The Development of a Standardized Oral Fat Tolerance Test (OFTT) for Research and Clinical Purposes

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RESEARCH

Please cite this paper as: Bodell NG, Navalta JW, Bungum T, Kawi J. The development of a standardized oral fat tolerance test (oftt) for research and clinical purposes. J. Food Nutr. Sci. [2026] 7(1): 1-12.

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ABSTRACT

Cardiovascular disease is the leading cause of death and can be accelerated with an unhealthy lifestyle. A diet high in fat and sugar yields chronic elevated triglycerides (TG) that cause vascular damage. An oral fat tolerance test (OFTT) is a common tool employed by researchers and clinicians to observe active lipid metabolism. There is currently no agreement in the literature upon a reliable OFTT and researchers have used a variety of high-fat meals to stimulate TG elevation. To compare postprandial TG responses to three OFTT fat loads (50g, 100g, 150g) in healthy adults. **Methods:** Data were analyzed through a three (condition: 50, 100, 150), by five (time: pre, 1h, 2h, 3h, 4h) repeated measures ANOVA. **Results:** Significantly lower plasma TG were observed in the 50g OFTT with no difference seen among the 100g and 150g loads. No differences with respect to TG were observed between the 100g and 150g loads ($p>0.05$). Each test found significantly increased plasma TG above baseline at the 1h, 2h, 3h, and 4h time points ($p<0.001$). **Conclusion:** The 150g load is unnecessarily high, therefore the 100g OFTT load is recommended as a reliable tool for assessing active TG metabolism for research and clinical purposes. This OFTT contains a higher proportion of polyunsaturated and monounsaturated fatty acids and a lower proportion of saturated fat relative to OFTTs based primarily on heavy whipping cream.

Keywords: Triglycerides, plasma triglyceride metabolism, diseases, atherosclerosis, metabolic studies, lipoproteins, metabolism.

ABBREVIATIONS:

OFTT - Oral Fat Tolerance Test

TG – Triglycerides

ACSM - American College of Sports Medicine

BIA - Bioelectrical Impedance

CHO - Carbohydrate

CV - Coefficient of Variation

ICC - Intraclass Correlation Coefficient

INTRODUCTION

Cardiovascular disease is the leading cause of premature death in the United States [1]. Primary

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contributors to this effect are vascular diseases, such as atherosclerosis. Prolonged elevation of circulating blood lipids is known to lead to vascular damage [2,3]. Chronic elevation of blood lipids is exacerbated by frequent high dietary fat intake, which is commonly observed in the Western diet. A primary mover of lipids is small fatty acid particles called triglycerides (TG). TG allows for the transport of fatty acid substrates throughout the body. The circulating triglycerides can accumulate within the bloodstream and damage the endothelial lining. This damage leads to a hardening of the vessels, which causes narrowing and stiffness, the hallmarks of coronary artery disease [4].

Traditionally, fasting TG has been the primary focus when evaluating a patient's blood lipid panel. However, it has become evident in recent years that the active metabolism of TG is more indicative of the development of chronic disease conditions [5-8]. Fasting TG values may not correctly identify those at risk for disordered postprandial lipid metabolism as this baseline value could still be below the established 150mg/dL which is a historic marker for intervention [7,8]. Disordered postprandial lipid metabolism is defined as having normal fasted values with an exaggerated elevation following a high-fat meal. Persons with fasting TG values between 89-180mg/dL are at an increased risk for disordered postprandial lipid metabolism [8]. Therefore, it would be beneficial for clinicians to administer a standardized oral fat tolerance test (OFTT) for people whose fasting TG values are in that range to reveal the early stages of disease progression. This would identify those who may be at an increased risk for developing chronic dyslipidemia and proactively manage them with nonpharmacological and pharmaceutical treatments.

The OFTT-fat load variations in the literature range from 16.32g of fat to as much as 140g [9-15]. This clearly indicates the necessity for a standardized OFTT. The present investigation seeks to determine an OFTT with a fat concentration eliciting an ideal metabolic curve over four hours. An ideal OFTT displays a peak elevation of TG followed by a return towards baseline at hours two and three. It has been shown that four hours is sufficient time to observe an effect on TG among healthy individuals [16]. One such investigation analyzed data from an 8-hour observation period and found that 89-96% of variance is accounted within four hours [16]. Further investigation into fatty acid metabolism observed varying fatty acid composition (saturated, monounsaturated, and polyunsaturated) and the respective rate of absorption. The findings revealed that the metabolic effect of fat type was stable within a four-hour time period, but not when extended over 8 hours, with polyunsaturated fat displaying an increased clearance over saturated fat [17]. Therefore, a four-hour observation period was deemed to be ideal when examining postprandial effects of an OFTT, as this time frame limits the variability which may be brought on via fat substrate metabolism.

In summary, there is a clear lack of consistency in which an OFTT is developed and administered in research studies, highlighting a need for further investigation into the development of a standardized lipid challenge test[18].The effects of lipid metabolism should be examined utilizing an unambiguous OFTT; to avoid errors arising from inappropriate generalization of previous findings. Therefore, the purpose of this investigation was to examine the effect of three OFTT loads (50, 100, 150g) on active TG metabolism. We hypothesized that one OFTT would present itself as being ideal for research and clinical usage within a 4-hour observation, displaying the greatest magnitude of change at the 3rd and 4th hour.

METHODOLOGY

Participants

A purposeful sample of college aged males and females were recruited from a university in a western part of the United States and surrounding communities. This study employed a randomized, counterbalanced crossover intervention design. Reporting was guided by established methodological standards for crossover trials where applicable. Inclusion criteria were between 18-39 years of age who are stratified as "low" or "moderate" risk according to the American College of Sports Medicine algorithm. Exclusion criteria: stratified as "high" risk, have an

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implantable device (such as a Pacemaker), or have orthopedic, cardiovascular, respiratory, or metabolic conditions. There were 15 males and 15 females who completed this investigation (Table 1). A total of 32 participants were initially recruited. Data from the two participants who failed to complete all the required trials were not analyzed. One participant withdrew from the investigation due to personal circumstances and the other participant dropped because of unrelated health concerns.

Participants completed a health history questionnaire and were classified as low risk as outlined by the 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 non-smokers or had ceased smoking for longer than six months. Prior to initiation of the test protocol, an informed consent form was completed notifying participants as to any potential risks, benefits, and confidentiality concerns. It was explicitly written that participation was voluntary and that participants could drop out for any reason at their discretion. This protocol was approved by the Institutional Review Board for Human Subjects Research at the University of Nevada, Las Vegas (#1157372-8).

Following completion of the health history questionnaire and informed consent, height was recorded using a stadiometer. Weight and body composition were estimated through the SECA mBCA 515 bioelectrical impedance (BIA) devices (Hamburg, Germany). The SECA BIA has been shown to be 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 on the day prior to data collection. This journal served as a reference for individual meal replication prior to subsequent trials; however, meals were not standardized. As such, residual dietary variability may have contributed to within-subject variation in postprandial triglyceride responses and is acknowledged as a limitation of the present study. Participants were required to adhere to an overnight fast of 12-16 hours prior to data collection. Additionally, exercise was prohibited on the day prior to data collection (defined as any repetitive activity elevating the heart rate over resting for greater than 20 minutes). Alcohol consumption was also prohibited on the day prior to and on the day of data collection. Participants were also advised to abstain from caffeine intake on the day of OFTT administration.

Blood Sampling

Capillary blood plasma TG analysis was undertaken prior to the consumption (baseline) of an OFTT. The time of completion of the OFTT was recorded and subsequent blood analysis was collected at 1, 2, 3, and 4-hours post-consumption. 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 demonstrated acceptable validity for clinical screening; however, capillary triglyceride assessment may exhibit greater variability than venous laboratory assays. This methodological choice may have reduced sensitivity for detecting subtle between-condition differences and is acknowledged as a limitation [21]. The four-hour observation time has been shown to be a reliable time constraint for lipid analysis among healthy participants [16,22].

OFTT

Following fasting blood analysis, one of three OFTT of varying fat concentrations were administered in randomized-crossover design. The OFTT employed has previously been tested for reliability and validity [23]. Participants were given 20 minutes to consume the OFTT. 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 49.5g, 99g, and 148.5g and represented a small, medium, and high fat load. For clarity and ease of reading, these concentrations are referred to as 50g, 100g and 150g loads throughout this investigation. Carbohydrates concentrations among the 50g,

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100g, and 150g loads were 19.13g, 38.25g, and 57.38g respectively (Table 2). These fat concentrations were selected to replicate similar OFTT concentrations seen among the literature [10,13,24,25]. Previous investigations have employed concentrations ranging from 16g to 140g of fat [12,15]. 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 adverse effects was low it was recommended that participants limit high-fat intake for the duration of 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. The majority of fat originates from the heavy dose of Benicalorie® (Nestle©, Vevey, Switzerland) high-fat food additive. 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. Caution was taken to ensure all remaining remnants of the OFTT were removed from the measuring cup following pouring. As the Benicalorie® consists of primarily sunflower oil it tends to harden. Therefore, the sealed Benicalorie® cup was placed on a heating pad for approximately two minutes prior to mixing to allow it to liquefy. Additionally, the Benicalorie® and Ensure® Plus was shaken prior to mixing.

The OFTTs were volume matched, with the small dose receiving 300ml of water, the medium dose receiving 150ml of water, and the large dose receiving no additional water. Water measurement was accomplished via graduated measuring cup and poured into the respective disposable cups. All participants ingested three OFTT in a counter balanced order corresponding to three different fat concentrations, separated by seven days. The OFTTs were matched by dilution with water; however, absolute fluid volumes differed across conditions. Variations in fluid volume may have influenced gastric emptying and postprandial triglyceride kinetics independently of fat dose and are therefore acknowledged as a limitation.

Statistical Analysis

To determine the effect of fitness on OFTT concentrations over time, data were analyzed through a three (condition: 50, 100, 150), by five (time: pre, 1h, 2h, 3h, 4h) repeated measures ANOVA, with an alpha level of $p \leq 0.05$. The assumption of sphericity was examined using Mauchly's test of sphericity. If sphericity was violated ($p \geq 0.05$), the more conservative F-test of Huynh-Feldt was adopted when determining the significance level. If a significant interaction was present pair wise comparisons were examined using one-way ANOVA at each time point between each concentration. If a significant interaction was present, exploratory pair wise comparisons were examined at each time point. This approach increases the potential for Type I error and was used descriptively rather than inferentially. Future investigations should consider mixed-effects modelling to more robustly account for repeated measures and within-subject variance. These statistical analyses were modelled from similar investigations examining the effects of an OFTT and TG response [26,27,28,29,30,31,32,33,34,35]. A similar investigation by Cohen et al. was utilized to determine statistical power a priori [15]. A power analysis of the aforementioned investigation determined that a sample n=6 would be sufficient to observe an effect, if an effect is present (80%, $\alpha=0.05$); with an effect size of f=0.59 (G*Power v.3.1.9.2, Bayern, Germany) [15]. The present investigation observed a power of 70% (f=0.501, $\alpha=0.05$, n=30) between the three OFTT concentrations. The present investigation observed an achieved power of approximately 70%, indicating that the study may have been underpowered to detect small between-condition differences, particularly between the 100g and 150g OFTT loads. Null findings between these conditions should therefore be interpreted with caution. A partial eta squared values of approximately .01, .06, and .14 indicate small, medium, and large effects, respectively [36].

RESULTS

OFTT Metabolism

The 50g, 100g, and 150g OFTT load TG revealed a significant interaction with time (F=1.508, p=0.033,

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ηp2=.075). Significantly lower plasma TG concentrations were observed in the 50g load compared to both the 100g and 150g loads at time points 1h, 2h, 3h, and 4h postprandially (p=0.001, p=0.003; p=0.01, p<0.001; p=0.004, p=0.002; p=0.003, p=0.001, respectfully) (Figure 1). No differences with respect to TG were observed between the 100g and 150g loads (p>0.05). Each test found significantly increased plasma TG above baseline at the 1h, 2h, 3h, and 4h timepoints (p<0.001).

OFTT Tolerance

Overall, the OFTT was generally tolerated; however, tolerance outcomes were assessed informally without validated gastrointestinal symptom scales. These observations should therefore be interpreted descriptively rather than quantitatively. The primary contribution of this investigation is methodological rather than mechanistic, providing empirical guidance for OFTT implementation while highlighting areas requiring further quantitative refinement. No adverse effects related to digestion were observed (e.g. vomiting, diarrhea, gastrointestinal discomfort). A common complaint was that the large (150g) dose was too great. Few participants had a difficult time ingesting it within the allotted 20min time frame. Nausea and the feeling of fullness were cited as inhibitory factors. Conversely, the meal was enjoyed by some who verbally reported that they “looked forward to it,” and “would eat it again.” The participants were all able to consume their respective OFTT concentrations with minimal discomfort. Some minor discomfort was reported among the high-fat load (150g OFTT) and manifested in the form of satiety or over-fullness.

DISCUSSION

OFTT Concentration Comparison

This investigation sought to identify a pragmatic OFTT fat load that produces a robust postprandial triglyceride response while remaining feasible and well-tolerated within a four-hour testing window. These data indicate that the 100g OFTT produces triglyceride responses comparable to the 150g load, while eliciting significantly greater responses than the 50g load within a four-hour observation period (F=1.508, p=0.033). As predicted, the 50g OFTT appears to be too low of a lipid challenge. This was displayed in participants that had consistently favorable TG levels at all concentrations. Among these participants TG levels remained below a 3-digit mg/dL value. Individual participant plots are provided for illustrative purposes only and do not reflect group-level inference (Figure 2). Conversely, it is likely the 150g OFTT was too great a challenge to observe the metabolism of TG within the established 4-hour observation period (Figure 3). Among those participants with consistently unfavourable lipid values (having 3-digit values at baseline), this extremely high lipid challenge was too great to observe the desired trend toward baseline at the 3 to 4-hour time point [37]. When considering the entire sample, there was no significant difference (p=0.676) in TG metabolism between the 100g and 150g OFTT load concentrations. At 1h there was a 14% difference between 100g and 150g OFTT: 14% at 2h, 13% at 3h, and 16% at 4h. Based on comparable triglyceride responses between the 100g and 150g loads and greater participant burden associated with the highest fat dose, the 100g OFTT represents a practical and sufficient lipid challenge for research applications within a four-hour testing window.

The National Cholesterol Education Program (NCEP), and American Heart Association (AHA) agree with the health guidelines for elevated TG (Table 3) [37,38]. The cut-off for healthy fasting TG is <150mg/dL. However, increasing evidence points toward the administration of a postprandial OFTT to identify potential metabolic and cardiovascular abnormalities [7,17,37]. These tests would aid in diagnosing those with disordered lipid metabolism, and hidden postprandial lipemia [7,37]. Postprandial TG values less than 220mg/dL over a four-hour observation period is ideal [37]. People with fasting TG values between 89-180mg/dL may potentially have hidden postprandial lipemia [7,37]. Those with hidden postprandial lipemia are at greater risk for developing early atherosclerosis [37]. As such, it would be beneficial for those patients with elevated fasting values to undergo an OFTT to evaluate active TG

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metabolism. The tested 100g concentration is a reliable and valid OFTT which can be used in for the aforementioned application. Clinical treatment decisions were beyond the scope of the present investigation. The current findings are intended to inform methodological considerations for OFTT implementation rather than guide pharmacological management. It would be prudent that early intervention of disordered TG metabolism be prescribed a combination of exercise, dietary restrictions, and possibly statins, fibrates, and nicotinic acid [37]. To our knowledge, this practice is not yet reflected among health care institutions. This practice would involve at risk individuals, those having elevated fasting TG values, to be assessed following ingestion of an OFTT [7]. Since current guidelines doing Lipid Panel for patients do not appear sufficient in identifying at risk individuals.

A common ingredient in the development and implementation of many OFTT consists, in large majority, of heavy whipping cream [12,14,39-41]. Other articles have claimed to use standardized OFTT, yet no such test has been identified, and methodologies are vague as to how such a test can be reproduced [42]. The common use of commercially available heavy whipping cream in research and clinical endeavours is not without limitations. The first and primary limitation would be the macronutrient concentration of heavy whipping cream. It has long been accepted that long-term heavy doses of saturated fat are linked to an increased risk for CVD [43]. Another focus of the present investigation was to create an OFTT which 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 breakdown more rapidly than saturated fats, however this difference in breakdown efficiency does not present itself until eight-hours postprandially. Therefore, the process by which the different fat substrates are broken down should not confound data collection under an eight-hour observation window [17]. The tested OFTT, having approximately 4% saturated fat, and containing roughly 43% of PUFA and MUFA is a healthier option for patients or research participants (Table 4). There also exists potential for confounding results based on the distribution of fatty acid isoforms. There is evidence which supports that the ingestion of PUFA and MUFA, concurrent with saturated fat, attenuates the elevation in TG [44]. As such, a standardized meal of known fat concentrations should be adopted, instead of utilizing the ambiguous milk shake or high fat meal. A detailed explanation on how to construct the reliable OFTT employed herein can be found in the article. The implementation and testing of a reliable and valid oral fat tolerance test for research and clinical purposes [23].

The present investigation isolated the 100g concentration as being ideal for research and clinical purposes. The 100g concentration is similar to that proposed by Kolovou et al. in a meta-analysis expert panel review [37]. The panel proposed having 70-80g (68%) fat, 25g of CHO (22%), and 10g of protein (9%). The 100g concentration utilized within this present investigation contains 99g of fat (60%), 19.13g of CHO (23%) and 14.5g of protein (17%) (Table 4). The percentage fat and CHO subfractions are similar between these OFTT. However, the protein concentration in the proposed OFTT is nearly double that of the macronutrient recommendation set forth by Kolovou et al. [37]. Additionally, the proposed OFTT contains a similar percentage of CHO, with a lower overall percentage of fat. It is the opinion of the authors that the proposed OFTT contains a more favourable macronutrient distribution considering participant health.

It is advisable to limit carbohydrates (CHO) when developing an OFTT.A 1:1 ratio or greater of CHO creates a competition for enzymatic activity and can convolute lipid metabolism [45]. The tested OFTT contains approximately 23% of CHO and 60% total fat. This fat to CHO ratio is much less than comparative investigations in which an OFTT was employed. This low ratio should further isolate the lipid metabolism effects following consumption of an OFTT. Protein does not appear to interfere with lipid metabolism [46]. As such, a greater ratio of protein (over that of CHO and saturated fats) is encouraged when developing an OFTT. The proposed 100g OFTT consists of roughly 17%

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protein, which is favourable to the heavy whipping cream base which contains a negligible volume of protein. This concentration of protein is superior to that commonly observed within commercially available heavy whipping cream, which ranges from 0-1g of protein per tablespoon. Consequently, the implementation of the proposed OFTT retains further health benefits over varieties holding to a traditional base of heavy whipping cream.

CONCLUSION

The composition of a specific ratio of Benicalorie® and Ensure® Plus, totaling 99g of fat, 38.25g of CHO, and 29g of protein, is more appropriate than the 50g and 150g loads. The 100g load demonstrated reproducible postprandial triglyceride responses and may serve as a practical OFTT option for research applications. Clinical application requires further validation in larger and metabolically diverse populations.Benicalorie® and Ensure® Plus are commonly administered in clinics and hospitals, consisting of standardized ingredients. The use of these commercially available products is encouraged as they are easily repeatable, converse to other OFTT consisting of an unspecified combination of ingredients. These practices will limit potential errorand lend 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. Additionally, it would be of benefit to the scholarly body of knowledge to re-visit previous investigations utilizing a previously established OFTT.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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PEER REVIEW

Not commissioned. Externally peer reviewed.

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TABLES

Table 1: Participant demographics.

	Males		Females	
	Mean	**SD**	**Mean**	**SD**
N	15		15	
Active	8		9	
Sedentary	7		5	
Height	173.36	12.15	163.44	5.32
Weight	84.69	18.1	61.08	11.13
Age	27.6	4.61	25.53	4.52

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

Table 3: Participant Demographics, (*) significant difference between height and weight (p=0.024 and p=0.001, respectively).

	Males		Females	
	Mean	**SD**	**Mean**	**SD**
Height	173	12	163	5
Weight	85	18	61	11
Age	28	5	26	5
n	15		15	
Active	8		9	
Sedentary	7		5	

Table 4: TG Classifications, as outlined by the National Cholesterol Education Program.

Classification	**Fasting serum TG**
Normal	<150 mg/dL
Borderline High	150-199 mg/dL
High	200-499 mg/dL
Very High	≥500 mg/dL

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Table 5: Comparison of macronutrients between proposed 100g OFTT and heavy whipping cream equivalent.

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

FIGURES

Figure 1: Mean TG metabolism (mg/dL) among the 50g, 100g, and 150g OFTT loads, (*) Significantly different than 100g and 150g time points.

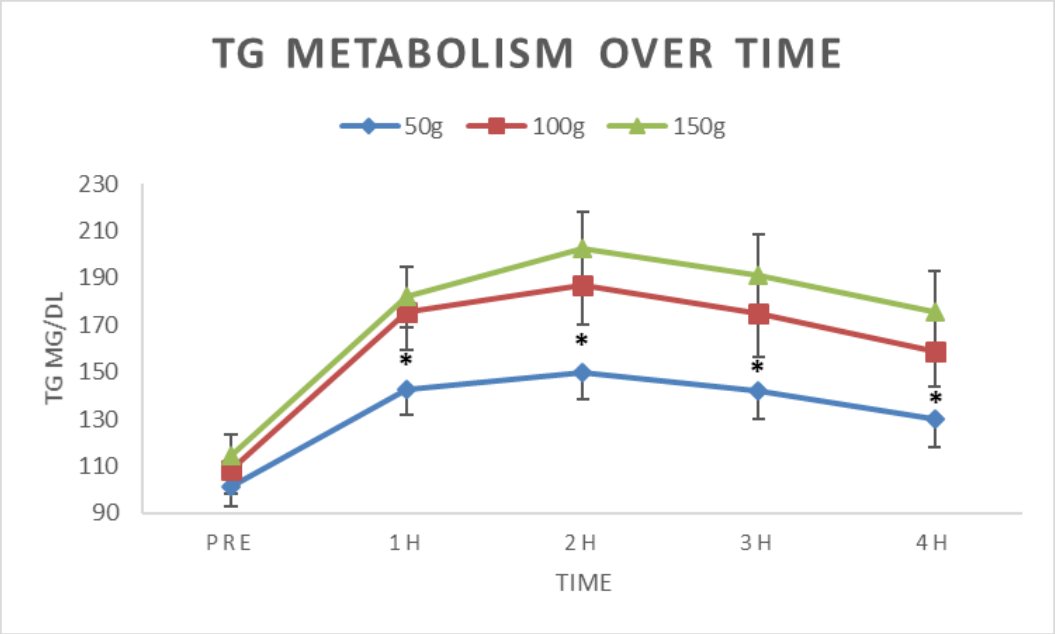

Figure 2: Mean TG metabolism (mg/dL) among the 50g, 100g, and 150g OFTT loads, (*) Significantly different than 100g and 150g time points.

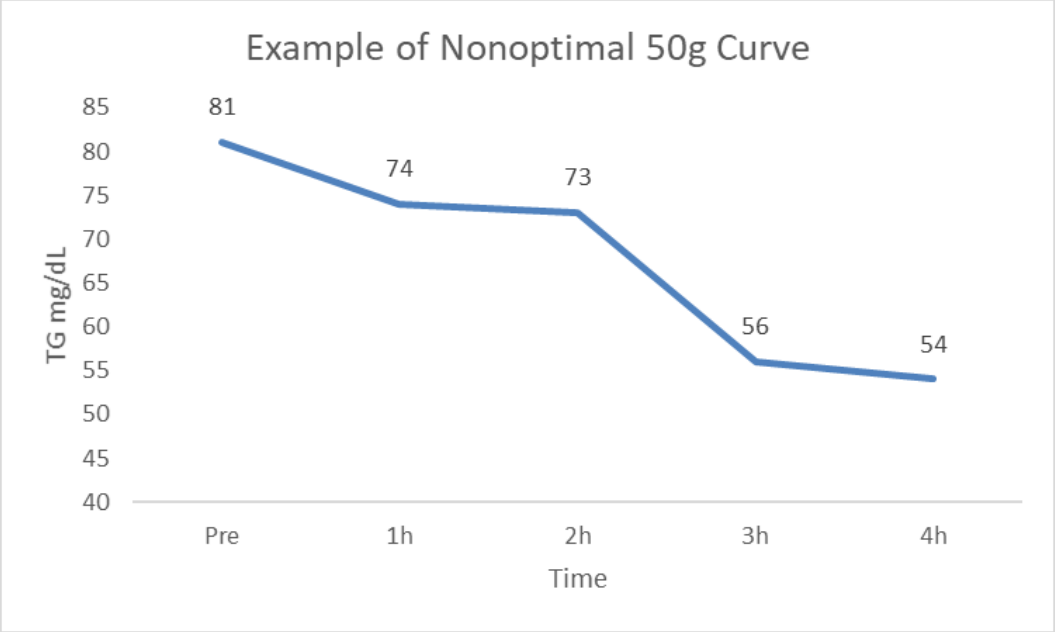

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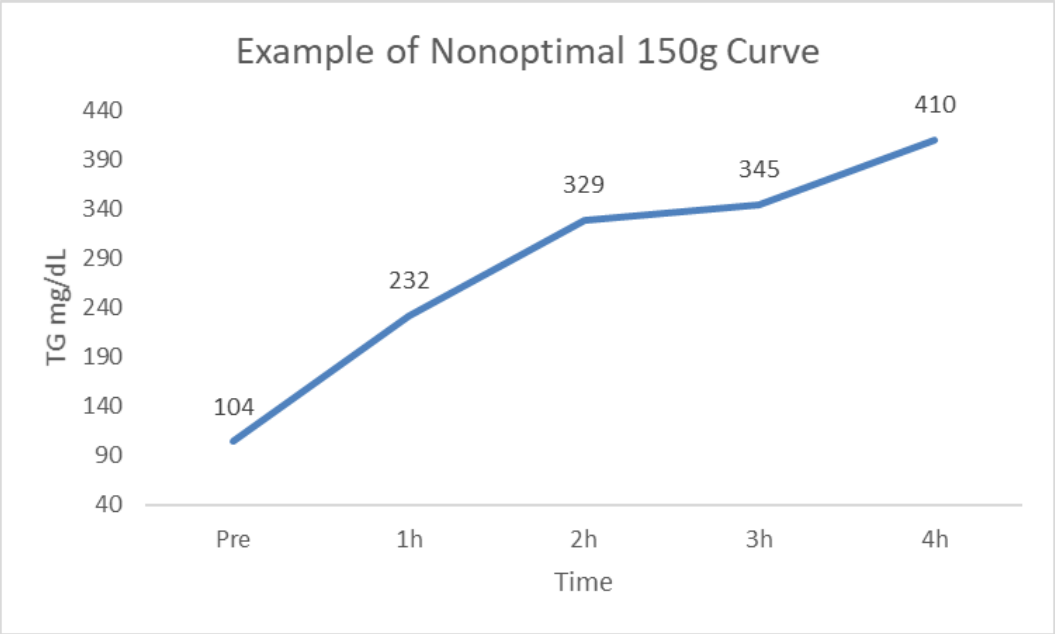
Figure 3: TG values of participant #2 over time following a 150g load (male, 69.5kg, 180.34cm, 31 years).



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