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Consumption of a high-fat breakfast on consecutive days alters the area-under-the-curve for selected cardiovascular diseases biomarkers

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ABSTRACT

Background: One of the primary causes of obesity-associated disease is the habitual consumption of a high-fat, high-calorie diet. The purpose of this study was to determine the effect of consumption of a high-fat, high-calorie breakfast on consecutive days in the area under the curve (AUC) for 22 emerging cardiovascular disease biomarkers in young women ($n = 8$). **Materials and Methods:** Subjects consumed a high-fat (70% of daily calories, 100% of daily fat) breakfast meal on 2 consecutive days. Blood samples were collected pre-meal, 1 h, 2 h, 3 h, 4 h, and 5 h post-meal. Cholesterol, triglycerides, and glucose were measured using an enzymatic assay. Cardiovascular diseases (CVD) biomarkers were analyzed simultaneously in serum using a multiplex method, and AUC was calculated separately for each biomarker on each day. A paired t-test with significance set at $P < 0.05$ was used to compare the outcomes. **Results:** We observed similar AUC for total cholesterol, triglycerides, and glucose between the 1st and 2nd day of the meal. In contrast, endocan-1, CXCL6, CXCL16, fibrinogen, placental growth factor, and adipsin exhibited statistically greater AUC on the 2nd compared to the 1st day. **Conclusion:** Further evaluation of these targets revealed that the largest changes were in endocan-1, CXCL6, and CXCL16, which have been linked to atherogenesis. These findings support the notion that chronic CVD risk may result from the cumulative effects associated with eating high-fat foods on a habitual basis.

KEY WORDS: Inflammation, nutrition, meal-response, multiplex, post-prandial

INTRODUCTION

There has been an increase in the consumption of high-fat meals over the past decade due to improved palatability, accessibility, and the low cost for these foods compared to fresh, healthy foods [1]. Habitual consumption of high-fat, high-calorie foods is one of the many causes of obesity in today's society [2,3]. The largest problem associated with the accumulation of excess adiposity is the development of metabolic and cardiovascular diseases (CVD) [4,5]. While the disease risk implications of habitual high-fat food consumption have been established, it was only recently that researchers found that single, high-fat meals can result in transient changes in disease risk as well [6-10]. Our lab and others have reported a variety of changes that take place within the first 5 h postprandial [6-11]. Of the changes we have previously observed, we found that consumption of a high-fat meal alters the concentration of circulating endothelial microparticles [11], which are believed to play a role in the accumulation of atherosclerotic plaques. Recently a focus in the literature has been on identifying novel disease risk factors that may serve as future therapeutic targets. According to the existing literature, validating a novel biomarker against a traditional disease risk factor is common (i.e. glucose, lipids, etc.) [12-18].

To our knowledge, no published study has compared the area under the curve (AUC) response on 2 consecutive days following consumption of a high-fat breakfast for the proposed 22 emerging CVD. Rather than discuss the physiological role of each biomarker in the introduction to this study, we

opted to discuss those biomarkers that were significant within the discussion section. The primary purpose of the present study was to determine how the AUC for CVD biomarkers is altered following the consumption of a high-fat breakfast on consecutive days.

Experimental Methods

Ethical approval

All procedures in the current study were reviewed and approved by the University of Houston Committee for Protection of Human Subjects, where the primary samples were collected. Analysis of CVD biomarkers was completed at the University of North Texas on de-identified samples. All subjects provided their verbal and written consent prior to participating in the study. All procedures were completed according to the standards set by the latest revision of the declaration of Helsinki.

Subjects

Women aged 18-30 year and of normal body mass index (20-24.9 kg/m^{-2}) were recruited for the study [n = 10; Table 1]. Of the original 10 recruited, 8 subjects completed all study requirements, and their data were used to prepare this report. We opted to use normal weight individuals to delimit the presence of obesity-associated disease, which may have created variability in our observations. We also opted to use only women because women are a traditionally understudied population. Exclusion criteria included a better than average VO_{2peak} (based on the American College of Sports Medicine Guidelines) [19], greater than low-moderate intake of alcohol (>1 drink per day, no binge drinking patterns), tobacco use in the previous 6 months, weight loss of >1.9 kg during the previous month, specific prescription medication use (i.e., statins, anti-diabetes medications, antidepressants, and insulin), metabolic disorders (i.e. Type II diabetes mellitus, etc.), or diagnosed CVD. We also excluded subjects who were vegan or vegetarian since the test meal included an animal protein source. If subjects were currently consuming any over-the-counter dietary supplements (vitamins, minerals, probiotics, etc.), they were asked to discontinue their use at least 7-days prior to their first high-fat meal trial.

Table 1: Subject characteristics

Characteristic	Value
Age (y)	19±1.3
Height (cm)	163.0±7.6
Weight (kg)	59.4±7.0
BMI (kg/m^2)	22.5±2.3
RMR (kcal/day)	1597±166
VO_{2Peak} (mL/kg-min)	26.6±2.1
Lean mass (kg)	36.1±2.9
Fat mass (kg)	16.8±5.3
Bone mineral content (kg)	1.6±0.2
Body fat (%)	30.3±6.1
Total cholesterol (mg/dL)	125.0±38.1
Triglycerides (mg/dL)	77.3±27.6
Glucose (mg/dL)	132.5±91.8

Values represent the mean±SD, SD: Standard deviation, BMI: Body mass index, RMR: Resting metabolic rate

Subject laboratory screening/evaluation

Subjects were asked to report to the laboratory at least 7-days before completing their first meal trial. Height and weight were measured using a stadiometer and analog weight scale (Detecto; Webb City, MO). Resting metabolic rate (RMR) was measured using a canopy and an air dilution technique via a metabolic cart (Parvomedics Vmax; Sandy, UT) to calculate each subject's daily caloric needs. A graded, maximal aerobic fitness test was completed on an electronically braked cycle ergometer (Velotron; Portland, OR). Expired air was collected and analyzed by automated methods using a metabolic cart (Parvomedics) to determine oxygen consumption (VO_2). A standard, whole-body dual-energy X-ray absorptiometry scan (DXA; Hologic Discovery-W; Hologic Corporation; Bedford, MA) was used to measure body composition.

Experimental Meal Timing/Blood Collection

Subjects were scheduled to report to the laboratory on 2 consecutive days between 0500 and 0800 following an overnight fast (>8-h) and abstention from exercise (>24-h) and extending through the end of the study protocol. Subject trials were scheduled to occur at approximately 7-days following the onset of menses to ensure that subjects were in a similar phase of their menstrual cycle. On the mornings of days 1 and 2, subjects were fitted with an intravenous catheter to collect blood samples. The catheter was maintained by a constant flow of sterile saline. Venous blood (30 mL per sample) was collected at 6 time points: Pre-meal, 1 h, 2 h, 3 h, 4 h, and 5 h post-meal. During blood to collection, the 1st 2 mL of blood was discarded, and the remaining 28 mL was used for subsequent analysis after either treating with ethylenediaminetetraacetic acid or being allowed to clot. Serum was isolated from clotted blood by centrifugation (20-min at 1000 × G) and stored at −80°C until analysis for enzymatic or multiplex analysis.

Experimental Meal Composition

After collection of the pre-meal blood samples, subjects were provided with a commercially prepared fast food breakfast meal (breakfast sandwich and hash browns) consisting 70% of their daily caloric needs and 100% of their daily fat needs (based on RMR). Subjects were given 20-min to consume the entire meal and were allowed 500 mL water to drink during meal consumption. After collection of the 5-h post-meal blood sample, subjects were provided with low-fat meal replacement bars and drinks (Slim Fast; Unilever; USA) designed to provide the remaining 30% of their daily caloric needs (based on RMR). Subjects were also allowed to drink water *ad libitum*, but were asked to refrain from consuming any other food or beverages.

Serum Lipid/Glucose Measurement

Serum samples were analyzed in duplicate for the concentration of total cholesterol, triglycerides, and glucose using an automated clinical chemistry analyzer (Chemwell-T; Palm City, FL) and commercially available enzymatic assays (Pointe Scientific; Canton, MI). Manufacturer-supplied controls and

standards were used to evaluate and confirm assay performance. All post-meal values were combined to generate a single AUC for each day based on a change from the pre-meal blood value [20].

Cardiovascular Risk Factors

Serum samples were analyzed in duplicate using two Milliplex MagPix CVD kits (CVD1; HCVD1MAG-67K; CVD3; HCVD3MAG-67K; EMD-Millipore; Billerica, MA) to determine the concentrations of brain natriuretic peptide (BNP), CK-MB, CXCL6/GCP-2, CXCL16, Endocan-1 (ESM-1), heart-type fatty acid binding protein 3 (FABP3), Adipose-type FABP4, LIGHT, Oncostatin (OSM), placental growth factor (PLGF), Troponin I (Tnl), α-2-Macroglobulin, Adipsin, Alpha-1 acid glycoprotein, C-reactive protein, Fetuin A, Fibrinogen, Haptoglobin, sL-Selectin, platelet factor 4 (PF4), serum amyloid P, and von Willebrand Factor. Analysis was completed as described by the manufacturer [21]. Sample intra-assay CV was <8% for all biomarkers. All sample analysis was completed on the same day to eliminate inter-assay variability as a source of error. The MagPix analyzer (Luminex Corp; Austin, Texas) was checked for calibration prior to analysis using Fluidics Verification and Calibration bead kits (Luminex Corp). A minimum of 50 beads for each targeted biomarker were acquired using Luminex xPonent software and analyzed using Milliplex Analyst software (v.5.1; EMD-Millipore). All post-meal values were combined to generate a single AUC for each day based on a change from the pre-meal blood value [20].

Statistical Analysis

Statistical analyses were completed using SPSS v. 21.0 (Chicago, IL). Variables were individually examined to ensure that they were normally distributed using the explore function in SPSS. Any non-normally distributed data were log transformed prior to analysis and noted in the results section. Days 1 and 2 AUC values for each variable were compared using a paired *t*-test. Significance was set at $P < 0.05$.

RESULTS

Serum Lipids and Glucose

With respect to pre-meal, AUC was similar between days 1 and 2 for total cholesterol [Figure 1a], triglycerides [Figure 1b] and glucose [Figure 1c]. For total cholesterol, the AUC response was negative on both days while the response for triglycerides and glucose was positive on both days. These responses are consistent with that of the typical response to consuming a meal.

CVD Biomarkers

Of the measured CVD biomarkers, we found six whose the AUC response was significantly different between days 1 and 2. In each of these instances, the 2nd day of consuming the high-fat breakfast resulted in a larger AUC. This response was observed for endocan-1 [$P = 0.037$; Figure 2a], CXCL6 [$P = 0.045$; Figure 2b], CXCL16 [$P = 0.010$; Figure 2c], PLGF [$P = 0.031$; Figure 2d], fibrinogen [$P = 0.030$; Figure 2e], and adipsin [$P = 0.028$; Figure 2f]. With the exception of fibrinogen, each of these AUC responses was very similar in that day 1 presented a negative response while days 2 demonstrated a positive response. In order to compare all of the AUC responses between days a radial fold was generated [Figure 3]. The radial plot demonstrates that the largest magnitude of difference for days

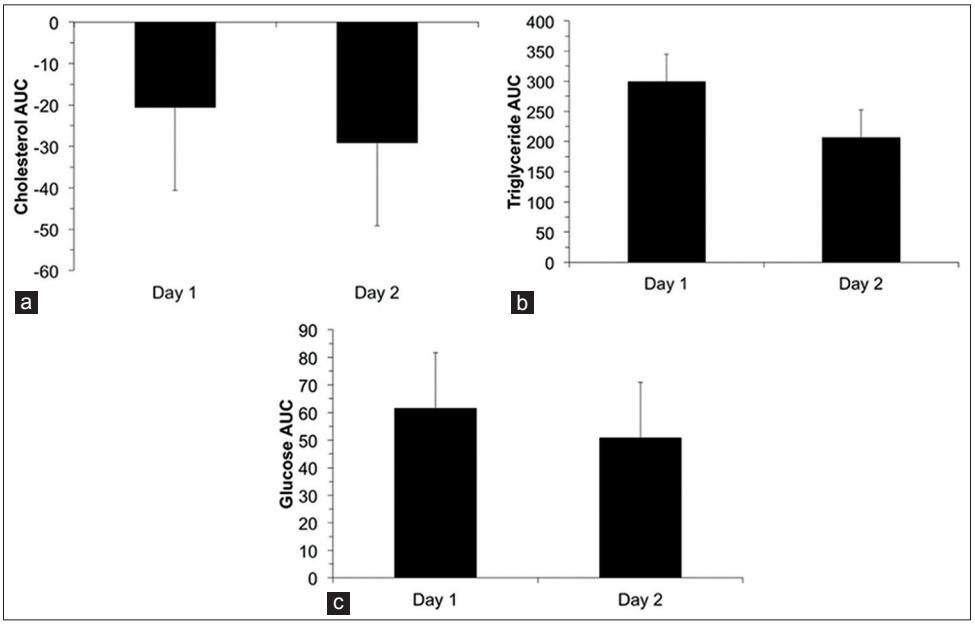

Figure 1: Postprandial response expressed as area under the curve (AUC) for meal (a) total cholesterol (b) triglycerides, and (c) glucose. Subjects were allowed 20-min to consume a high-fat, high-calorie breakfast (70% of kcal from saturated fat). Postprandial blood samples were collected over a period of 5-h biomarkers and were measured using a clinical chemistry analyzer. AUC was calculated according to previous methods. Values represent the mean± standard error of the mean. There was no significant difference in the AUC response between days.

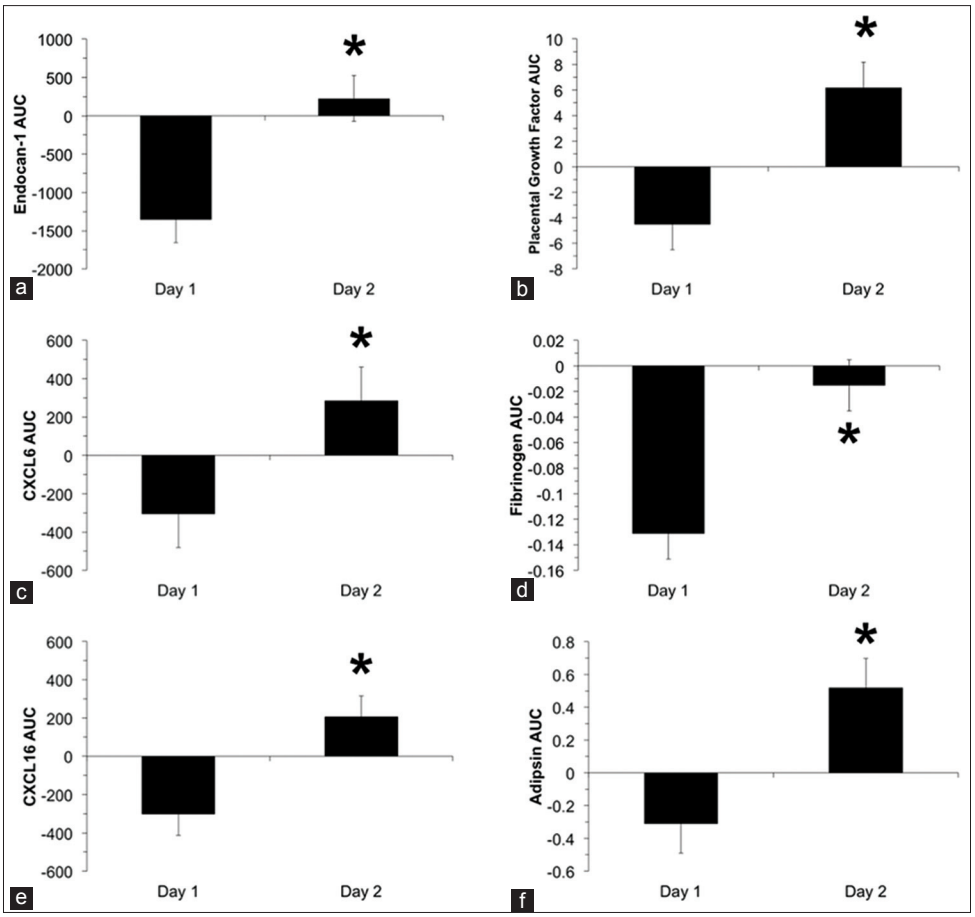

Figure 2: Postprandial response expressed as area under the curve (AUC) for Meal (a) endocan-1 (b) CXCL6, (c) CXCL16, (d) placental growth factor, (e) fibrinogen, and (f) adipsin. Subjects were allowed 20-min to consume a high-fat, high-calorie breakfast (70% of kcal from saturated fat). Postprandial blood samples were collected over a period of 5-h biomarkers and were measured using a milliplex bead-based multiplex assay. AUC was calculated according to previous methods. Values represent the mean ± standard error of the mean. * indicates day 2 AUC significantly greater than day 1 AUC ($P < 0.05$).

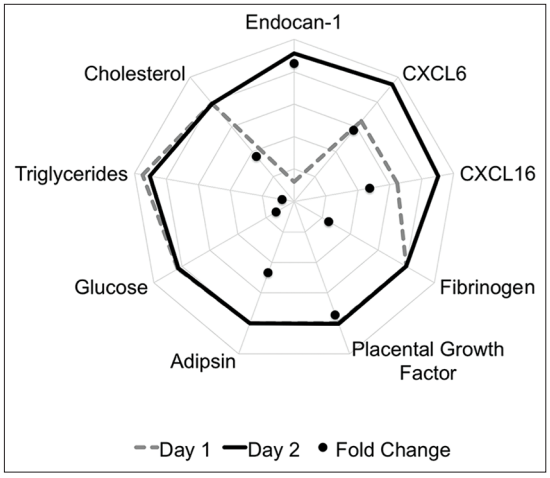

Figure 3: The radial plot compares day 1 (dashed grey line) and day 2 (solid black line) AUC responses for all the variables presented in Figures 1 and 2. The radial axis scale for the grey and black lines is that each bar represents 400 units of AUC response. Furthermore, the fold change between the 2 days is presented as black dots for each variable. The radial axis for the fold change is that each bar represents a change of 50 fold.

1 and 2 AUC occurred for endocan-1, CXCL6, and CXCL16. The magnitude of the shift for fibrinogen, PLGF, and adipsin, while significant was much smaller.

DISCUSSION

The key objective of the present study was to compare the post-prandial AUC response for emerging CVD biomarkers following consumption of a high-fat meal on consecutive days. From our initial panel of 22 biomarkers, we identified 6 CVD biomarkers (CXCL6, CXCL16, endocan-1, adipsin, fibrinogen, and PLGF) whose AUC response was greater on day 2. Of these, the magnitude of change associated with endocan-1, CXCL6, and CXCL16 was greater than adipsin, fibrinogen, and PLGF. Consistent with previous findings, there was no difference in cholesterol, triglyceride, or glucose AUC between the 2 days [7,8,10,11]. It is important to note that the observed changes were transient, and the post-prandial meal response was resolved to baseline at 24-h post-prandial. It is important to consider that our subjects were healthy and did not have any active form of CVD or diabetes mellitus. Thus, the reported changes are likely classified as pre-clinical risk.

CXCL6, CXCL16, endocan-1, and adipsin have similar inflammation-promoting properties [22-27]. The resultant chronic inflammation caused by these substances has been linked to arterial plaque accumulation, arterial plaque index, and angiogenesis [22-25]. Furthermore, adipsin has also been reported to stimulate β-cell production of insulin in patients with diabetes mellitus [26]. In the present study, we found that the AUC for CXCL6, CXCL16, endocan-1, and adipsin was significantly greater on day 2 compared to day 1. It is also important to note that the AUC on day 1 was negative while the AUC on day 2 was positive. If the observed response remained elevated on day 2 and beyond, this may over time increase the risk of arterial plaque accumulation, angiogenesis, and disrupted pancreatic insulin production [22-26]. According to the literature, these disease outcomes are largely classified as chronic diseases that take months or years to develop [14,20]; however, the key findings of the present study support the notion that chronic responses are potentially caused by the accumulation of a series of daily meal choices. The present findings contribute to the hypothesis that chronic disease results from the cumulative effects of consuming high-fat meals on consecutive days.

We also found AUC differences for fibrinogen and PLGF, and while significant the magnitude of change may not be as clinically relevant as observed for endocan-1, CXCL6, and CXCL16. Under normal physiological conditions, fibrinogen plays a role in blood clotting; however, in obese individuals, elevated serum fibrinogen was associated with an increased vascular inflammation and CVD [14,16]. Following a single meal, fibrinogen changes range between 3 and 6% over a 4-h post-prandial period and the magnitude of response positively correlated to the macronutrient fat content of the meal [6-8,10]. The days 1 and 2 AUC response for fibrinogen of the present study fell within reported post-prandial ranges. A 27% increase in PLGF was associated with diabetes mellitus, elevated CVD risk, and increased all-cause mortality [28,29]. Comparing days 1 and 2, we observed an overall 10% rise in PLGF, which is below the threshold of change reported for patients with active disease. Other than the observation that our 2-day post-prandial shift was less than half of the change reported in patients with disease, it is under clear what the exact clinical relevance is.

To our knowledge, the present study is the first to report post-prandial AUC for six emerging CVD risk biomarkers following consumption of a high-fat breakfast on consecutive days. Prior to the study, we had hypothesized that when comparing day 1 and day 2 AUC we would find a significantly larger change on day 2 compared to day 1. While, we found the hypothesized difference, the magnitude of change was not what we expected. For example, all of the significant variables had a negative AUC on day 1, and the majority had a positive AUC on day 2. Unfortunately, the design of the present study did not allow us to determine the underlying cause of a negative AUC on day 1, which should be the focus of future studies. We found statistically significance day differences for endocan-1, CXCL6, CXCL16, adipsin, fibrinogen, and PLGF. Further evaluation and interpretation of these changes suggests that the response for endocan-1, CXCL6, and CXCL16 may be the most clinically relevant because they had the largest change between days. These biomarkers have been demonstrated to be elevated in humans with active CVD [22,24,25]; however, the present study is the first to demonstrate that they are also transiently changed during the post-prandial period following consumption of a high-fat meal.

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