diabetes Federation, AHA, and others have recently projected a synchronized classification. By

this definition, risk factors to diagnose CMS include the following: elevated fasting blood glucose ≥100 mg/dL, triglycerides (TG) ≥150 mg/dL, HDL cholesterol <40 mg/dL in males or <50 in females, waist circumference of >40 inches in males or >35 inches in females, or elevated blood pressure of ≥130 mmHg systolic or ≥85 mmHg diastolic (Emelia et al., 2017). If an individual presents with at least 3 of the 5 criteria above they are considered to have metabolic syndrome. Notably, blood tests are necessary for determining 3 out of the 5 criteria for metabolic syndrome and are commonly used in a clinical setting. Of these, two blood tests – glucose and triglycerides – are standardized to be examined in a fasted state, as they are recognized to be altered by recent dietary intake.

Metabolic Risk Assessment

Fasting Triglycerides

While fasting TG is traditionally a criterion for CMS, there has been controversy regarding whether or not it is actually a risk factor for CVD. Traditionally, serum TG is measured after an overnight fast and a desirable level is generally classified as <150 mg/dL. There are different definitions as to what values are considered high TG. Mild-to-moderate hypertriglyceridemia ranges from 150-885 mg/dL, with severe hypertriglyceridemia being greater than 885 mg/dL (Boren et al., 2014). According to the AHA guidelines (2018), mild to moderate hypertriglyceridemia is 174-499 mg/dL.

A strong correlation exists between TG levels and levels of chylomicron remnants and very low-density lipoproteins (VLDL). Due to their large size, VLDL particles cannot penetrate the endothelium wall as efficiently as smaller LDL particles. However, similar to LDL, TG- and cholesterol-rich remnants can penetrate the arterial intima and accumulate in the endothelial space, ultimately leading to the development of atherosclerosis (Varbo et al., 2014; Freiberg et al., 2008). Thus elevated levels may still lead to accelerated development of CVD through similar and alternative mechanistic pathways. For example, remnants are associated with endothelial

dysfunction, including reduced vasodilation and increased inflammatory response (Varbo et al., 2014).

Several meta-analyses have been conducted investigating the association between fasting TG and CVD (Boren et al., 2014). In one meta-analysis including 17 prospective studies with 2,900 coronary heart disease (CHD) endpoints, fasting TG was found to be modestly, independently associated with CVD, and demonstrated that there was a 14% increased risk in CVD for each 1 mmol/L (~89 mg/dL) increase in fasting TG. Conversely, another prospective study (Emerging Risk Factors Collaboration study) with over 300,000 people determined comparable hazard ratios (HRs) but did not conclude that TG was an independent risk factor for CVD. After adjustment for non-lipid risk factors, HDL cholesterol, and non-HDL cholesterol, this study revealed a hazard ratio for CHD with fasting TG of 0.99 (95% CI, 0.94-1.05).

Sarwar et al. (2007) conducted two separate nested case control comparisons based on two population-based cohorts analyzing the relationship between fasting TG and risk of coronary heart disease (CHD). Measurements were made from over 6,000 controls and over 3,500 incident cases of CHD selected from over 44,000 individuals from the EPIC-Norfolk studies. The results suggested long-term stability of log TG values over 4 and 12 years with within-person correlation coefficients of 0.64 [95% confidence interval (CI), 0.60–0.68] and 0.63 [95% CI, 0.57-.70], respectively. The odds ratio after adjusting for risk factors (1.72 [95% CI, 1.56-1.90]) was similar to the adjusted odds ratio found in an updated meta-analysis including over 10,000 CHD cases from over 260,000 individuals in 29 studies (Sethi et al., 2007). Together, these studies indicate a moderate yet significant correlation between fasting TG values and risk of CHD.

It is important to note that a weakness of some of these studies is over-adjustment for potential confounders that are associated with elevated TG. An example is adjustment for HDL cholesterol. HDL is reduced when TG is elevated and vice versa (Freiberg et al, 2008). This consideration has caused several researchers to overlook the association between fasting TG and

CVD, and is partly why a more appropriate means of characterizing TG was explored: analyzing TG elevation following a HFM.

Non-fasting Triglycerides

One of the earliest studies examining postprandial TG was a case-control study conducted by Patsch et al. (1992). Male participants with severe coronary heart disease (CHD) were included in the study and compared to subjects without CHD. At baseline and after consuming a fatty meal containing 65 g of fat, TG was measured at 2, 4, 6, and 8 hours. Findings indicated that TG area under the curve (AUC) and maximal TG increase was significantly higher in the CHD group compared to the controls. TG levels at 6 and 8 hours after the test meal showed a 68% accuracy in predicting the presence of CHD through logistic-regression analysis. This study concluded that non-fasting TG is an independent predictor of CHD in a multivariate analyses including HDL cholesterol and provided a basis for several future studies.

Multiple prospective epidemiologic studies have been conducted exploring the relationship between non-fasting TG and CVD. For example, the Copenhagen City Heart study observed that postprandial TG levels above 5 mmol/L were associated with a 17-fold increased risk in women and a 5-fold increased risk in men for myocardial infarction in 13,000 Danish individuals followed-up for 26-31 years (Boren et al, 2014). From this same study, Freiburg et al. (2008) showed that highest levels of postprandial TG (≥443 mg/dL) were associated with a 3- and 4-fold increased risk of ischemic stroke in men and women.

The Women's Health Study (WHS) was a large prospective cohort study comprised of over 26,000 initially healthy U.S. women followed for over 11 years. The WHS started the debate over the use of fasting TG in determining CVD risk. A subset of over 6,300 of women in the WHS provided non-fasting blood samples at baseline. From this subset, Bansal et al. (2007) observed that there was a strong independent association between postprandial TG and CVD events, even after fully adjusting for other factors including total cholesterol and HDL. On the

other hand, there was little association between fasting TG levels and CVD events. This was an important finding in the debate between fasting and postprandial TG.

In a recent longitudinal study (Kats et al., 2017), the association of postprandial lipemia (PPL) as an independent predictor of CVD was reexamined using a population-based cohort. TG was measured at 3.5 and 8 hours following a fat-tolerance meal in a population without CVD (n = 559) from the Atherosclerosis Risk in Communities Study (ARIC) (1990-1993). Incident CVD events associated with postprandial change was estimated through 2012. In the 23% of participants that developed CHD, severity of PPL was not shown to predict CVD risk. However, the small cohort, relative to other prospective studies, needs to be considered in interpreting this study's findings. Although this is the largest study to examine the relationship between PPL and CVD risk using a postprandial test meal, more highly powered prospective studies are needed to elucidate this association. Notably, two clear challenges to conducting large prospective studies utilizing PPL as an outcome of interest are the unfeasibility of conducting traditional oral fat tolerance tests in a widespread fashion, and the absence of an established abbreviated fat testing protocol.

According to Warnick et al. (2008), the Copenhagen study showed that peak TG values were around 4 hours postprandial and concluded that these peaks were due to cholesterol remnant particles that had not yet cleared. This was calculated by subtracting LDL and HDL cholesterol from total serum cholesterol. An impairment of remnant clearance is likely the cause of increased CVD risk, and is supported by a retrospective analysis of the Copenhagen study that concluded that the distinguishing factor in patients with CVD was elevated remnant levels (Sethi et al., 2007). Remnants have more atherogenic properties compared to nascent particles, and a postprandial TG measurement may be a more accurate indicator of impaired remnant clearance (Warnick et al., 2008).

Because TG levels are normally evaluated in the fasted state, the majority of the lipoprotein remnants that likely promote atherosclerosis are omitted from the measurement.

Additionally, it is important to note that with the exception of before breakfast, people spend the majority of their day in a postprandial state. Therefore, a non-fasted measurement is likely more informative and representative than a fasted measurement when it comes to detecting cardiometabolic risk. As supported by several research studies and reviews, it is well-established that non-fasted TG levels are a significant risk marker for cardiovascular disease (Nordestgaard et al., 2007, Langsted et al., 2008, Jackson et al., 2012, Boren et al., 2014, Pirillo et al., 2014).

Fasting vs Non-fasting Glucose

Unlike TG, there is precedence for consideration of both fasting and postprandial glucose in a clinical setting. Diabetes may be diagnosed using either fasting or postprandial blood glucose levels. Individuals with a fasting glucose level of 70-99 mg/dL are considered to have normal glucose regulation, according to the American Diabetes Association (ADA 2016). A fasting glucose level of 126 mg/dL or higher is typically a criteria for diabetes, while anything in between indicates prediabetes. Due to variability in fasting glucose concentrations, individuals must have 2 fasting levels greater than 126 mg/dL to classify diabetes. Diabetes can also be diagnosed postprandially through the oral glucose tolerance test (OGTT). This test involves drinking 75 grams of pure glucose and then checking glucose levels at the 2-hour time point. The ADA states that a level less than 140 mg/dL is considered normal, 140-199 mg/dL indicates prediabetes, and 200 mg/dL or greater is a diabetes diagnosis.

Although a diabetes diagnosis can be determined both ways, the influence of postprandial versus fasting blood glucose on diabetes complications has been a controversy (Cavalot et al., 2006). It is known that individuals affected by type 2 diabetes are at increased risk for cardiovascular morbidity and mortality. According to Horwich et al. (2010), elevated fasting glucose is a risk factor for heart failure independently of whether or not an individual has diabetes. This prospective cohort study involving over 31,000 people at high risk for CVD found that fasting glucose was an independent predictor of hospitalization for heart failure. However, the role of postprandial blood glucose as an independent contributor to diabetes complications is

shown to be more predictive of CVD events than fasting levels, as supported by studies carried out in the general population (Cavalot et al., 2006).

In a prospective epidemiological study of over 500 individuals with type 2 diabetes in a 5-year follow up, CVD risk factors and events were examined. Baseline blood glucose was measured at 4 time points: after fasting, after breakfast, after lunch, and before dinner. Associations between glucose and CVD events were different between men and women. The authors concluded that postprandial blood glucose, but not fasting levels, was an independent risk factor for CVD events in those with type 2 diabetes. They also observed that this relationship was stronger in women compared to men (Cavalot et al., 2006).

Because CVD is the leading cause of death in patients with diabetes, it is important to know which screening method is best with regard to early detection and prevention. In a recent study conducted by Jiang et al. (2017), fasting glucose, postprandial glucose, and glycated hemoglobin (HbA1c) were all assessed as screening tools for coronary heart disease (CHD). The study population included 1,852 Chinese patients who completed coronary angiography and tested positive for CHD. After undergoing all 3 screening procedures, patients were classified into normal glucose tolerance, impaired glucose regulation, or diabetes groups. Glucose metabolism and insulin resistance were correlated with the Gensini score, a means of quantifying atherosclerosis. A score of zero indicates the absence of atherosclerosis, while a higher score indicates the sum of all lesion scores, taking into account geographical location, degree of luminal narrowing, and the cumulative effect of having multiple lesions (Kashani et al., 2016).When all 3 tests were compared, only postprandial blood glucose was consistent with the Gensini score. This indicates that postprandial glucose procedure rather than procedures for fasting glucose or HbA1c was most correlated with the presence and severity of CHD. These results suggest that CHD screening should be based on postprandial glucose levels, although this finding needs to be validated in additional populations.

Postprandial Assessment Methods

Oral Fat Tolerance Test

Standard Protocol

The oral fat tolerance test (OFTT) is a generic test used to assess postprandial lipemia (PPL) by examining TG responses to a high-fat meal (HFM). The OFTT is a burdensome procedure that lasts 6 hours or longer and requires serial blood sampling, typically each hour (Maraki et al., 2011). According to an expert panel statement, an OFTT should consist of a single fat load following an 8 hour fast and measure total TG at the 4-hour postprandial mark (Kolovou et al., 2011). There are no official protocols or guidelines when it comes to administering the OFTT. Standardization and consensus is still needed.

In the research setting, subjects normally arrive at the laboratory following a 10-12-hour fast. They may be asked to avoid alcohol consumption, refrain from physical activity, and keep their dietary habits consistent for several days before the test. A baseline blood draw is taken, after which the high-fat test meal is consumed within 15-20 minutes. An IV catheter may be inserted into the forearm for the ease of blood sampling. Most postprandial studies utilize an IV catheter for blood sampling as opposed to repeated single venipunctures (Emerson et al., 2018). The catheter is flushed with heparin-free 0.9% NaCl to keep the port viable. Subjects then remain in a rested state throughout the remainder of the postprandial period. No other foods or beverages are typically allowed during this period besides water. In consideration of the time commitment for both the participant/patient and researcher/clinician, it is not surprising that this test is not regularly performed in clinics.

Abbreviated Protocols

In order to increase the clinical feasibility of PPL testing, a few studies have aimed to evaluate the validity of a simplified postprandial TG assessment protocol. This idea was initially investigated by Guerci and colleagues (2001). In this cross-sectional study, a statistical 3-point fat tolerance test was carried out in three different groups: obese individuals with normal lipids

compared to normal weight individuals, normolipidemic individuals with type 2 diabetes compared to a normolipidemic control without diabetes and individuals with type 2 diabetes and hyperlipidemia studied before and after lipid-lowering therapy. Blood was drawn 7 times over the course of the 8-hour post-meal period. TG responses were measured by utilizing conventional area under the curve (AUCc) and comparing 5-7 lipid samples to 3 samples (taken at baseline, 4, and 8 hours) to determine the predicted area under the curve (AUCp). The AUCc and AUCp for TG were found to be highly correlated in each of the groups. This study demonstrated that a 3-point protocol for lipid testing may be suitable for determining postprandial lipemia in both healthy subjects and those with altered postprandial metabolism (Guerci et al., 2001).

This idea was further explored by Weiss and colleagues (2008). With a repeated-measures design, 5 lean and 4 obese participants underwent 4 consecutive postprandial fat tests separated by 1 week each. Blood sampling was taken every hour after the OFTT for 8 hours, and the first 4 hours were interpreted as abbreviated PPL results. In both lean and obese groups, total plasma TG concentrations peaked from 3 to 4 hours postprandially and returned to baseline by 8 hours. The authors determined that the 4-hour lipemic responses accounted for 89-96% of the variance of the full-length 8-hour test, demonstrating that the TG results were highly related to the overall postprandial response.

Shortly thereafter, these results were validated by Rector et al. (2009) and Maraki et al. (2011). Rector et al. (2009) looked at the PPL response in over 100 males and 90 females from 8 previous studies. The purpose of their study was to determine whether a single-point TG concentration was predictive of the overall PPL response. TG concentrations were determined at baseline and at several time points up to 8 hours. The relationship between the 8-hour AUC for TG and peak TG were examined against various single time points. Bootstrap simulations and stepwise multiple regression were utilized to determine that TG concentration at 4 hours accounted for over 90% of the variance in AUC and peak TG levels throughout the postprandial testing period. The authors concluded that the 4-hour TG value can be used to accurately measure

PPL in both healthy individuals and in those at-risk for cardiometabolic disorders (Rector et al., 2009).

The retrospective study by Maraki and colleagues (2011) compared 3 different OFTT protocols: a single TG response at 3 or 4 hours postprandially, a shortened OFTT lasting less than 6 hours, and a reduced-sampling OFTT. Over 70 subjects underwent a conventional 6-hour OFTT in previous studies. Predictability of the single TG and shortened OFTT was analyzed via linear regression while the reduced-sampling OFFT was assessed using Pearson's correlation coefficients. Results from the single TG protocol showed that TG concentration at 3 or 4 hours postprandially were moderately to highly predictive of both total and incremental AUC in the overall sample, obese, and lean groups. However, this was not true for the exercise, energy restriction, and hypertriglyceridemia groups. In the abbreviated protocol, reducing the OFTT to 3 hours resulted in lesser predictability, while reducing the test to 4 hours was moderately to highly predictive of the corresponding values of the conventional OFTT in every group besides the hypertriglyceridemia group. For the reduced sampling protocol, 3 blood draws at 0, 3, and 6 hours was shown to be moderately to highly correlated with the conventional 6-hour OFTT with hourly blood draws in all groups, as well as the overall sample. The authors concluded that a reduced-sampling OFTT may be a convenient alternative to the conventional test, but an abbreviated OFTT may only be appropriate in healthy individuals.

Thus far, the aforementioned research studies have shown that a single TG measurement at 4 hours post-meal or an abbreviated OFTT in small samples is statistically valid. It is important to point out that so far, these studies are merely examining the usefulness of an abbreviated protocol through statistical methods. A limitation is that they are not empirically testing the tests' usefulness or accounting for variability that may occur in a real life situation. Although this is an improvement to the conventional OFTT, the procedure still requires empirical evaluation. Additionally, these studies have required participants to remain in the laboratory between the

baseline and postprandial blood draws, which would be a barrier to feasible implementation in a clinical setting.

Our laboratory group recently completed a randomized cross-over pilot study in which participants could leave the lab after the baseline blood draw and return 4 hours later, and hypothesized that this would not significantly alter TG results from when participants stayed in the lab for 4 and 6 hours (Sciarrillo et al., 2019). Participants (n = 18) completed 3 different HFM trials: a conventional 6-hour OFTT, an abbreviated fat tolerance test (AFTT) with a single postprandial TG measure at 4-hours wherein participants remain in the laboratory, and an AFTT in which participants left the laboratory between the meal and 4-hour measurement. There was no statistical difference between the 3 trials, supporting the validity of an AFTT and suggesting that allowing patients to leave the lab may be implemented clinically, greatly increasing the test's feasibility. This study empirically tested the single-point PPL response and allowed subjects to leave the lab, neither of which had been previously tested. However, a limitation of this study was its small sample size and inclusion of only heathy individuals.

Reliability of OFTTs

Few studies have examined the reliability of the OFTT (i.e., within a given individual, the consistency of the TG response following multiple OFTTs). In the previously mentioned study conducted by Weiss et al. (2008), the primary purpose was to determine the reproducibility (i.e., reliability) of the OFTT in both lean and obese individuals in 4 different OFTT administrations separated by 1 week. Group comparisons were performed by utilizing independent t-tests, and the mean of all 4 tests was used for each participant. Results indicated that total AUC for TG had low within-subject coefficient of variation (WCV) (8%), indicating high reliability. On the other hand, incremental AUC had 2-fold greater WCV values (20-31%), indicating poor reliability.

A recent repeated-measures study examined the repeatability of an abbreviated OFTT in 26 healthy males (Tentolouris et al., 2017). Each individual completed the test twice (1 week apart), and blood samples were collected hourly until 4 hours post-meal. Bland-Altman plots,

correlation coefficients, and coefficients of variation (CV) were conducted to examine agreement, precision, and accuracy between the two visits as well as reliability. The tests showed high agreement and accuracy, and the CV value at 4 hours was 17.21%, indicating moderate to low reliability.

Similarly, in another recent randomized cross-over study conducted by O'Doherty et al. (2018), the repeatability of an in-lab abbreviated 4-hour OFTT was examined. Ten healthy males underwent 4 OFTTs in either a rested state or immediately after aerobic exercise with blood samples taken hourly for 4 hours. Bland-Altman analyses were utilized in order to determine agreement between repeated measures. Results demonstrated that 4-h TG response to the OFTT was repeatable in the rested group, while repeatability in the OFTT given after aerobic exercise was poor. An implication of this finding is the importance that exercise before the test be avoided in order to ensure accurate and consistent results (O'Doherty et al., 2018).

A weakness to consider is that two of these aforementioned studies only looked at healthy males. This may be due to the possible influence of menstrual cycle on OFTT reliability. A study conducted by Gill et al. (2005) examined the effect of menstrual cycle phase on PPL. Women underwent two OFTTs – one during the luteal phase and one during the follicular phase. The postprandial TG response was found to be lower in the luteal phase than the follicular phase, and the WCV was 23.2% (Gill et al., 2005). In contrast, Weiss et al. (2008) found high reliability despite the fact that most of the participants were women and menstrual phase was not accounted for.

All of these studies also had relatively small sample sizes. Because most of these studies utilized full/traditional OFTTs, testing a large number of participants was not reasonable. This is another area where research utilizing the AFTT can be valuable. There is a necessity for the reliability of the AFTT to be studied in larger populations that include both males and females. Because there also seems to be a discrepancy in the reliability between exercise and inactive groups, this also needs to be explored and validated further.

Composition of Test Meal

Due to the lack of standardization, there is much discrepancy in the test meal composition utilized in an OFTT. The variability ranges from a calorie-rich mixed breakfast meal to a dessert-like shake, making it challenging to compare different studies (Boren et al., 2014). Many test meals consist of dairy products, including ice cream and heavy whipping cream. However, this would not be tolerated in individuals with a dairy allergy or lactose intolerance. Many other test meals contain commercial-grade ingredients that cannot normally be obtained at a store, and hence are not foods that would be regularly consumed. Thus, a meal that is more inclusive for those with dietary restrictions and also something that people would realistically consume would be beneficial. Table 1 below shows that test meals vary tremendously in terms of macronutrient and total energy distribution. Additionally, many of the test meals are not clinically appropriate or feasible. For example, a test meal of over 1,000 kcal is extremely energy-dense and may be difficult to consume. An Expert Panel comprised of scientists and clinicians recommended that a single OFTT meal should be comprised of 75 g fat, 25 g carbohydrate, and 10 g protein. They also recommended a mixed meal, as fat partly needs carbohydrates in order to be metabolized, and this is also more representative of a normally consumed meal (Kolovou et al. 2011).

Table 1

OFTT Meal Compositions

Study	Ingredients	Fat	Carbs	Protein	Total Energy
Mohanlal et al., 2004	Strawberry-flavored fat emulsion, maltodextrin	50 g	50g	0 g	656 kcal/200 mL drink
Gill et al., 2005	Whipping cream, fruit, cereal, nuts, chocolate	1.2 g	1.2 g		16.73 kcal/kg of body mass
Weiss et al., 2008	Whipping cream, vanilla ice cream	71%	23%	6%	2.84 kcal/g of meal
Maraki et al. 2011	Whipping cream, vanilla ice cream, syrup	79 ± 1 g	70 ± 1 g	14 ± 0 g	1043.75 ± 11.94 kcal
O'Doherty et al., 2018	Dairy products, chocolate powder	75.4 g	21.7 g	13.7 g	822.8 kcal
Sciarrillo et al., 2019	Marie Callender's Chocolate Satin Pie	63%	34%		12 kcal/kg of body mass

This table shows the variability of OFTT meal compositions used within a research setting assessing its utility and/or reliability.

Oral Glucose Tolerance Test

Reliability of OGTT

Although the OGTT remains the gold standard with regard to the diagnosis of type 2 diabetes, there is ongoing debate with regard to its reliability. McDonald et al. (1965) explored the reproducibility of a 100 g OGTT in over 400 male subjects. The subjects underwent 6 different OGTT administrations over the course of 1 year with approximately 2 months between each test. Blood draws were taken 1, 2, and 3 hours postprandially after the test. Results showed stable glucose levels for the total group over time, but tremendous variation among individual readings. Many individuals would vary from normal to abnormal readings between tests. Toeller et al. (1973) looked at the OGTT's reliability with 3 differing glucose loads (50 g, 75 g, and 100 g) in 20 men. Each individual underwent 15 OGTTs at intervals of 3-4 days apart. Glucose was measured at 1, 2, and 3 hours post-test. The administration of the 100 g OGTT was found to have significantly lesser individual variation in blood glucose compared to both the 50 and 75 g tests. The authors concluded that the 100 g OGTT was the most reliable, meaning it showed the least variation.

Ganda and colleagues (1978) developed a methodology for measuring the reproducibility of the OGTT as well as the intravenous glucose tolerance test (IVGTT). Similarly to an OGTT, the IVGTT is used to evaluate glucose tolerance, but is not used to diagnose diabetes. The IVGTT procedure in this article entailed rapid glucose infusion into the vein (0.5 g glucose/kg body weight) for three minutes. Two groups of subjects (healthy individuals without diabetes and offspring of married diabetic parents) completed two 100 g OGTTs and two IVGTTs. Seventeen of the healthy individuals and nine of the offspring completed both tests twice. Glucose samples were taken 12 times during the 2 hour postprandial period for the IVGTT, and 9 times during the 5 hour postprandial period for the OGTT. Change in blood glucose was measured for each subject at each time interval, and the changes within each time interval were categorized into quartiles. In this way, a reproducibility index was created. If a participant fell in quartiles 3 or 4, they had higher reproducibility than those in lower quartiles. There were no significant differences found

between the OGTT and IVGTT reproducibility or between normal subjects versus the offspring. However, only 50% of the tests were considered reliable.

A retrospective epidemiological study assessed over 200 Chinese subjects who underwent two OGTTs in order to revisit the test's reliability. The overall reliability was 65.6%, meaning 139/212 fell into the same category (normal glucose tolerance, impaired glucose tolerance, or diabetes) after completing both tests. The authors concluded their results were in agreement with most studies up until that point. Subjects with one abnormal OGTT were found to be at risk for cardiovascular disease compared to those who had two normal OGTTs, suggesting that 2 OGTTs may be needed in order to confirm a diagnosis (Ko et al., 1998).

The reliability of repeated OGTTs was analyzed further in 2011 by Gordon and colleagues. Ten inactive healthy individuals repeated the OGTT 4 times over 4 consecutive days, and found no significant differences. The CV was 7.8-14.4%, indicating good reliability. This finding disputed previous studies that found poor reliability in apparently healthy subjects who repeated the OGTT within days. However, this may not be the case for those with impaired glucose metabolism (Gordon et al., 2011).

A recent study evaluated the reliability of the OGTT for the diagnosis of gestational diabetes in an 84 African women. The women completed the test twice. The results were reliable in only 74.2% of pregnant women, meaning they had the same result after both tests, and the kappa statistic was 0.46. Only 48.6% of women had negative results in both tests, and 25.7% tested positive both times. This indicates that test interpretation needs to be taken with caution, and more than 1 OGTT is likely needed for a more accurate diagnosis (Munang et al., 2017).

Considering these studies, there are drawbacks to the OGTT. Because there is so much individual variability, diabetes diagnosis is challenging. Another problem is that glucose results following an OGTT cannot be directly inferred to glucose results following a mixed meal (Cavalot et al., 2006). However, the hyperinsulinemic euglycemic clamp notwithstanding, the OGTT is still considered the gold standard method for assessing glucose tolerance in a clinical

setting. Thus, the OGTT is an imperfect but still generally accepted and utilized metabolic challenge. Although assessing different aspects of metabolic capacity, the OGTT nevertheless provides a model for determining reliability and clinical utility of a dynamic metabolic test. Specifically, if the AFTT presents a similar or higher reliability than the OGTT, a widely used standardized clinical test, then this will greatly enhance the AFTT's outlook as a trustworthy asset in the clinical setting.

CHAPTER III

METHODS

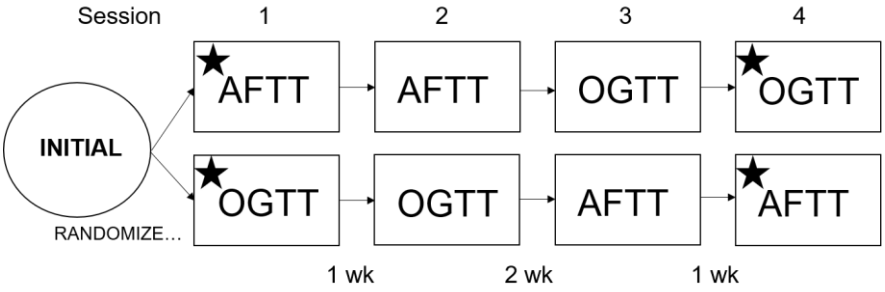
Research Participants

We aimed to recruit 20-30 healthy men and women to participate in this study. Inclusion criteria included 18-45 years of age, free of chronic disease, fasting TG <150 mg/dL, and fasting glucose <100 mg/dL. Exclusion criteria were presence of pacemaker or other electrical implant, pregnancy, use of tobacco products, and use of dietary supplements or medications that could potentially influence the primary outcome (birth control excluded). Participants were recruited through flyers, personal contacts, and snowballing methods. All participants gave their informed consent before partaking in the study. They were compensated $50 at the completion of the study as an incentive for their participation.

Study Design

With a randomized-crossover design, participants were randomized to 1 of 2 tracks: 2 AFTTs separated by 1 week followed by 2 OGTTs separated by 1 week or vice versa. There was a minimum of a 2-week washout period separating the tests to diminish the impact of any carry-over effects (See Figure 1 below).

Figure 1. Study protocol. The stars indicate measurement of fasting/baseline flow-mediated dilation and HbA1c, as well as 3-day food record and physical activity assessment. AFTT, abbreviated fat tolerance test; OGTT, oral glucose tolerance test.



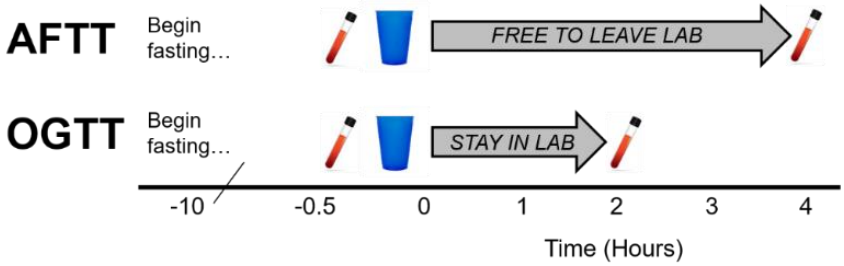
The initial assessment involved anthropometric measures such as height, weight, waist circumference, and body composition using bioelectrical impedance analyzer (Seca mBCA 514; Hamburg, Germany). Blood pressure was measured using an automated cuff (Omron 5 Series BP742N; Kyoto, Japan). A fasting blood draw was taken to ensure healthy glucose and triglyceride values. Subjects also completed short questionnaires involving their general health history and physical activity level. A physical activity tracker (Actigraph GT3XP; Pensacola, FL, USA) was worn on the wrist for 5-7 days, a 3-day food record was completed, and HbA1c was measured before the first and last tolerance tests. We measured these outcomes to ensure consistency in these factors across the 4-week trial period. Before each tolerance test, a 210-kcal whole-grain peanut butter cracker snack was consumed as the last food item before fasting for at least 10 hours. This snack ensured consistency in fasting among both participants and trials. Subjects were asked to avoid planned exercise for 1 day before each meal test.

For blood draws, a 21-gauge needle was used to draw blood via single venipuncture from a forearm vein into 3 mL lithium heparin vacutainer tubes at baseline and 2 or 4 hours post-challenge for the OGTT and AFTT, respectively. Approximately 4 mL of blood was taken each time for a total of 8 mL of blood per session and 32 mL of blood over the course of the entire study.

The AFTT entailed a fasted blood draw followed by consumption of a gluten-free, dairy-free, high-fat test shake consisting of coconut milk, chocolate syrup, and vegan protein powder (73% fat, 26% carbohydrate, 1% protein; 9 kcal/kg body weight). After completing the test meal in 20 minutes or less, participants were instructed to vacate the lab for approximately 4 hours,

abstaining from additional food intake or planned exercise. Participants then returned for a follow-up blood draw 4 hours later. Similarly to the AFTT, the OGTT entailed a fasted blood draw followed by consumption of a 75 g pure glucose drink (Azer Scientific). Participants then remained in the lab for 2 hours, at which point a follow-up draw was taken. Whole blood was inserted into a comprehensive metabolic panel (OGTT) or lipid panel plus (AFTT) reagent disc and processed utilizing the Piccolo Xpress chemistry analyzer (Abaxis Inc.; Union City, CA, USA). Schematics of the AFTT and OGTT protocols are displayed below.

Figure 2. Protocols for the AFTT and OGTT. Each participant completed two AFTTs and two OGTTs. The cup indicates consumption of the meal challenge beverage.



Flow-Mediated Dilation

During the first and last assessment, flow-mediated dilation (FMD) was measured via Doppler ultrasound imaging in order to measure each participant's vascular function (Mindray Z5 Portable Ultrasound Machine; SonoScape Medical; Shenzhen, China). We utilized this technique because FMD has been shown to be a strong indicator of CVD risk (Daniel et al., 2011; Maruhashi et al., 2013; Ras et al., 2013; Shechter et al., 2014). A 12 MHz linear probe (Linear Array Z5; SonoScope) was used to image the participant's brachial artery, and baseline imaging was collected for 2 minutes. A blood pressure cuff was then used to occlude blood pressure at the wrist for 4 minutes. The pressure of the cuff was 220 mmHg, with the intent of inflating the cuff to a sufficient pressure to occlude the vessel. The pressure was then released, and the degree of vascular dilation was measured for an additional 4 minutes, for a total of 10 minutes of imaging.

Statistical Analysis

In order to assess the reliability of the AFTT and the OGTT, we calculated the within-subject coefficient of variation (WCV) based on each participant's two trials of the two metabolic challenges. WCV represents variability of a particular value for individuals in a sample, and is calculated using the mean and within-subjects standard deviation. It has been recommended that a WCV of 10-20% or less represents "good" reliability (Quan and Shih, 1996). Weiss et al. (2008) chose to use the strict end of this range (i.e., <10%) as the criterion for "high" reliability. Thus, a WCV of <10% would be considered as "highly reliable", a WCV of 10-20% as "moderately reliable", and a WCV >20% as "low reliability". To compare the AFTT and the OGTT with regard to reliability, we simply compared the WCV and associated classification of each test. Additionally, we used a paired t-test to compare the CV for the AFTT and OGTT for the total sample.

Along with WCV, another method of analyzing reliability used was intraclass correlation coefficient (ICC). This method examines test-retest reliability (i.e. variation in measurements taken on same subjects under same conditions). The ICC is calculated as between-subjects variability divided by between-subjects variability + within-subjects variability. The closer the ICC value to 1, the more reliable. For example, an ICC of 0.95 means 95% of the observed variance is due to SD (Weir et al., 2005). There are several different models of ICC; we chose 2-way random due to our crossover trial and to generalize results outside the current study. In addition to ICC, the standard error of the mean (SEM), minimal difference to be considered real (MD), and between-subjects coefficient of variation (CV) was calculated.

Outliers in the data were removed using the robust regression and outlier removal (ROUT) method. This method works by fitting a curve that is not influenced by outliers. Any residuals are examined to identify outliers, removed, and then ordinary least-squares regression is performed on the remaining data (Moltusky and Brown, 2006).

In addition to comparing the AFTT and OGTT, we explored dietary and physical activity behaviors to assess their possible influence on test-retest variations in TG change and 4-hour TG. Correlations using Pearson's r were performed to test for associations between variations in diet and physical activity leading up to each metabolic test against variations in metabolic results. Dietary outcomes assessed included differences between the two AFTTs in total calories, total fat calories and percentage of fats in the diet, saturated fat, unsaturated fat, fiber, sugar, and added sugar intake. For activity level, we included differences in steps per day and time spent in moderate- to vigorous-intensity physical activity (MVPA) per day.

We further considered the potential impact of variation in dietary and activity factors by performing a median split based on test-retest differences in 4-hour TG or TG change. We then compared dietary and physical activity behaviors between the "low variation group" and "high variation group" using unpaired t-tests.

CHAPTER IV

RESULTS

Participant Characteristics

A total of 22 participants completed the current study. Using the formal outlier analysis (ROUT method), two outliers were identified and removed from data analysis due to extreme differences in baseline and 4-hour TG concentrations (154 and 196 mg/dL, respectively). These large deviations likely reflect noncompliance with lifestyle controls (i.e. not following 10-hour overnight fast or abstaining from food intake during AFTT postprandial period). Thus, twenty individuals (10 male and 10 female) were included in the final analysis (**Table 2**). With regard to participant characteristics, fasting metabolic outcomes were determined by averaging results from the initial assessment. Paired t tests were utilized to compare differences in baseline characteristics between males and females. In our sample, males had greater height, weight, skeletal muscle mass, visceral adiposity, and fat mass percentage compared to females. Males also exhibited a greater HbA1c compared to females. There were no differences in fasting metabolic markers between men and women.

Table 2

Participant Characteristics

Participants	Total	Men	Women	*P*-value
Number in Sample	20	10	10	
Age (years)	23.9 ± 6.8	25.7 ± 6.0	22.0 ± 7.3	0.140
Height (m)	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0	0.003**
Weight (kg)	70.9 ± 12.6	79.9 ± 9.0	61.9 ± 8.6	<0.001***
BMI (kg/m²)	23.4 ± 3.6	24.6 ± 3.8	22.1 ± 3.1	0.065
Fat Mass (%)	24.4 ± 8.0	20.8 ± 8.0	28.0 ± 6.5	0.016*
Skeletal Muscle Mass (kg)	25.9 ± 5.8	31.0 ± 2.9	20.8 ± 2.5	<0.001***
Visceral Adiposity Tissue (kg)	1.0 ± 1.0	1.4 ± 1.3	0.5 ± 0.3	0.046*
HbA1c (%)	5.2 ± 0.0	5.3 ± 0.0	5.0 ± 0.0	0.002**
Fasting Triglycerides (mg/dL)	88.2 ± 26.7	88.6 ± 31.1	87.8 ± 23.2	0.945
Fasting Glucose (mg/dL)	95 ± 5.9	97.0 ± 5.4	92.9 ± 5.9	0.135
Fasting Total-C (mg/dL)	160.7 ± 25.7	155.2 ± 23.0	166.2 ± 28.3	0.434
Fasting HDL-C (mg/dL)	54.7 ± 12.0	51.1 ± 9.8	58.2 ± 13.4	0.066
Fasting LDL-C (mg/dL)	88.2 ± 22.1	86.1 ± 26.1	90.3 ± 18.5	0.741
Steps per Day (average)	10896 ± 2735	11929 ± 2789	9734 ± 2300	0.200
Time in MVPA	183.1 ± 46.7	187.3 ± 52.8	178.3 ± 42.0	0.995
AFTT Test Meal Energy (kcal)	639.3 ± 113.5	720.3 ± 81.6	558.3 ± 77.2	<0.001***

Note. Using paired t tests, we compared characteristics between men and women. Means are expressed plus or minus standard deviation. MVPA; moderate to vigorous physical activity.
**p <0.05 **p <0.01 ***p <0.001*

AFTT Comparisons and Correlations

Data regarding reliability of TG data in the AFTT are displayed in **Table 3**. The mean difference in fasting TG between AFTT 1 and AFTT 2 was 5.9 mg/dL (95% CI: [-7.7, 19.5]) (**Figure 3A**). The mean difference in 4-hour TG level was -0.8 mg/dL (95% CI: [-21.4, 19.9] (**Figure 3B**), and the mean change in difference between baseline and 4-hour TG was -6.8 mg/dL (95% CI: [-26.9, 13.4]) between AFTT 1 and AFTT 2 (**Figure 3C**). In the total sample, there were no differences observed between the two AFTTs for fasting, 4-hour, or change in TG when compared with a paired t-test. Correlations between the two tests were also significant for fasting, 4-hour, and change in TG.

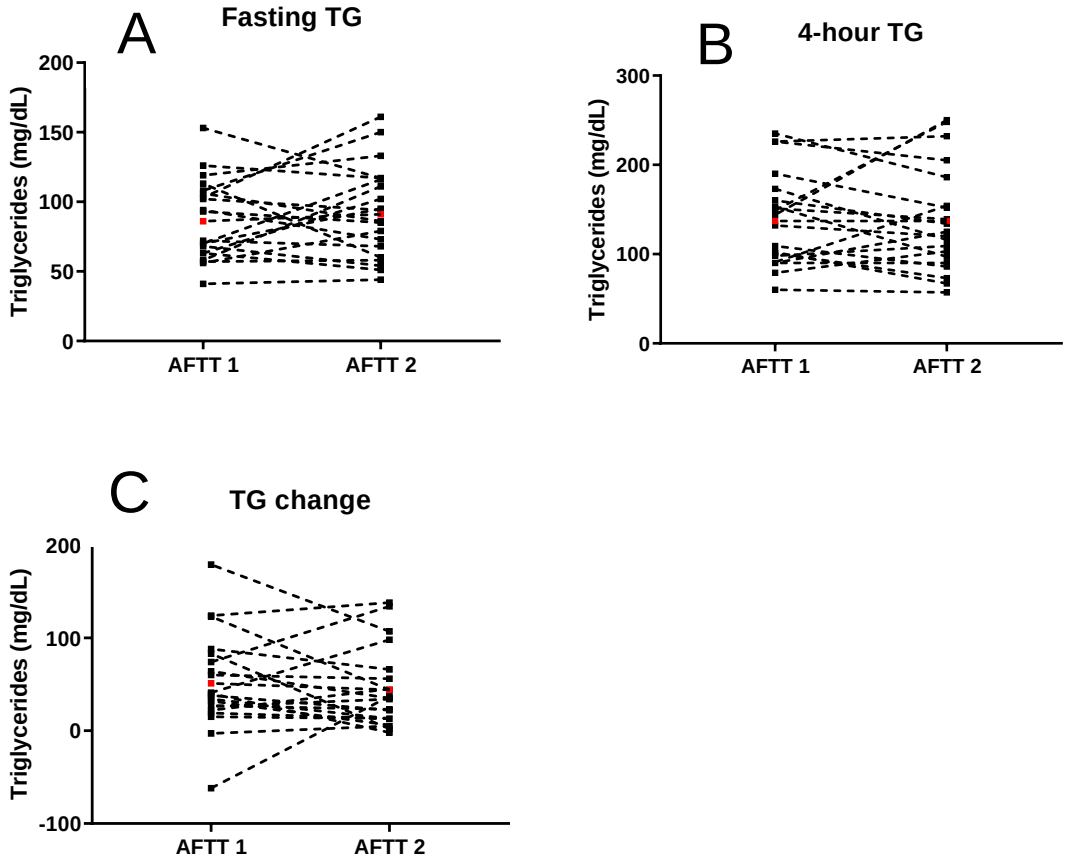
Table 3

AFTT Comparisons and Correlations

	AFTT1	AFTT 2	*P*-value	Correlation *(r)*	*P*-value
Fasting TG (mg/dL)					
Total	86.8	92.7	0.37	0.52	0.007**
Men	93.2	107.6	0.29	0.15	0.339
Women	80.3	77.8	0.58	0.89	<0.001***
Low BMI (< 22.8 kg/m²)	89.7	78.7	0.15	0.73	0.008**
High BMI (> 22.8 kg/m²)	83.8	106.7	0.03*	0.56	0.046*
Younger (< 21.5 years)	94.7	81.0	0.07	0.72	0.009**
Older (> 21.5 years)	78.8	104.4	0.01*	0.74	0.007**
4-hour TG (mg/dL)					
Total	138.0	137.2	0.94	0.66	<0.001***
Men	156.4	174.5	0.34	0.49	0.073
Women	119.5	99.8	0.02*	0.84	0.001**
Low BMI (< 22.8 kg/m²)	142.7	129.0	0.26	0.78	0.004**
High BMI (> 22.8 kg/m²)	133.2	145.3	0.50	0.61	0.029*
Younger (< 21.5 years)	127.8	112.5	0.22	0.44	0.102
Older (> 21.5 years)	148.1	161.8	0.43	0.71	0.011*
TG Change (mg/dL)					
Total	51.2	44.5	0.49	0.56	0.004**
Men	63.2	66.9	0.84	0.54	0.054
Women	39.2	22.0	0.08	0.45	0.098
Low BMI (< 22.8 kg/m²)	53.0	50.3	0.85	0.75	0.007**
High BMI (> 22.8 kg/m²)	49.4	38.6	0.50	0.29	0.204
Younger (< 21.5 years)	33.1	31.5	0.91	0.20	0.286
Older (> 21.5 years)	69.3	57.4	0.45	0.63	0.025*

Note. Using paired t tests, we compared triglyceride levels between test 1 and test 2, as well as calculated correlation using Pearson's coefficient r. Results are further stratified by sex, BMI, and age respectively.
**p <0.05 **p <0.01 ***p <0.001*

Figure 3. Triglyceride concentrations between two AFTTs, shown as fasting levels (A), 4-hour postprandial (B), and change from baseline to 4-hours (C). Lines represent individual responses to the two tests, while red points depict the average.



Results were dichotomously stratified by sex, BMI, and age using the median values for the group. For fasting TG, there was a difference between the two tests in the high BMI group (p = 0.03) and the older age group (p = 0.01). Women's 4-hour TG were significantly different between AFTT 1 and AFTT 2 (p = 0.02). Otherwise, TG results for the consecutive AFTTs did not differ within subgroups.

OGTT Comparisons and Correlations

Data regarding reliability of glucose data in the OGTT are displayed in **Table 4**. The mean difference in fasting glucose level was 2.2 mg/dL (95% CI: [-1.2, 5.6]) between OGTT 1

and OGTT 2 (**Figure 4A**). The mean difference in 2-hour glucose was -7.5 mg/dL (95% CI: [-16.7, 1.7]) (**Figure 4B**), while the mean change in difference between baseline and 2-hours was -9.7 mg/dL (95% CI: [-19.4, 0.02]) from OGTT 1 to OGTT 2 (**Figure 4C**). In the total sample, no significant differences were observed in fasting, 2-hour, or change in glucose concentrations between the two OGTTs. Also, there was a significant positive correlation between the consecutive tests for fasting, 2-hour, and change in glucose concentrations.

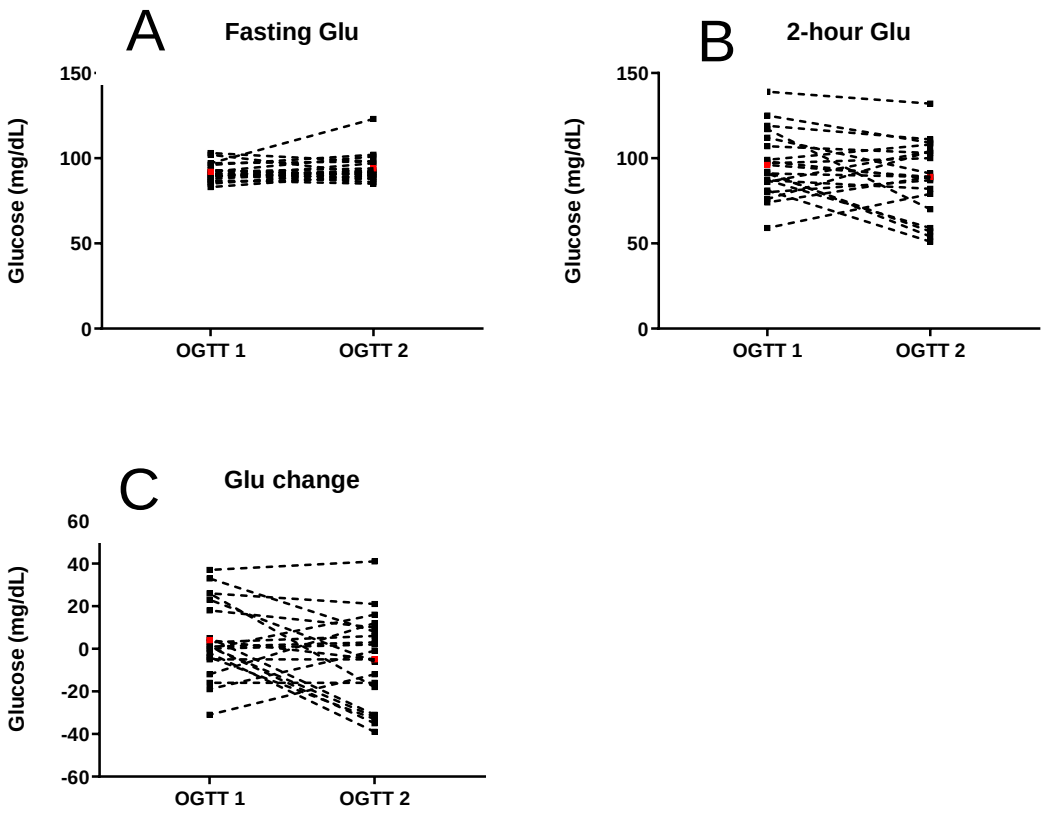
Table 4

OGTT Comparisons and Correlations

	OGTT 1	OGTT 2	*P*-value	Correlation (*r*)	*P*-value
Fasting Glu (mg/dL)					
Total	91.6	93.8	0.19	0.45	0.020*
2-hour Glu (mg/dL)					
Total	95.9	88.4	0.11	0.49	0.012*
Glu Change (mg/dL)					
Total	4.25	-5.45	0.05	0.40	0.037*

Note. Using paired t tests, we compared triglyceride levels (mg/dL) between test 1 and test 2, as well as calculated correlation using Pearson's coefficient r.
**p < 0.05 **p < 0.01 ***p < 0.001*

Figure 4. Glucose concentrations between two OGTTs, shown as fasting levels (A), 2-hour postprandial (B), and change from baseline to 2-hours (C). Lines represent individual responses to the two tests, while red points depict the average.



Reliability of the AFTT vs OGTT

Raw subject-level differences between the pre-/post- changes for both TG and glucose are displayed in **Figure 5**. Although the standard deviation of the OGTT is smaller than the AFTT, more data points fall within one standard deviation for the AFTT compared to the OGTT. The mean 4-hour within-subjects coefficient of variation (WCV) was 12.6% for the AFTT, while the mean 2-hour WCV was 10.5% for the OGTT (**Figure 6**). Thus, the WCV for both of these tests were similar, with both WCVs indicating moderate reliability for both tests.

ICC values for both 4-hour TG and TG change were > 0.7, indicating good reliability (**Table 5**). Notably, the ICC values for non-fasting TG were generally higher than fasting TG,

especially in the total sample. Interestingly, when stratified by sex, men had a considerably lower ICC value for fasting TG compared to women. When stratified by age, those in the younger group had a low ICC value for both 4-hour TG and TG change compared to the older group. Those with a higher BMI also had a lower ICC for TG change compared to the lower BMI group. The minimal differences (MD), calculated from ICC and SEM, was determined to be approximately 70 mg/dL for both 4-hour TG and TG change. The SEMs for total 4-hour TG and TG were both found to be approximately 25 mg/dL. Both CV and WCV percentages were comparable for total fasting and 4-hour TG. ICCs for glucose were lower than ICCs for TG for the total sample (**Table 6**). Minimal differences were considerably lower for glucose (approximately 35 mg/dL) compared to TG.

Figure 5. Differences between pre-/post- changes in triglycerides and glucose in response to two consecutive AFTTs and OGTTs, respectively. Points within the colored bars fall within 2 standard deviations away from the mean.

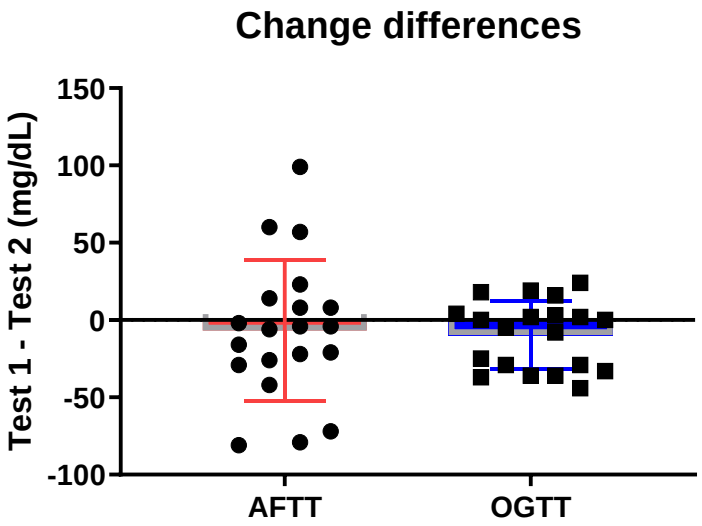


Figure 6. Within-subjects coefficient of variation (WCV) for the AFTT and OGTT. Each dot represents an individual's CV in response to the two consecutive tests. Points within the colored bars fall within 2 standard deviations away from the mean.

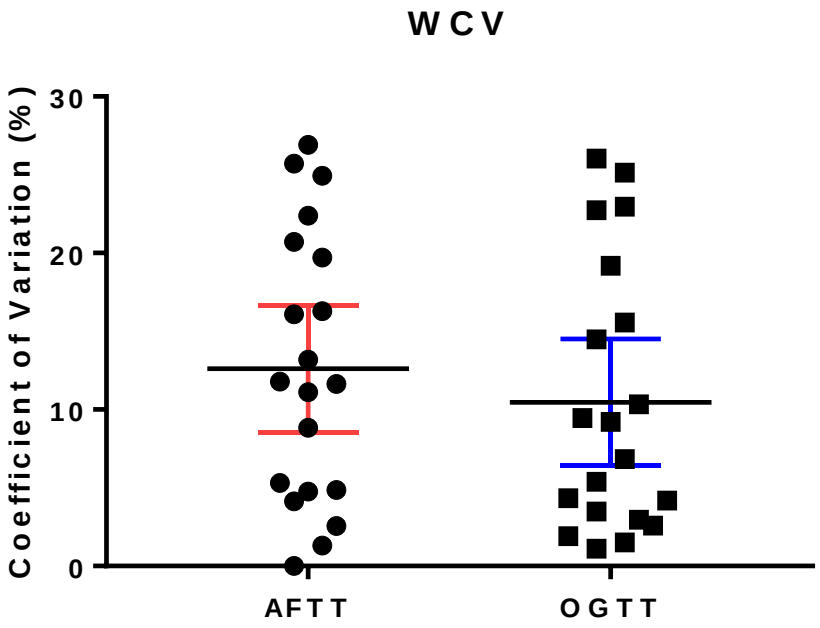


Table 5

AFTT ICCs and SEMs

	ICC	SD	SEM	MD	CV (%)	WCV (%)
Fasting TG (mg/dL)						
Total	0.684	30.9	17.4	48.1	34.4	13.2
Men	0.262	31.0	26.6	73.7	30.8	19.3
Women	0.937	27.7	7.0	19.3	35.0	7.1
Low BMI (< 22.8 kg/m²)	0.840	29.8	11.9	33.0	35.4	10.9
High BMI (> 22.8 kg/m²)	0.709	31.8	17.2	47.5	33.4	15.5
Younger (< 21.5 years)	0.823	27.4	11.5	32.0	31.2	10.5
Older (> 21.5 years)	0.819	34.7	14.8	40.9	37.9	16.0
4-hour TG (mg/dL)						
Total	0.788	54.9	25.3	70.1	39.9	12.6
Men	0.662	56.1	32.6	90.4	33.9	14.1
Women	0.897	37.6	12.1	33.4	34.3	11.1
Low BMI (< 22.8 kg/m²)	0.874	52.9	18.8	52.0	38.9	13.3
High BMI (> 22.8 kg/m²)	0.742	58.2	29.6	81.9	41.8	11.9
Younger (< 21.5 years)	0.585	34.0	21.9	60.7	28.3	13.4
Older (> 21.5 years)	0.824	66.4	27.9	77.2	42.9	11.9
TG Change (mg/dL)						
Total	0.708	47.2	25.5	70.7	N/A	N/A
Men	0.678	57.0	32.3	89.7	N/A	N/A
Women	0.588	26.5	17.0	47.1	N/A	N/A
Low BMI (< 22.8 kg/m²)	0.814	54.2	23.4	64.8	N/A	N/A
High BMI (> 22.8 kg/m²)	0.449	40.1	29.8	82.5	N/A	N/A
Younger (< 21.5 years)	0.273	34.1	29.1	80.6	N/A	N/A
Older (> 21.5 years)	0.775	53.9	25.6	70.9	N/A	N/A

Note. Intraclass correlation coefficient (ICC), standard deviation (SD), standard error of the measurement (SEM), minimal difference (MD), between-subjects coefficient of variation (CV), and within-subjects coefficient of variation (WCV). Results are further stratified by sex, BMI, and age respectively.

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Table 6

OGTT ICCs and SEMs

	ICC	SD	SEM	MD	CV (%)	WCV (%)
Fasting Glu (mg/dL)						
Total	0.578	7.0	4.5	12.5	7.5	2.8
2-hour Glu (mg/dL)						
Total	0.658	20.7	12.1	33.6	22.5	10.5
Glu Change (mg/dL)						
Total	0.562	20.1	13.3	36.9	N/A	N/A

Note. Intraclass correlation coefficient (ICC), standard deviation (SD), standard error of the measurement (SEM), minimal difference (MD), between-subjects coefficient of variation (CV), and within-subjects coefficient of variation (WCV).

FMD, Diet, and Physical Activity

FMD percentages were not statistically different between the first and last test meal sessions. The average FMD level was 8.22% and responses ranged from 0.25-33.94%. The average difference in FMD from session 1 to session 4 was 0.27%. There was no association between FMD and 4-hour TG ($r = -0.12$; $p = 0.60$), TG change ($r = -0.11$; $p = 0.64$), 2-hour glucose ($r = -0.09$; $p = 0.69$), or glucose change ($r = -0.21$; $p = 0.37$).

When exploring dietary factors and physical activity levels to assess its possible influence on test-retest variations in TG change and 4-hour TG, we observed a few significant correlations using Pearson's r. There was a significant correlation between difference in total kcals consumed and difference in 4-hour TG (p=.028, r=.50). There was also a significant correlation between differences in total saturated fat intake and 4-hour TG (p=.039, r=.47). Lastly, there was a significant correlation shown between difference in percent of unsaturated fat and difference in TG change (p=.042, r=.47). There were no significant correlations between variation in physical activity and metabolic results.

When comparing dietary and physical activity behaviors between the "low variation group" and "high variation group" using unpaired t-tests, we saw the group exhibiting more variation in 4-hour TG also exhibited significantly more variation in steps/day preceding the AFTTs. This variation in steps/day was significantly different compared to the low 4-hour TG variation group (p=.02). There were no other significant differences due to variation of dietary or physical activity factors.

Test Meal Tolerance

Our test shake consisting of coconut cream, chocolate syrup, and vegan protein powder was generally well-accepted. When asked about preferences regarding the AFTT versus the OGTT, approximately half of the participants stated they preferred the taste of the fat shake compared to the glucose drink. The thick volume and richness of the shake made it more challenging to eat compared to the liquid nature of the OGTT. Adverse effects were minimal,

with a few complaints about indigestion. One participant reported vomiting after the test meal, and another reported diarrhea. While we cannot be certain that the shake caused these effects, we speculate it may be the high fat content. The most common symptom was a feeling of fullness. Allowing participants to drink water with the shake seemed to increase palatability. Since the volume of the shake was specific to each individual's body weight, the factor of someone ingesting a larger or smaller amount than someone else was eliminated. No adverse effects were observed with the OGTT aside from a dislike for the taste.

CHAPTER V

DISCUSSION

Summary of Main Findings

The primary purpose of this study was to determine whether or not the AFTT is reliable and compare its reliability to the OGTT in a group of healthy adults. The present study is important with regard to establishing the clinical utility of postprandial TG testing, and consequently increasing early detection of cardiometabolic risk. We hypothesized that the AFTT would demonstrate similar or better reliability compared to the OGTT, a clinically accepted postprandial metabolic test. This hypothesis was supported by our results, as we observed: 1) a similar WCV between the AFTT and OGTT, demonstrating both tests had moderate reliability in our sample; and 2) ICC values for the AFTT that were generally higher than ICCs for the OGTT, indicating similar or even better reliability in the AFTT.

Reliability and Clinical Utility of the AFTT

As previously mentioned, there are very few studies studying the reliability of fat tolerance testing. In a previous study by Weiss et al. (2008) in lean and obese individuals, the authors found the 4-hour total AUC WCV to be 17.3% and 4-hour incremental AUC WCV to be 26.4%. Similarly, our average 4-hour WCV value was 12.6%, showing alike reliability. Our ICC value for 4-hour TG was 0.79, compared to an ICC for 4-hour total AUC of 0.91 and incremental AUC of 0.71 observed by Weiss and colleagues. The authors considered an ICC of greater than 0.75 as highly reliable, and thus concluded that PPL tests are highly reliable when total TG response is calculated using total AUC and much less reliable when incremental AUC is used.

With regard to ICC, our results agree that the absolute TG response (4 hour TG) is more reliable than the incremental response, as can be seen from our ICC for TG change of 0.71. It is important to note that the study by Weiss et al. (2008) included four separate OFTTs, while our study only utilized two AFTTs. Overall, our results are generally in agreement with those of Weiss et al. and demonstrate empirically-tested reliability of the AFTT despite the fact that participants were permitted to vacate the laboratory. This finding is an important contribution in addressing the reliability of the AFTT.

In addition to Weiss et al., few other studies have investigated the reliability of fat tolerance testing. A trial conducted by O'Doherty et al. (2018) assessed the repeatability of the abbreviated OFTT with and without the influence of prior aerobic exercise in healthy males. Triglyceride AUC was observed to have correlations of 0.9 and 0.42 in the control and exercise conditions, respectively. Our correlation value for 4-hour TG for the total sample was 0.66. Thus, it appears that allowing participants to leave the lab lowered the repeatability of postprandial TG results, but not to the same extent as preprandial exercise. Further, the difference in postprandial TG repeatability between our results and the control trial results of O'Doherty and colleagues may be explained by our larger sample size and inclusion of females. Our WCV value was similar to the 4-hour TG CV in another repeated-measures study examining the abbreviated OFTT in healthy males (Tentolouris et al., 2017). After completion of two tests, the CV for TG at 4 hours was 17.2% and the ICC value for 4-hour TG was 0.88; this is agreeable to the present study's findings of a 12.6% WCV value and ICC value for 4-hour TG of 0.79. Thus, we observed similar reliability compared to previous studies focused on oral fat tolerance tests administered in a laboratory.

Our results also suggest that assessment of postprandial TG is more reliable than fasting TG. In the total sample and across different subgroups, non-fasting TG ICCs were generally higher than fasting TG, supporting the finding that postprandial TG is a more reliable biomarker compared to fasting values. Since postprandial TG is also more strongly associated with CVD

risk (Freiburg et al. 2008, Boren et al., 2014), our finding further supports the use of postprandial TG in the clinical setting. Specifically, 4-hour TG values exhibited higher ICCs for the total sample and every stratified category except for women and the younger age group when compared to fasting TG. When comparing the 2 AFTTs for the total sample, the correlation coefficient was shown to be higher for 4-hour TG and TG change compared to fasting TG values. Based on our data, postprandial TG would be a more valuable and reliable biomarker in a clinical setting than fasting TG.

This study was an important follow-up to the work by Sciarrillo et al. (2019) that examined the validity of the AFTT compared to the standard PPL testing protocol. Consistent with their findings, our study revealed the AFTT to be an innovative and feasible approach to assessing PPL. Allowing participants to leave the lab for 4 hours before returning is practical, saving time, money, and several blood draws compared to the standard protocol for PPL testing. This protocol opens up the door for improved study designs in research that may have previously been too expensive or burdensome. Not only does this reduce hassle for both the researchers and the participants, the contribution of this research determining the reliability of the AFTT and comparing it to the OGTT strengthens its potential use in the clinical setting.

Much like the OGTT can help individuals with pre-diabetes acquire support to reduce their chance for full disease development, the AFTT may be able to help at-risk individuals lessen their CVD risk. If more individuals can be assessed for CVD risk through the AFTT, this may allow for appropriate lifestyle intervention or pharmacological action to take place earlier, potentially leading to more effective treatment.

Comparison of Reliability between the AFTT and OGTT

Our study brings novelty in that it is the first to compare the AFTT to another commonly used meal tolerance test: the OGTT. To our knowledge, this is the first study to compare fat and glucose tolerance test reliability. Several studies examining reliability of the OGTT used percentage of participants who fell into the same diagnostic category (normal, pre-diabetic, or

diabetic glucose value) to define reliability. For example, Ko et al. (1998) found the overall reliability of the OGTT to be 65.6% in subjects who underwent 2 tests. In other words, ~65% of the time, participants were placed in the same risk classification based on consecutive OGTT results. A similar study examining reliability in pregnant women found the OGTT reliable in 74.2% of the sample. Because the AFTT is currently not a diagnostic test (recommended cut-points still need to be established), it cannot be directly compared to the OGTT in this manner. However, we can nevertheless infer from these studies that the OGTT is by no means perfectly reliable and there is considerable individual variability, potentially resulting in misclassification of a substantial proportion of individuals.

A study investigating the reliability of the OGTT in ten healthy individuals who completed the OGTT 4 times in 4 days observed the CV to be 7.8-14.4%, indicating good reliability (Gordon et al., 2011). Our sample of twenty healthy individuals who completed the OGTT twice separated by one week showed the average CV to be 10.5%, which is similar to the findings of Gordon and colleagues. Given that our OGTT findings demonstrate similar reliability compared to previously published findings, we are well-positioned to compare the OGTT and AFTT with regard to reliability.

In the current study, there were no significant differences for fasting, 2-hour, or change in glucose between the two OGTTs – just as there were no differences in fasting, 4-hour, or change in TG for the AFTT. The correlations for the AFTT were overall higher for fasting TG, 4-hour TG, and TG change compared to fasting, 2-hour, and glucose change for the OGTT, respectively. These initial comparisons reveal that the AFTT is not grossly less reliable than the OGTT and even has stronger correlations between results from consecutive tests. In addition, the WCVs for both tests demonstrated moderate reliability, as shown by a WCV of 12.6% for the AFTT and 10.5% for the OGTT. The ICC values for total fasting, 4-hour, and TG change were also higher than ICC values for total fasting, 2-hour, and glucose change.

Overall, our data suggest similar reliability between the OGTT and AFTT. In fact, there are some indices in which the AFTT outperforms the OGTT. Because the OGTT is the gold standard for determining glucose tolerance despite its imperfect reliability, our data suggest that it would be reasonable to consider the AFTT a sufficiently reliable metabolic test for determination of the postprandial lipid tolerance.

Factors that May Modify Fat Tolerance Test Reliability

When determining the reliability of a clinical test, it is important to ascertain whether there are individual factors that may modify the reliability of the outcome. In the present study, we stratified our sample by age, sex, and BMI. There were a few noteworthy differences discovered when comparing and correlating the two AFTTs within these subgroups. In older participants and those with a greater BMI, we observed significant differences in fasting TG between the two repeated AFTTs. However, these differences were not present with regard to non-fasting TG outcomes. On the other hand, sex may affect reliability of 4-hour TG levels, as females showed a significant difference between the two tests and males did not. Because we examined the reliability of 2 tests in both males and females, the results are broadened as many previous studies have studied only males.

Although we observed moderate reliability for the AFTT, it was clearly not perfectly reliable, indicating variation in repeated tests in the same individuals over one week. This variation could be due in part to differences in sex, age, and BMI in our sample. For example, men had a considerably lower ICC value for fasting TG compared to women. Interestingly, the results indicate that the test was more reliable in the older group compared to the younger group for predicting 4-hour and TG change, as well as in those with a higher BMI compared to a lower BMI for predicting TG change.

In addition to individual factors that may modify AFTT reliability, we also assessed acute behavioral factors that may explain variation in postprandial TG results. Specifically, we sought to determine whether dietary behavior, measured via three-day food record, or daily steps,

measured with accelerometers, leading up to each AFTT were partially responsible for postprandial TG differences between the two tests. The difference in total kcals consumed and absolute saturated fat intake from test 1 to test 2 was significantly positively correlated with variability of 4-hour TG. Similarly, difference in the percent of unsaturated fat intake from test 1 to test 2 was significantly positively correlated with variability of TG change. In other words, the amount of total calories consumed and proportion of unsaturated fat intake before administration of an AFTT may influence the reliability of the PPL response. We also found greater variation in daily steps prior to the AFTT in participants that exhibited more variable 4-hour TG results. Therefore, our results indicate that both diet and activity behaviors prior to administration of a fat tolerance test likely contribute to variability in postprandial TG results.

Strengths and Limitations

There are multiple strengths in this study. First, we utilized a randomized cross-over design and controlled for several lifestyle variables such as physical activity and diet. Providing the same snack before every session also safeguards the level of fasting between trials and within participants. Our AFTT test challenge is an allergy-friendly mixed meal containing a realistic amount of calories and fat that an individual might typically consume. Additionally, allowing participants to leave the lab is an innovative approach that would increase the feasibility of fat tolerance testing for application to a clinical setting.

This study is not without limitations. One limitation is that our sample population is limited to healthy adults and does not take into account individuals with metabolic abnormalities or chronic illness. Another limitation is the inability to control for actions taken by participants when they leave the lab during the AFTT postprandial period. Although they are instructed to not eat, drink, or exercise during this time, we may be unaware of variabilities between participants. Lastly, it is important to note that our study was insufficient to address the effectiveness of the AFTT in determining CVD risk. This was likely due to our small sample size and low variation in FMD between participants, weakening our ability to determine associations.

Future Directions

Future studies should test the effectiveness of the AFTT in wider populations across the CVD spectrum. Additionally, larger sample sizes are needed to elucidate the utility of the AFTT in revealing risk compared to the OGTT. Future studies should also further investigate factors that influence variation of postprandial TG (i.e. kcals, fat intake, and activity level) to establish their significance when implementing an AFTT. The utilization of FMD as a risk factor may be better examined in a larger population with a variety of age groups. Different approaches to FMD may need to be considered, such as visceral adiposity as an alternative risk marker. The establishment of a standardized test meal is also a necessary next step in the utilization of the AFTT in a clinical setting (i.e., determining a recipe and formula that can be used by all). Finally, clinical trials integrating the AFTT into practice are needed to elucidate its utility as a clinical test for revealing risk early and prompting more timely and effective intervention.

Conclusions

This study demonstrated that the AFTT is reliable, similar to the OGTT, in a small sample size of healthy individuals. Variation in TG results are likely explained by variation in dietary and physical activity factors. The AFTT is a more feasible and convenient method than the outdated OFTT to determining post-meal lipemia, which is an important CVD risk factor. The AFTT has potential to be used in the clinical setting as a routine assessment tool. However, future clinical studies are needed to standardize the test meal, as well as establish the AFTT's true utility. If incorporated as a routine clinical test, the opportunity to prevent or implement obligatory treatment of PPL will be augmented.

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APPENDICES

Oklahoma State University Institutional Review Board

Date:	08/20/2018
Application Number:	HS-18-44
Proposal Title:	The Reproducibility and Clinical Utility of an Abbreviated Fat Tolerance Test
Principal Investigator:	Sam Emerson
Co-Investigator(s):	Nathaniel Jenkins
Faculty Adviser:	
Project Coordinator:	
Research Assistant(s):	Christina Sciarrillo, Madison Krehbiel, Nick Koemel
Processed as:	Full Board
Status Recommended by Reviewer(s): Approved	
Approval Date:	**08/20/2018**
Expiration Date:	**08/19/2019**

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

The final versions of any recruitment, consent and assent documents bearing the IRB approval stamp are available for download from IRBManager. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be approved by the IRB. Protocol modifications requiring approval may include changes to the title, PI, adviser, other research personnel, funding status or sponsor,

subject population composition or size, recruitment, inclusion/exclusion criteria, research site, research procedures and consent/assent process or forms.

2. Submit a request for continuation if the study extends beyond the approval period. This continuation must receive IRB review and approval before the research can continue.
3. Report any unanticipated and/or adverse events to the IRB Office promptly.
4. Notify the IRB office when your research project is complete or when you are no longer affiliated with Oklahoma State University.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact the IRB Office at 223 Scott Hall (phone: 405-744-3377, irb@okstate.edu).

Sincerely,

Hugh Crethar, Chair Institutional Review Board

VITA

Madison D. Dixon

Candidate for the Degree of

Master of Science

Thesis: THE RELIABILITY OF AN ABBREVIATED FAT TOLERANCE TEST: A COMPARISON TO THE ORAL GLUCOSE TOLERANCE TEST

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