Predicting Postprandial Oxidative Stress Using Serum Triglycerides Following Oral Fat Tolerance Testing

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ABSTRACT

Background: The topic of postprandial oxidative stress continues to receive considerable attention, with elevations in oxidative stress biomarkers associated with human disease (e.g., insulin resistance, atherosclerosis). The predictable rise in serum triglyceride (TAG) following high fat meal ingestion is strongly correlated to the increase in circulating oxidative stress biomarkers. Our intent with the present study was two-fold: 1) To further characterize the postprandial response to high fat feeding and 2) to develop regression models that could be used to predict the oxidative stress response to high fat feeding.

Methods: 154 men and women reported to the lab in the morning hours following an overnight fast and consumed a high fat liquid meal. Blood samples were collected before meal ingestion and at 2 and 4 hours following meal ingestion. Samples were analyzed for TAG and oxidative stress biomarkers (hydrogen peroxide \([H_2O_2]\), malondialdehyde [MDA], advanced oxidation protein

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products [AOPP]). Simple linear regression analyses were used for model development to predict the oxidative stress response to feeding. In addition, biochemical variables were analyzed using an analysis of variance (ANOVA) to present response to feeding data across time.

**Results:** In all of the regression models, TAG explained a significant portion of the outcome variables (H$_{2}$O$_{2}$, MDA, and AOPP) and predictor models were created for each variable. In addition, values for TAG and all oxidative stress biomarkers increased following meal ingestion. A time effect was noted where all values were higher post meal ingestion as compared to pre meal for all biochemical variables (p<0.05).

**Conclusion:** Obtaining serum TAG values in response to a high fat meal challenge may provide investigators with the ability to predict postprandial oxidative stress using regression equations.

**Keywords:** Triglycerides; reactive oxygen species; postprandial; lipids; nutrition.

1. INTRODUCTION

Postprandial oxidative stress describes a condition in which the production of reactive oxygen species (ROS) increases in response to feeding, leading to the oxidation of molecules such as lipids and proteins [1]. The increased oxidation potential, coupled with a decrease in antioxidant defense, leads to an elevation in circulating oxidative stress biomarkers, including hydrogen peroxide, lipid peroxidation products (e.g., malondialdehyde), and protein oxidation products (e.g., advanced oxidation protein products). Such findings have been noted from work originating in our lab [2-4], as well as from the labs of others [5-7]. The magnitude of increase in these biomarkers is dependent on the type and size of the meal, with high fat feeding promoting a far greater increase than carbohydrate- or protein-rich meals [8], and larger meals promoting a greater increase than smaller meals [9].

It is well documented that following ingestion of high fat meals of adequate size, a transient (2-4 hour) state of hypertriglyceridemia results, evident by an increased concentration of circulating triglycerides (TAG). The increased availability of substrate (in this case free fatty acids [FFA] which are oxidized and generate NADH and FADH$_{2}$) appears to promote an increased “leak” of electrons within the mitochondrial respiratory chain, resulting in the increased generation of superoxide anion [10]. The production of superoxide may lead to the downstream events of increased inflammation [11], hyper-coagulability [12,13], sympathetic hyperactivity [14], and endothelial dysfunction [15]. The above may be associated with further superoxide generation and oxidative damage. Indeed, oxidative stress has been associated with human disease [16,17], including various forms of cancer, obesity, endothelial dysfunction, and neurodegenerative diseases. Considering the rampant increase in metabolic disease such as obesity and type II diabetes, methods to minimize oxidative stress both in a fasted state and following feeding are being investigated currently.

While the oxidative stress response to high fat loads is routinely evaluated in a research setting, to our knowledge, this is not done clinically. However, oral fat tolerance testing (OFTT) is performed clinically [18], typically with the sole inclusion of serum TAG data as the outcome measure of interest—with the possible inclusion of FFA. In fact, the study of postprandial metabolism, involving either serum triglyceride response or serum glucose response, has great clinical relevance [19,20]. Investigations from our laboratory have yielded a strong correlation between the postprandial increase in blood TAG and a variety of oxidative stress biomarkers [8,9,21]. It is possible that the lone measure of serum TAG could be used to predict the more complex cascade of oxidative stress response that routinely occurs following high fat meal ingestion. If so, this would provide both clinicians and researchers with the ability to conduct OFTT using only TAG data, with the goal of predicting a patient’s oxidative stress response. Such information may prove helpful in determining a patient’s overall health status and might also guide clinical decisions regarding the use of treatments aimed to attenuate oxidative stress (e.g., antioxidant therapy).

Collectively considering the above, the purpose of the present study was two-fold. First, we sought to further characterize the postprandial response to high fat feeding in men and women. Second, we sought to develop regression models using serum triglyceride values that could be used to predict the oxidative stress response to high fat feeding.
2. MATERIALS AND METHODS

2.1 Subjects

A total of 154 men and women participated (49 men and 105 women). All subjects completed a health history form prior to enrollment and were in good overall health. All but three subjects (all men) presented with fasting serum TAG values <150 mg·dL⁻¹. No subject was a current smoker and most subjects were engaged in a program of regular exercise—they were physically active. Subjects' height, weight, heart rate, and blood pressure were measured and recorded. Subject descriptive characteristics are provided in Table 1, demonstrating mean values for heart rate and blood pressure that were in the "normal" range. Following the initial screening procedure, subjects were scheduled for testing. All experimental procedures were performed in accordance with the ethical standards of the Helsinki Declaration, and approved by the University Human Subjects Review Board. Subjects provided verbal and written consent prior to participating.

Table 1. Descriptive characteristics of men and women

| Variable                  | Men (n=49)      | Women (n=105)     |
|---------------------------|----------------|-------------------|
| Age (yrs)                 | 27.8±1.4        | 29.2±1.1          |
| Height (cm)               | 179.2±0.9       | 165.4±0.7         |
| Weight (kg)               | 82.6±1.3        | 72.4±2.1          |
| BMI (kg·m⁻²)              | 25.7±0.4        | 26.3±0.7          |
| Waist (cm)                | 86.5±1.1        | 82.1±2.0          |
| Hip (cm)                  | 100.4±1.0       | 104.9±1.5         |
| Waist:Hip                 | 0.86±0.01       | 0.78±0.01         |
| Resting heart rate (bpm)  | 64.3±1.2        | 70.0±1.1          |
| Resting SBP (mm Hg)       | 117.2±1.4       | 111.5±1.2         |
| Resting DBP (mm Hg)       | 72.3±1.4        | 72.1±1.0          |

Values are mean±SEM
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure

2.2 Test Meals

All testing was performed in the morning following an overnight fast. Subject data used for the present analysis was pooled from investigations using slightly different meal compositions, although all meals involved the intake of a relatively high amount of dietary fat. Specifically, 67 subjects consumed a meal consisting of 0.8 grams of fat, 1.0 gram of carbohydrate, and 0.25 grams of protein per kilogram of body weight. The remaining 77 subjects consumed a meal consisting of 1.2 grams of fat and carbohydrate, and 0.25 grams of protein per kilogram of body weight. All mixed meals were made with whole milk, Breyers® "all natural" vanilla ice cream, and heavy whipping cream. The mixed meals contained a moderate amount of carbohydrate, mainly in the form of simple sugars. In all meals, the fat content was comprised of approximately 65% saturated fat. All subjects were allowed 15 minutes to consume their meal. Subjects remained in the laboratory (or in close proximity) during the 4-hour postprandial period and rested (i.e., watched movies, worked on a computer, read). No additional meals or calorie containing beverages were allowed, although water was allowed ad libitum.

We have noted in our prior work that a 4-hour postprandial measurement time is sufficient when studying the TAG and oxidative stress response to feeding in healthy men and women, as peak TAG response to feeding is observed prior to and by this time [3,8,9,21]. This agrees with the timeframe for clinically administered OFTT [22]. It should be noted that although intravenous administration of lipid has been done previously using Intralipid® [23], a fat emulsion product consisting of various mixtures (i.e., soybean oil, egg yolk phospholipids, glycerin, and water), more recent clinical assessments use oral ingestion of lipids (i.e., OFTT). Hence, the present study involved the oral ingestion of high fat meals.

2.3 Blood Sampling and Biochemistry

Approximately 10mL of venous blood was taken from a forearm vein of each subject via needle and collection tube pre meal (0 hour) and at 2 and 4 hours post meal. Therefore, a total of three blood samples were obtained from subjects. In some cases, blood was not available for a particular subject or for a particular assay. The exact numbers used for each analysis are included within the Results section. Blood collected in tubes containing EDTA was centrifuged immediately at 4°C and the plasma stored in multiple aliquots at -70°C until analyzed. Blood collected in tubes with no additive was allowed to clot for 30 minutes at room temperature and then centrifuged at 4°C.
The serum was stored in multiple aliquots at -70°C until analyzed.

Triglyceride was analyzed in serum following standard enzymatic procedures as described by the reagent provider (Thermo Electron Clinical Chemistry). Advanced Oxidation Protein Products were analyzed in plasma using the methods as previously described [24]. Malondiadehyde was analyzed in plasma using a commercially available colorimetric assay (Northwest Life Science Specialties, Vancouver, WA), using similar methods as previously described [25]. Hydrogen peroxide was analyzed in plasma using the Amplex Red reagent method as described by the manufacturer (Molecular Probes, Invitrogen Detection Technologies, Eugene, OR). In the reaction mixture, hydrogen peroxide, in the presence of horseradish peroxidase, reacts with Amplex Red reagent to generate the red-fluorescence oxidation product, resorufin.

2.4 Dietary Records and Physical Activity

Subjects were instructed to maintain their normal diet and physical activity levels leading up to the test day, with the exception of refraining from strenuous activity during the 24 hour period prior to testing. This was important in order to control for any acute effects of physical activity on postprandial oxidative stress [7]. In our work, compliance is confirmed with subjects regularly throughout study periods, primarily through the use of email, phone, and text message reminders sent to subjects in an attempt to improve adherence to the protocol (e.g., message sent to subjects the night before testing to confirm that subject arrives to the lab fasted).

2.5 Statistical Analysis

Simple linear regression analyses were performed in an attempt to develop models for predicting the oxidative stress response to feeding. Due to potential differences in TAG and oxidative stress response between men and women [2], separate regressions were conducted for men and women where TAG was the predictor variable and AOPP, MDA, and H₂O₂ were the criterion variables in their respective models. In addition to the regression analysis, biochemical variables were analyzed using an analysis of variance (ANOVA) to present response to feeding data over time. The method of Tukey was used for post-hoc testing, comparing values across time. The data are presented as mean ± standard error of the mean. Statistical significance was set at p ≤ 0.05.

3. RESULTS

3.1 AOPP Regression Models

For men (n = 72), AOPP was significantly positively correlated with TAG (r = 0.710, p<0.001). In addition, TAG explained 50.3% of the variance in AOPP (F (1,70) = 70.98, p<0.001). The resulting regression equation is AOPP (µmol·L⁻¹) = 15.861 + 0.202 (TAG) (See Fig. 1A).

For women (n = 81), AOPP was significantly positively correlated with TAG (r = 0.555, p<0.001). In addition, TAG explained 30.8% of the variance in AOPP (F (1,79) = 35.21, p<0.001). The resulting regression equation is AOPP (µmol·L⁻¹) = 16.350 + 0.233 (TAG) (See Fig. 1B).

3.2 MDA Regression Models

For men (n = 144), MDA was significantly positively correlated with TAG (r = 0.637, p<0.001). In addition, TAG explained 40.5% of the variance in MDA (F (1,142) = 96.82, p<0.001). The resulting regression equation is MDA (µmol·L⁻¹) = 0.622 + 0.006 (TAG) (See Fig. 2A).

For women (n = 314), MDA was significantly positively correlated with TAG (r = 0.630, p<0.001). In addition, TAG explained 39.7% of the variance in MDA (F (1,312) = 205.27, p<0.001). The resulting regression equation is MDA (µmol·L⁻¹) = 0.403 + 0.010 (TAG) (See Fig. 2B).

3.3 H₂O₂ Regression Models

For men (n = 132), H₂O₂ was significantly positively correlated with TAG (r = 0.532, p<0.001). In addition, TAG explained 28.3% of the variance in H₂O₂ (F (1,130) = 51.37, p<0.001). The resulting regression equation is H₂O₂ (µmol·L⁻¹) = 5.778 + 0.064 (TAG) (See Fig. 3A).

For women (n = 307), H₂O₂ was significantly positively correlated with TAG (r = 0.489, p<0.001). In addition, TAG explained 30.8% of the variance in H₂O₂ (F (1,305) = 96.08, p = p<0.001). The resulting regression equation is H₂O₂ (µmol·L⁻¹) = 4.921 + 0.110 (TAG) (See Fig. 3B).
Fig. 1A. AOPP ($\mu$mol·L$^{-1}$) and TAG (mg·dL$^{-1}$) scatterplot for men

Fig. 1B. AOPP ($\mu$mol·L$^{-1}$) and TAG (mg·dL$^{-1}$) scatterplot for women
Fig. 2A. MDA ($\mu$mol·L$^{-1}$) and TAG (mg·dL$^{-1}$) scatterplot for men

Fig. 2B. MDA ($\mu$mol·L$^{-1}$) and TAG (mg·dL$^{-1}$) scatterplot for women
Fig. 3A. \( \text{H}_2\text{O}_2 \) (µmol·L\(^{-1}\)) and TAG (mg·dL\(^{-1}\)) scatterplot for men

Fig. 3B. \( \text{H}_2\text{O}_2 \) (µmol·L\(^{-1}\)) and TAG (mg·dL\(^{-1}\)) scatterplot for women
3.4 Effect for Each Variable over Time

Values for TAG and all oxidative stress biomarkers increased following meal ingestion. Specifically, a time effect was noted for TAG (\(p=0.0007\)), AOPP (\(p<0.0001\)), MDA (\(p<0.0001\)), and \(\text{H}_2\text{O}_2\) (\(p<0.0001\)), with values higher at 2 and 4 hours following meal ingestion as compared to pre meal, for all biochemical variables (\(p<0.05\)). Data are presented in Figs. 4 and 5.

![Figure 4](image_url)

*Fig. 4. Serum triglycerides (A) and advanced oxidation protein products (B) before and following intake of a lipid meal*

*Values are mean±SEM*

*Time effect for TAG (\(p=0.0007\)) and AOPP (\(p<0.0001\)); *values higher at 2 and 4 hours following meal ingestion as compared to pre meal (\(p<0.05\)); only blood samples from 51 subjects were available for AOPP analysis*
Fig. 5. Plasma malondialdehyde (A) and plasma hydrogen peroxide (B) before and following intake of a lipid meal

Values are mean±SEM. Time effect for MDA (p<0.0001) and \( \text{H}_2\text{O}_2 \) (p<0.0001); *values higher at 2 and 4 hours following meal ingestion as compared to pre meal (p<0.05).

Note: Values for MDA were not available for 1 subject; values for \( \text{H}_2\text{O}_2 \) were not available for the 2 and 4 hour post meal time points for 8 subjects.

4. DISCUSSION

In support of our prior work and the work of others, our data clearly indicate that high fat meal ingestion leads to an increase in TAG and oxidative stress biomarkers during the acute postprandial period. With regards to the main aim of the present study, our data indicate that TAG is a significant predictor of AOPP, MDA, and \( \text{H}_2\text{O}_2 \) for men and women. However, for men, TAG has the most influence on the variance in AOPP (50.3%) compared to MDA (40.5%) and \( \text{H}_2\text{O}_2 \) (28.3%). For women, TAG has the most influence on the variance in MDA (39.7%).
compared to AOPP (30.8%) and H$_2$O$_2$ (30.8%). These regression equations can be used to help predict postprandial oxidative stress using serum TAG data.

While we believe that our findings will have relevance to a wide variety of individuals, including those within research and clinical environments, some limitations of this work and the potential applications should be considered. First, it should be understood that different populations of individuals (e.g., those with known cardiovascular or metabolic disease) may experience varying responses as compared to those individuals included in the present study. Second, although we used common assay procedures for all analyses, differences in assay format and techniques may result in slightly different findings than those noted here. Third, and related to the actual meals provided, while consistent with prior work involving oral fat tolerance testing (inclusive of approximately 65% saturated fat), it is possible that test meals with a lower amount of saturated fat may yield different findings. In addition, liquid meals may not be as commonly consumed as solid food meals—which are also known to influence oxidative stress biomarkers [26]. Future research within varying populations, inclusive of different test meals, may be done to extend these initial findings.

We believe that our findings may have clinical application, as the determination of postprandial oxidative stress may provide relevant information pertaining to an individual’s overall health. For example, postprandial lipemia leads to neutrophilia [27] and is positively correlated with leukocyte production of superoxide [10]. In support of this, it has been noted that blood lipids contribute to platelet-derived superoxide production, via the activation of phospholipase A2 and NADH/NADPH enzymes [28]. This increased superoxide in the presence of nitric oxide can generate the toxic reaction product peroxynitrite [29], which may lead to a loss in nitric oxide bioavailability and an increase in lipid peroxidation. An increase in oxidative stress coupled with lipemia may have a direct detrimental effect on endothelial function, while playing a mechanistic role in the development of atherosclerotic disease [6].

Considering that most individuals living in Western civilization exist in a constant postprandial state, it appears important to determine the oxidative stress response to feeding. That is, values obtained following a single feeding are likely duplicated many times throughout the day—assuming a similar food intake. Of course, the Western diet is high in both saturated fat and processed carbohydrate, nutrients that elicit the greatest amount of oxidative stress [8]. Moreover, these diets are often rich in cholesterol, which may exacerbate the health consequences of increased dietary fat intake [30,31]. In addition, meals are often consumed in large portions leading to excess substrate accumulation and a potentially greater impact on ROS production and oxidation (9). Indeed, individuals in the Western world often consume frequent, high calorie, fat- and processed carbohydrate-rich meals. This creates a scenario that best leads to massive increases in serum TAG. Therefore, prediction equations of postprandial oxidative stress using TAG data may be a valuable tool if used clinically, as well as in a research setting where oxidative stress biomarkers are not included, as they may provide important information pertaining to general health and wellbeing of individuals being assessed.

The proposed equations presented within this paper may allow for an estimation of the oxidative burden borne by cells following ingestion of high fat meals. Although TAG data alone may be of interest, the simple measure of serum lipids outside of the context of oxidative stress measures does not provide a comprehensive overview of the health status of the system. Additional information gathered from the estimation of postprandial oxidative stress may serve to influence treatment options, such as the inclusion of antioxidant therapy, which has been shown to be useful in lowering postprandial oxidative stress in both healthy and diseased populations [32]. It is possible that this additional information may also aid in determining other items to focus on concerning the patient, including the measure of endothelial function.

Aside from clinical practice, the estimation of oxidative stress from TAG data may prove beneficial from a research standpoint. For example, these prediction equations may make it possible to gather additional information on the particular subject population, without the requirement of additional oxidative stress assays and analyses, thus saving time and expense. In addition, they may allow for the option of looking more closely at previous studies that have included the measurement of TAG, while attempting to determine estimated oxidative stress levels. It is possible that this approach
would unveil new information and potentially fuel new research ideas.

It is apparent that increased and persistent oxidative stress can have a detrimental impact on certain aspects of human health. For example, elevated oxidative stress may impair vascular function and may increase susceptibility to a variety of cardiovascular diseases (10,15). We have shown that indicators of oxidative stress increase in response to high fat feeding and can be estimated using the single measure of serum TAG. The proposed regression equations described herein can be used to estimate oxidative stress following high fat feeding; however, it should be noted that our subjects were relatively young, in good overall health, and presented with relatively low fasting TAG values. We are uncertain if the equations presented within will work well for individuals with very high fasting TAG values—as such individuals will also likely experience a concomitant increase in TAG in response to high fat feeding. Moreover, while statistical significance was noted for each variable, the prediction equations are in no way perfect. This needs to be considered when using these models.

We conclude that obtaining serum TAG values in response to a high fat meal challenge may provide investigators with the ability to predict postprandial oxidative stress using regression equations. These regression models may provide an enhanced view of physiological occurrences following high fat meal ingestion, which may yield further insight into the overall health status of the individual. Specifically, these equations may prove helpful for both researchers and clinicians during times when oral fat tolerance testing is performed, in an attempt to determine an individual’s ability to clear triglycerides from the circulation. Understanding the importance of oxidative stress to the overall disease process, clinicians may then seek to use our proposed prediction equations in order to provide information related to their patient’s oxidative stress response to high fat feeding. Doing so may prove to be a simple method of gaining additional insight into the overall metabolic health of the individual.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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