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ORIGINAL RESEARCH

The Implementation and Testing of a Reliable and Valid Oral Fat Tolerance Test for Research and Clinical Purposes

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ABSTRACT

Background: Increased fat intake leads to a greater risk of atherosclerosis due to the increase in circulating triglycerides (TG), which damages the endothelial lining. An oral fat tolerance test (OFTT) is usually employed to evaluate postprandial lipid metabolism. However, there has yet to be a standardized procedure for doing an OFTT. This investigation assists in the determination of a standardized, reliable, and valid OFTT.

Methods: All participants (n=20) were free of any known cardiovascular or metabolic disease. Participants were allotted 20 minutes to complete the OFTT. Baseline and subsequent blood analyses postfeeding were collected at 1, 2, 3, and 4 hours. One of the three OFTT loads of varying fat concentrations was administered to each participant in a randomized crossover design, containing fat loads of 150 g, 100 g, and 50 g of fat. A single-measure consistency intraclass correlation coefficient was used to determine significance ($r>0.75$).

Results: The test-retest reliability of the OFTT loads (150 g, 100 g, and 50 g) were all significant ($p<0.001$), with ICC at 0.745, 0.923, and 0.715, respectively. Face Validity was confirmed upon repeat analysis.

Conclusions: The 100 g OFTT load was the most reliable and valid measure for observing TG elevation. It is proposed that the 100 g load would be a reliable tool for further research investigation and eventual use for clinical purposes.

KEYWORDS: Triglycerides, Plasma triglyceride metabolism, Diseases/atherosclerosis, Metabolic studies, Lipoproteins/metabolism.

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INTRODUCTION

Cardiovascular disease is the leading cause of premature death in the United States [1]. Atherosclerosis is one of the primary contributors to mortality and morbidity and has an etiology that includes chronic prolonged elevation of circulating blood lipids leading to vascular damage [1]. Chronic elevation of blood lipids is exacerbated by frequent high dietary fat intake, commonly observed in the Western diet. Triglycerides (TGs) are small fatty acid particles that facilitate the transportation of fatty acid substrates throughout the body. In a disordered condition, the circulating TGs accumulate within the bloodstream and damage the endothelial lining, leading to a hardening of the vessels, causing narrowing and stiffness and increasing the risk of developing coronary artery disease [2].

Traditionally, fasting TGs have been the primary focus when evaluating a patient's blood lipid panel. However, it has become clear in recent years that the active metabolism of TGs is more indicative of the development of a chronic disease condition [3, 4]. Fasting TG values do not correctly identify those at risk for disordered postprandial lipid metabolism, as their baseline value is still below the established 150 mg/dL marker for intervention [3, 4]. Persons with fasting TG values between 89 and 180 mg/dL are at an increased risk for disordered postprandial lipid metabolism, having normal fasting values and

accompanying exaggerated elevation following a high-fat meal [4]. It would be beneficial for clinicians to administer a standardized oral fat tolerance test (OFTT) for persons with fasting TG values between 89 and 180 mg/dL to reveal any metabolic abnormalities and potentially highlight the onset of a diseased process for early intervention. The administration and evaluation of an OFTT would identify those with an increased risk for developing chronic dyslipidemia and requiring prompt management to include pharmaceutical intervention.

The problem lies in the lack of a universally recognized, standardized OFTT. Although attempts have been made to create a repeatable OFTT, these attempts rarely test for reliability and lack clarity in the development of high-fat meals (5–7). These considerations make it impossible to replicate an OFTT. Additionally, these OFTTs often contain various substrates that metabolize differently, such as fat sources of varied saturations (saturated, monounsaturated, and polyunsaturated) and meals high in carbohydrates [8].

The OFTT fat load variations in the literature range from 16.32 g of fat to as much as 140 g of fat, with multiple values therein clearly indicating the necessity for a standardized OFTT [5, 12–17]. The present investigation seeks to determine a reliable OFTT with a fat concentration eliciting an ideal metabolic curve over four hours. An ideal OFTT displays a rapid peak elevation of TG, followed by a return to baseline at hours two or three; it has been determined that four hours is a valid time frame to observe the metabolic effects of an OFTT among TG in healthy individuals [18]. One such investigation analyzed data from an eight-hour observation period and found that 89–96% of the variance was accounted for within four hours [18]. Additional studies into fatty acid metabolism examined varying fatty acid compositions (saturated, monounsaturated, and polyunsaturated) and their respective absorption rate. Findings revealed that the metabolic effect of fat type was absent within four hours. A time effect was present at eight hours, with polyunsaturated fat displaying an increased clearance over saturated fat (SMD -2.28, 95% CI) [9]. Therefore, a four-hour observation period is ideal when examining the postprandial effects of an OFTT, as it limits the variability brought about via fat substrate metabolism.

Given the current equivocal findings in the literature and the lack of consistency in which an OFTT is created and administered, there exists a clear need for further investigation into the development of a standardized and reliable lipid challenge test. Therefore, this investigation aimed to examine the reliability of three OFTT loads (50, 100, 150 g) on TG metabolism. It was hypothesized that all three concentrations of an OFTT would be reliable (r>0.70), with one OFTT eliciting greater reliability.

MATERIALS AND METHODS

These methods aim to determine the reliability of three high-fat loads in a test-retest design. Volunteer college-aged males and females, totaling 10 males and 10 females, were recruited to participate in this investigation (n=20) (Table 1). Recruiting efforts included verbal announcements and posted flyers. A similar investigation by Cohen et al. was utilized to determine statistical power *a priori* [12]. A power analysis of the aforementioned investigation determined that a sample size of six (n=6) is a sufficient population, with an effect size of f=0.59 (80%, α=0.05) (G*Power v.3.1.9.2, Bayern, Germany) [12]. The present investigation observed a power of 70% (f=0.501, α=0.05, n=20) among the three OFTT concentrations.

Table 1: ICC demographics (n=20).

	50g		100g		150g	
	Mean	SD	Mean	SD	Mean	SD
Age	27	6	26	4	28	3
Height (cm)	165	17	170	8	171	7
Mass (kg)	80	26	66	10	78	20
Male	3		4		3	
Female	3		3		4	

DISCUSSION

Participants completed a health history questionnaire and were classified as low-risk based on the American College of Sports Medicine (ACSM) algorithm [19]. All participants were free of any known cardiovascular disease, metabolic disorder, implantable device, liver or gallbladder complications (such as surgery, cirrhosis, or fatty liver) and were normolipidemic (fasting TG>150 mg/dL). All participants were nonsmokers or had ceased smoking for longer than six months. In total, 23 people were assessed for eligibility. Of these, three were excluded from participation: two due to preexisting conditions and one who declined to participate following the informed consent process. Before initiation of the protocol, an informed consent form was completed notifying participants of any potential risks, benefits, and confidentiality concerns. This protocol was approved by the Institutional Review Board for Human Subjects Research (#1157372-8). After completing the health history questionnaire and informed consent, height was recorded using a stadiometer, and weight and body compositions were estimated through the SECA bioelectrical impedance (BIA) device (Hamburg, Germany). The SECA BIA is a valid and reliable method of evaluating fat and lean mass in an investigation employing magnetic resonance imaging (MRI) as the criterion measure [20]. Participants were advised to keep a food journal documenting all food and beverage consumption 24 hours before data collection. This food journal would serve as a reference for individual meal replication before the ensuing trials. Participants were required to adhere to an overnight fast of 12–16 hours before data collection. Alcohol and caffeine consumption were prohibited 24 hours before data collection. The exercise was also prohibited 24 hours before data collection (defined as any repetitive activity elevating the heart rate over resting for greater than 20 minutes).

BLOOD SAMPLING

Capillary blood plasma TG analysis was undertaken immediately before the consumption of an OFTT. Participants were allotted 20 minutes to consume the OFTT. The time of completion of the OFTT was recorded, and subsequent blood analysis was collected at 1, 2, 3, and 4 hours postconsumption. Circulating blood plasma TG was analyzed using the CardioChek® point-of-care *in vitro* diagnostic system (Polymer Technology Systems, Inc., Indianapolis, IN, USA). This point-of-care system has been determined to be a valid and reliable tool for clinical application [21]. The four-hour observation time is a reliable time constraint for lipid analysis among healthy participants [1,18].

OFTT

Following fasting, blood analysis, one of the three OFTT fat concentrations was administered using a randomized crossover design. The participant's specific OFTT fat concentration was readministered upon subsequent trial. Water was given ad libitum on the first trial and was measured to replicate fluid consumption during subsequent trials. The three fat concentrations used were 49.5 g, 99 g, and 148.5 g, representing a small, medium, and high-fat load. For clarity, these concentrations were rounded to 50 g, 100 g, and 150 g loads and are referenced in this article as such. Carbohydrate content among the 50 g, 100 g, and 150 g loads was 19.13 g, 38.25 g, and 57.38 g, respectively (Table 2). These fat concentrations were selected to replicate similar OFTT concentrations in the literature [6, 13, 16, 22].

The consumption of an OFTT places the participant at or above the recommended daily allotment of fats (20–30% of dietary intake). Although the risk of an adverse effect was low, participants were recommended to limit fat intake for the testing day. The OFTT was composed of commercially available products and is easily replicated. Ensure® Plus (Abbott©, Abbott Park, IL) was used as a flavor base (chocolate) for the OFTT. Most fat originates from the heavy dose of Benicalorie® (Nestle©, Vevey, Switzerland) high-fat food additive. Both the Benicalorie® and Ensure® Plus should be shaken vigorously before mixing. Caution was taken to ensure all remaining remnants of the OFTT were removed from the measuring cup following pouring; Benicalorie® consists of primarily sunflower oil that tends to harden. Therefore, the sealed Benicalorie® cup was placed on a heating pad for approximately two minutes before mixing to allow for liquefaction. The components of the OFTT were poured into a mixing pitcher and mixed thoroughly with a silicone spatula. The individual portions were measured using a graduated measuring cup and poured into individual disposable cups.

Table 2: OFTT substrate breakdown, 11:3 ratio (Benicalorie® to Ensure®).

	50g	100g	150g
Milliliters	149	299	448
Fat (g)	50	99	149
Saturated fat (g)	5	9	14
Carbohydrates (g)	19	38	57
Sugar (g)	17	33	50
Protein (g)	15	29	44
Calories	585kcal	1,170kcal	1,755kcal

The OFTTs were volume-matched, with the small dose receiving 300 ml of water, the medium dose receiving 150 ml of water, and the large dose receiving no additional water. Water measurement was accomplished via a graduated measuring cup and poured into the respective disposable cups. Participants were randomly assigned an OFTT, in a systematic design, following the pattern of (150 g, 100 g, and 50 g). Following a minimum seven-day washout period, these participants returned and ingested an additional OFTT, of the same fat concentration as previously consumed, in a test-retest manner.

All three OFTTs were received well by most of the subjects. No adverse effects of digestion were observed (e.g., vomiting, diarrhea, and gastrointestinal discomfort). A common complaint was that the large (150 g) dose, with the greatest volume of liquid, was challenging to consume. All participants ingested the OFTT within the allotted 20-minute time frame. Two participants had a difficult time ingesting the OFTT within the allotted 20 min time frame, citing feelings of fullness. Nausea and the feeling of fullness were noted as limiting factors upon consumption. Conversely, the meal was enjoyed by some (n=3), regardless of volume, who verbally reported that they "looked forward to it" and "would eat it again."

STATISTICAL ANALYSIS

Intertest reliability was analyzed using a single-measure consistency intraclass correlation coefficient, ICC (3.1), with significance accepted at r>0.75 [23]. Additional reliability measures include the highest observed 95% confidence interval and the coefficient of variation (CV). Face validity will be confirmed by examining the metabolic response of mean TG following feeding among each OFTT concentration.

RESULTS

Reliability: When considering the test-retest reliability of the OFTT loads, the 150 g, 100 g, and 50 g were all significant (p<0.001), with ICC at 0.745, 0.924, and 0.715, respectively (Table 3). The 100 g load had a fourfold greater F-Value than that of the 150 g or 50 g loads. The 100 g load had the narrowest lower bound to upper bound margin.

Table 3: Single-measure intraclass correlation for each OFTT concentration (CV: coefficient of variation), revealing the 100 g OFTT as highly reliable with an ICC of 0.924.

	Single Measures Intraclass Correlation						
	N	CV (%)	ICC	Lower Bound	Upper Bound	F-Value	Sig.
150g	14	24.3	0.745	0.551	0.863	6.837	p<0.001
100g	14	16.7	0.924	0.855	0.961	25.332	p<0.001
50g	12	14.6	0.715	0.483	0.854	6.029	p<0.001

Face Validity: Findings showed a mean TG metabolism upper limit of μ = 202.6mg/dL at the two-hour observation among participants ingesting the 150 g load, with the 100 g and 50 g loads averaging 187 mg/dL and 150 mg/dL, respectively, for the same time point (Figure a). All three OFTT loads exhibited a rapid elevation from the fasted baseline, followed by a gradual return to baseline.

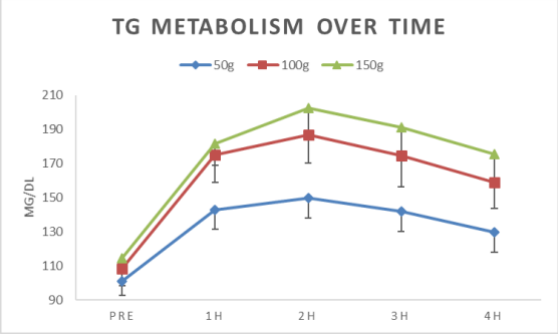

Figure a: Mean TG metabolism (mg/dL) over time among the 50 g, 100 g, and 150 g OFTT loads. All three OFTTs experienced the expected metabolic curve following an OFTT.

DISCUSSION

Reliability is the agreement of consistent results between different observations [24]. Results from the current study provide evidence supporting the 100 g OFTT concentration load as highly reliable with an ICC of 0.924 (Table 3) and a CV at 16.7%. In evaluating the CV threshold in clinical testing, Reed et al. determined that a

CV above 20% should not be considered reliable [25]. The narrow lower and upper bounds are noteworthy as it can be assumed that the true value of the parameter lies within [26].

Validity is defined as the extent to which the testing procedures apply to the desired outcome measurement [24]. The present investigation satisfies the parameters of construct validity as outlined via the homogeneity parameters, in which all persons were tested and retested exclusively for TG. Additionally, the progression of TG metabolism over time was consistent with all expectations, dramatically increasing circulated TG, followed by a gradual decline as these lipids are metabolized. Compared to the two concentrations examined, we can confidently report that the 100 g OFTT is the most reliable and valid for future research investigations. To the best of our knowledge, this is the first investigation to examine the reliability and validity of a novel OFTT for clinical and research purposes.

The current investigation aims to construct an OFTT that is both valid and reliable. A dearth of literature is focused on the test-retest reliability of plasma TG metabolism following the administration of differing OFTT loads. This lack of reliability analysis existing in the literature is prevalent despite the use of multiple OFTT configurations. Previously established guidelines of single-measure consistency intraclass correlation coefficients, ICC (3.1), categorize values as less than 0.5 being poor, between 0.5 and 0.75 as having moderate reliability, between 0.75 and 0.90 as having good reliability, and values greater than 0.9 as reflective of excellent reliability [23]. Moderate reliability was observed in the 150 g OFTT load measurements when considering both the ICC and the 95% confidence intervals. The 150 g ICC was 0.745 with a 95% confidence interval from 0.551 to 0.863 ($F=6.837$, $p<0.001$). Excellent reliability was found for the 100 g OFTT measurements when considering both the ICC and the 95% confidence intervals. The average measure ICC was 0.924 with a 95% confidence interval from 0.845 to 0.963 ($F=25.059$, $p<0.001$). Moderate reliability was seen for the 50 g OFTT measurements with an ICC of 0.715 and a 95% confidence interval (0.483 to 0.854, $F=6.029$, $p<0.001$). Therefore, these data identify the 100 g OFTT load as having the most desirable reliability effects of all the high-fat loads tested in the current study. This 100 g concentration is similar to other volumes seen in the literature, including an expert panel conclusion that 75–80g is an ideal concentration [27]. Further testing needs to be done on a larger sample size to determine precision measures.

A common practice when evaluating lipid values is to draw and analyze fasted blood. More recent evidence points toward administering an OFTT to evaluate active TG metabolism. These tests would aid in diagnosing those with disordered lipid metabolism and hidden postprandial lipemia [3,27]. Postprandial TG values less than 220 mg/dL over a four-hour observation period are ideal [27]. Persons with fasting TG values between 89-180mg/dL potentially have hidden postprandial lipemia [3,27]. These persons are at a greater risk of developing early atherosclerosis [27]. As such, it would be beneficial for those patients with elevated fasting values to undergo a valid, reliable, and standardized OFTT to evaluate active TG metabolism. The tested 100 g concentration is a reliable and valid OFTT that can be used for the aforementioned application. Early intervention of disordered TG metabolism should be initiated considering a combination of exercise, dietary restrictions, statins, fibrates, and nicotinic acid [27].

Heavy whipping cream (HWC) is a common ingredient in the development and implementation of an OFTT [14,17,28–31]. The use of commercially available HWC in research and clinical endeavors is not without limitations. The first and primary limitation would be the macronutrient concentration of HWC. It has long been accepted that long-term heavy doses of saturated fat are linked to an increased risk for CVD [2]. Another focus of the present investigation was to create an OFTT that minimizes the volume of saturated fats, supplanting it with healthier fats, such as polyunsaturated fat (PUFA) and monounsaturated fat (MUFA). There exist time discrepancies regarding the metabolism of these three fat substrates. PUFA and MUFA break down more rapidly than saturated fats; however, this difference in breakdown efficiency does not present itself until eight hours postprandially. Therefore, breaking down the different fat substrates should not confound data collection under an eight-hour observation window [9]. The tested OFTT, having approximately 4% saturated fat and roughly 43% of PUFA and MUFA, would be a healthier option for patients or participants (Table 4). There also exists potential for confounding results based on the distribution of fatty acid isoforms. There exists evidence to support that the ingestion of PUFA and MUFA, concurrent with saturated fat, attenuates the elevation in TG [10].

Table 4: Comparison of macronutrients between proposed 100g OFTT and heavy whipping cream (HWC) equivalent; note the disparities in saturated fat.

	100g OFTT	Low-fat HWC	High-fat HWC
Volume (ml)	299	325	244
Fat (g)	99	99	99
Saturated Fat (g)	9	66	58
Carbohydrates (g)	38	22	17
Protein (g)	29	0	0
Calories	1170kcal	990kcal	990kcal

As insulin activation from glucose also stimulates the activation of lipoprotein lipase (LPL), a primary motivator of lipid metabolism, it would appear imperative that carbohydrate substrates be limited in an OFTT [2]. Additionally, it is known that not all fats metabolize the same, and this variation exists across saturated, monounsaturated, and polyunsaturated fats, with saturated fat requiring the most time to metabolize [9,10]. One attempt at standardizing an OFTT developed 200 ml of a liquid containing equal parts fat and carbohydrates at 50g [5]. This OFTT was administered to diabetic and nondiabetic individuals aged 35–65. This use of an equal ratio of fats and carbohydrates is not ideal due to the competing effects on metabolism. In addition, the stimulating effects of insulin on LPL would aid in fat metabolism [1]. Furthermore, using an OFTT on diabetic persons is not recommended due to the commonality of impaired lipid values typically seen among this population [2,5,11].

Hence, it is advisable to limit carbohydrates (CHO) when developing an OFTT since a 1:1 ratio or greater of CHO creates competition for enzymatic activity and can convolute the observation of lipid metabolism [24]. The

tested OFTT contains approximately 23% CHO and 60% total fat. This fat-to-CHO ratio is much less than that in comparative investigations in which an OFTT was employed. This low ratio should further isolate the lipid metabolism effects following the consumption of an OFTT. In the case of protein, it does not appear to interfere with lipid metabolism [32]. As such, a greater ratio of protein (over that of CHO and saturated fats) is encouraged when developing an OFTT. The proposed 100 g OFTT consists of roughly 17% protein, which is favorable compared to the HWC base containing a negligible protein volume. The protein concentration in this study is superior to what is commonly observed within commercially available HWC, which ranges from 0 to 1g of protein per tablespoon. Consequently, the implementation of the proposed OFTT retains further health benefits over OFTT varieties holding to a base of HWC, which is high in saturated fat.

Potential limitations of the present investigation include the lack of pretest meal standardization. To minimize this potential effect, participants kept individual food journals. Although participants gave verbal confirmation of meal replication the day before testing, some variation may exist. Additionally, participants were advised to abstain from alcohol and vigorous exercise the day before data collection. These lifestyle interventions could not be tracked and validated by any member of the research team. Additionally, it is unknown how this valid and reliable 100 g OFTT will respond to populations outside the healthy, college-aged sample.

CONCLUSION

The current investigation provides evidence that the 100 g OFTT load is a reliable and valid measure for observing TG elevation over time. The composition of a specific ratio of Benicalorie® and Ensure® Plus totaling 99 g of fat, 38.25 g of CHO, and 29 g of protein in the 100 g OFTT was appropriate compared to the compositions in the 50 g and 150 g OFTT loads. Further, the 100 g load would be a reliable tool for research and clinical purposes. Benicalorie® and Ensure® Plus are commonly administered in clinics and hospitals and consist of standardized ingredients. The use of these commercially available products is encouraged as they are easily repeatable and converse with other OFTTs consisting of an unspecified combination of ingredients. These practices will limit error potential and lend to a more accurate representation of lipid metabolism over time. Future investigations should consider more rigorous diet and exercise constraints, such as standardized meals on the day preceding the administration of an OFTT. This is the only investigation to our knowledge examining the reliability and validity of an OFTT. Future investigations should employ a valid and reliable OFTT, such as the one presented herein, to limit the potential for Type-2 error.

ABBREVIATIONS

OFTT	Oral fat tolerance test
ACSM	American College of Sports Medicine
BIA	Bioelectrical impedance
CHO	Carbohydrate
CV	Coefficient of variation
ICC	Intraclass correlation coefficient.

DECLARATIONS

Ethics approval and consent to participate: This protocol, methodology, and informed consent had been approved by the Institutional Review Board for Human Subjects Research (#1157372-8) and were in accordance with the Declaration of Helsinki.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The author(s) declare(s) that they have no competing interests.

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