Evaluation of Oxidation Reduction Potential in Metabolic Syndrome Subjects Compared
with Matched Normal Subjects

by
Tora Jean Cobb

A THESIS

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Chemistry
(Honors Scholar)

Presented March 18, 2016
Commencement June 2016

AN ABSTRACT OF THE THESIS OF

Tora Jean Cobb for the degree of Honors Baccalaureate of Science in Chemistry presented on March 18, 2016. Title: Evaluation of Oxidation Reduction Potential in Metabolic Syndrome Subjects Compared with Matched Normal Subjects.

This study was conducted to assess whether healthy adults compared with those with Metabolic Syndrome (MetS) have different mean oxidative-reductive potentials. Static oxidation-reduction potentials and capacity oxidation reduction potentials (sORP and cORP, respectively) were measured in plasma samples. The RedoxSYS® measures sORP in millivolts (mV), which is proportional to the overall redox status of the sample, with higher sORP values relating to higher oxidation potential. The capacity of antioxidant reserves is measured by cORP in microcoulombs (μC), with lower values meaning the sample has below normal antioxidant reserve levels.

Plasma from healthy men and women (n = 5 M, 5 F/group; age: 18-40 y; BMI: 22.6 ± 0.7 kg/m^2) were compared with samples from age- and gender-matched individuals with MetS (n = 5 M, 5 F/group; age: 18-40 y BMI: 37.7 ± 3.0 kg/m^2). Heparin-anticoagulated plasma samples, which were collected in the fasting state at 0, 24, 48, and 72 h from each of four separate trials, were used for study. The sORP (136.2 ± 10.5 mV) measured in plasma from healthy subjects were significantly lower ($P<0.0001$) than in MetS subjects (154.5 ± 19.2 mV, mean±SD). By contrast, the cORP measured in plasma from healthy subjects were approximately double those from MetS subjects (0.50 ± 0.06 μC vs. 0.95 ± 0.06 μC; respectively, $P<0.0001$). Spearman correlations were conducted to find if characteristics of MetS could be linked to sORP and cORP values. The combined values of BMI, HDL cholesterol, HOMA, and uric acid explained 93% of the variation in cORP. Seven variables (BMI, waist circumference, total and HDL cholesterol, CRP, TNFα, and γ-tocopherol) explained 91% of the variation in sORP.

Assessment of ORP via this novel method appears to be a useful tool to rapidly assess metabolic status of a subject by using a fasting plasma sample. Higher sORP values appear to be a robust indicator of metabolic stress, while lower cORP values act as an indicator of lower metabolic resilience.

Abstract approved: _____

Maret G. Traber

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APPROVED:

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Toni Doolen, Dean, University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

Tora Jean Cobb, Author

INTRODUCTION

A current health problem that is faced by people in developed countries is the metabolic syndrome (MetS), which is diagnosed using a combination of three out of five of the following physiological abnormalities relative to healthy subjects: increased waist circumference, increased fasting blood triglycerides, increased fasting blood glucose, decreased HDL cholesterol levels, and increased resting diastolic and systolic blood pressures (13,1). Most individuals with MetS have a large abdominal circumference. A diagnosis of this syndrome is important because MetS is associated with an increased risk of cardiovascular disease (CVD), type 2 diabetes mellitus, stroke, and kidney disease (8, 32). Thus, MetS is a serious predictor of increased risk of additional chronic diseases.

MetS, or syndrome X due to the number of risk factors associated with it, has been steadily increasing in numbers because a third of the US population of men and women are obese (8) and obesity is the primary cause for developing MetS. Obesity, as measured by an increased body mass index (BMI), has been positively correlated with systemic oxidative stress markers and increased health issues (12). This oxidative stress can be measured through the oxidative reduction potential (ORP) of a biological solution. ORP is the transfer of electrons between each half reaction of redox species and is used to determine the overall potential of the electrochemical pairs in the sample and is an integrated measurement of both the pro-oxidants and antioxidants in a biological solution without relying on purification or processing for the isolation and measurement of any specific biomarkers.

Potentially, *in vivo* reactive oxygen species (ROS) generated from these cellular reactions could compromise cell integrity; dysregulation of these reactions is combated

through prooxidant/antioxidant pairs that are maintained the *in vivo* environment. In plasma, these pairs could include glutathione-glutathione disulfide, NAD^+ and NADH (and $NADP^+$ and NADPH), which can be assessed using the Nernst Equation (Figure 1). Disproportionate ROS results in an increase of damaged biomolecules that are higher in diseases such as traumatic brain injury, sepsis, stroke, myocardial infarction, Alzheimer's and atherosclerosis (6).

The RedoxSYS® meter measures static ORP (sORP) in millivolts (mV) and is proportional to the overall redox status of a plasma sample, with higher sORP values indicating a higher oxidation potential, which are often associated with higher instances of disease. The capacity of antioxidant reserves is measured by capacity ORP (cORP) in microcoulombs (μC), with lower values meaning the biological sample has below normal antioxidant levels.

Thus, we hypothesized that MetS compared with healthy adults would have higher sORP values and lower cORP values because obese and MetS patients are known to have higher oxidative stress (15). Indeed, higher levels of sORP have been shown in MetS subjects compared to healthy subjects using the RedoxSYS® (28). Therefore, the aim of the current study was to determine the standard redox potential of MetS plasma compared to healthy participant plasma using the RedoxSYS®.

MATERIALS AND METHODS

Materials

All disposable 3-electrode sensor strips and the RedoxSYS® Diagnostic System were provided as a gift from Luoxis' RedoxSYS® Diagnostic System (Englewood, CO).

Subjects and Study Design

Plasma samples were used from a collaborative study carried out by Drs. Maret Traber at the Linus Pauling Institute and Richard Bruno and his colleagues from The Ohio State University (1). The Oregon State University Institutional Review Board (IRB) and The Ohio State University IRB gave approval for the study.

Samples from healthy men and women (n = 5 M, 5 F/group; age: 18-40 y; BMI: 22.6 ± 0.7 kg/m^2) were compared with those from age- and gender-matched individuals with MetS (n = 5 M, 5 F/group; age: 18-40 y BMI: 37.7 ± 3.0 kg/m^2). Participants' waist circumference, weight, height, blood pressure, and fasting blood chemistries were measured and have been previously reported (1). MetS participants were categorized based on the presence of three of the five high risk factors for MetS (1): Waist circumference, fasting blood triglycerides, fasting blood glucose, HDL cholesterol levels, and resting diastolic and systolic blood pressures. Subjects were excluded from the study if they did not have a steady body weight for three months, smoked, had less than five hours of physical activity a week, drank more than three alcoholic beverages per day, had a history of gastrointestinal or lactose disorders, used medications that would affect lipid metabolism or ingested any dietary supplements for the past two months.

The parent study was designed to test the role of milk fat on vitamin E absorption. Participants consumed milk (whole, non-fat, reduced-fat, and soy) in random order with an encapsulated deuterium-labeled (d$_6$)-*RRR*-α-tocopherol to determine α-tocopherol

pharmokinetics in plasma and lipoproteins (1). All samples of blood and urine were collected from participants in the parent study at the Ohio State University Clinical Research Center from July 2013 to May 2014. Blood samples were collected from the antecubital vein at 0, 3, 6, 9, 12, 24, 36, 48, and 72 h; urine samples were collected in 8-hour intervals during the initial 24 hours of the intervention. Fasting subjects provided samples obtained at 0 24, 48 and 72 h.

The blood plasma used in the present study for analysis using the RedoxSYS[®] was collected into sodium heparin–containing evacuated tubes, then centrifuged and immediately frozen. Blood was centrifuged to obtain the plasma and then the plasma was immediately snap frozen in liquid nitrogen for subsequent storage at -80° C. Frozen samples were shipped on dry ice by overnight freight to Oregon State University for analysis.

Assessment of Plasma sORP and cORP using the RedoxSYS[®] Diagnostic System

To test the reliability of the outputs from the RedoxSYS[®], a control experiment was carried out. The oxidation-reduction potentials were analyzed in triplicate using the RedoxSYS[®] System using a plasma sample with or without added α-tocopherol. The samples for this test were prepared by adding known amounts of α-tocopherol to human recovered plasma (Valley Biomedical, Winchester, VA, #HP1051K3). The plasma sample was measured using our standard protocol for vitamin E analysis by high-performance liquid chromatography with electrochemical detection (20). The plasma contained 10 µM α-tocopherol; the added α-tocopherol (Supelco, Bellefonte, PA) increased the plasma α-tocopherol concentrations from 10 to 25 to 40 µM.

For the randomized control trial (described in the preceding section and (1)), we used plasma samples, which were collected in the fasting state at 0, 24, 48, and 72 h from each trial. The samples from one subject were thawed; a total of 16 samples, one from each time point (0, 24, 48, and 72 h) of the four study trials were analyzed. Each plasma sample was measured in a single run of four minutes to determine the sORP and the cORP, according to the manufacturer's instructions. Each analysis consists of 30 µL heparinized plasma, which is pipetted onto a disposable sensor after insertion into the RedoxSYS® unit. These disposable sensors are a three-electrode system comprised of a working, reference, and counter electrode. After four minutes of equilibration, the sORP (mV) value is established by the unit, which determines the mean electric potential measured during the last 10 seconds of the equilibration period. The cORP (µC) value, reported by the unit, is measured from the increased current between the counter and working electrodes. The cORP is calculated by the unit by integrating the results measured from the time at which the current begins to increase to the point of the maximum rate of change in the oxidation/reduction potential, as described (2).

STATISTICAL ANALYSIS

Data were analyzed with the MIXED Procedure with the Statistical Analysis System (SAS, version 9.4; SAS Institute). The author gratefully acknowledges the statistical analyses for this study, which were carried out by Dr. Gerd Bobe. In the control study, the variance of the effect of added α-tocopherol in a human pooled blood sample on cORP and sORP values was calculated in the MIXED Procedure: the fixed effect was sample α-tocopherol concentrations (10, 25, or 40 µM) and the random effect was from

the replicates (n=3). At each α-tocopherol concentration, the values were mean and the SD calculated and used to calculate the %CVs for the sORP and cORP values.

In the samples from the clinical trial, the variance components were estimated in the MIXED Procedure: the random effects in the model were sampling time (0, 24, 48, and 72 h); time period (day 1, 2, 3, or 4), their interaction; gender (female, male), health status (healthy, MetS), their interaction; and the subject nested in gender and health status. The fixed effect of health status was calculated in the MIXED Procedure: the fixed effects were sampling time, time period, milk source (whole milk, reduced fat milk, skim milk, soy milk), health status, gender, and the interactions of milk source, gender, and health status with sampling time.

Receiver operating characteristic (ROC) values and curves were calculated in GraphPad® Prism 6.02 (GraphPad Software, Inc., La Jolla, CA). Potential cut-off values were verified using Fisher's Exact test in PROC FREQ. Spearman correlations between sORP and cORP values and baseline concentrations of other variables were calculated in PROC CORR. To determine linear combinations of baseline concentrations with other variables that could explain the variation in sORP and cORP values, PROC REG was used.

All statistical tests were two-sided. Significance was declared at $P<0.05$.

RESULTS

Control Study

To determine the repeatability of the assays, the sORP and cORP values of pooled human plasma were measured in triplicate. The α-tocopherol concentrations of the final plasma solutions were 10 µM, 25 µM, or 40 µM. The mean %CVs for the sORP and cORP values were 5.3% and 5.7%, respectively, indicating the assays are repeatable (Table 1).

The pooled control sample study served also to determine the sensitivity of the assays, as sORP and cORP are designed to determine the oxidation-reduction potential of plasma samples, which is in part dependent on the increasing antioxidant, α-tocopherol, concentrations. Increasing the α-tocopherol concentration of the human plasma sample by 15 and 30 µM, decreased sORP values from baseline by 8.2% (P=0.008) and 12.5% (P=0.002), respectively, and increased cORP values from baseline by 17.3% (P=0.05) and 25.0% (P=0.01) (Figure 2a and b). Our results indicate the sORP and cORP values are sensitive to detect differences of oxidation-reduction potential of plasma samples.

Metabolic Syndrome Study

The next step was to determine the robustness of the assays. Plasma samples of 20 fasting, human subjects (10 men and 10 women, half of each gender, either healthy or having MetS) were obtained at 0, 24, 48, and 72 hours at four different occasions, each 2 to 4 weeks apart. The variability of sORP and cORP values (mean ± SD) measured in plasma from a healthy (Table 2) or a MetS (Table 3) subject four times per trial and over the four trials was assessed. These values demonstrated no significant changes within a single subject during the entire trial period. Representative subjects are shown; similar

findings were observed for all subjects. The %CVs among subjects were 12.1% and 49.7%, respectively, for sORP and cORP.

Importantly, sORP and cORP values differed between subjects that were healthy or had MetS (Figure 3 a and b). MetS subjects compared to healthy adults had 13.4% greater values of sORP (154.5±19.2 mV vs. 136.2±10.5 mV; $P<0.0001$) and 47.5% lower values of cORP (0.50±0.06 μC vs. 0.95±0.06 μC; $P<0.0001$) (Figures 3a and b).

Sampling time, sampling period and their interactions explained 0.2% of the variance for sORP and 0% of the variance for cORP. Thus, sORP and cORP are robust measurements within subjects (Figure 4).

The next step was to determine whether gender and health status affects sORP and cORP values (Table 4). Gender did not explain the variance observed between the MetS and healthy participants. Gender accounted for 0.9% of the variance for sORP and 0% of the variance for cORP, indicating that gender did not significantly affect sORP and cORP measurements (Figure 5). Similarly, the various milks administered in the four trials had no effect on these parameters in MetS and healthy participants (Figure 6 a and b).

In the next step, we wanted to determine whether sORP and cORP could be used to detect differences in health status between healthy and MetS subjects (Figure 7). Using the baseline values of the first time period, we calculated receiver operating characteristics (ROC) ± SEM values for sORP (0.86±0.09; 95% CI: 0.69-1.00; $P=0.007$) and cORP (0.94±0.06; 95% CI: 0.82-1.00; $P=0.001$) to identify MetS subjects. A cORP value of <0.50 μC could identify MetS with 90% sensitivity (9 out of 10 MetS syndrome subjects correctly classified) and 100% specificity (10 out of 10 healthy subjects correctly

classified; *P*-value for Fisher's Exact test: 0.0003). A sORP value of >140.0 mV could identify MetS with 90% sensitivity (9 out of 10 MetS subjects correctly classified) and 80% specificity (8 out of 10 healthy subjects correctly classified; *P*-value for Fisher's Exact test: 0.006).

There are several criteria for diagnosis of MetS that include anthropomorphic measures (i.e., large waist circumference), plasma lipid profile (i.e., high triglyceride and low HDL cholesterol concentrations), increased blood pressure, and elevated fasting blood sugar. Inflammation (i.e., elevated plasma CRP concentrations) has been recognized as independent risk factor for cardiovascular diseases. To determine whether sORP and cORP values were linked to one or several risk factors of cardiovascular disease, we calculated Spearman Correlations of these criteria with sORP and cORP values at baseline of the first period. cORP and sORP plasma values were linked to three cardiovascular risk factors: increased waist circumferences (cORP: *P*=0.002; sORP: *P*=0.02), elevated CRP concentrations (cORP: *P*=0.0009; sORP: *P*=0.002), and abnormal plasma lipid profile [i.e., high triglyceride (cORP: *P*=0.01; sORP: *P*=0.02) and oxidized LDL cholesterol concentrations (cORP: *P*=0.03; sORP: *P*=0.13) and low HDL cholesterol concentrations (cORP: *P*=0.0006; sORP: *P*=0.02)] but not to elevated blood pressure, abnormal glucose metabolism, and/or liver damage.

As cORP and sORP measure the oxidation-reduction potential of plasma samples, we also correlated cORP and sORP values to plasma concentrations of antioxidants. We observed that cORP and sORP values were not consistently linked to antioxidant status (not shown).

Additionally, we determined using multivariate-regression analysis, whether cORP and sORP values can be explained by a combination of our other baseline variables. Using as selection criterion the lowest parameter model that explained over 90% of the variation in cORP and sORP values, we observed that a linear combination of four variables (BMI, HDL cholesterol, HOMA, and uric acid) could explain 93.3% of the variation in cORP, and that a linear combination of seven variables (BMI, waist circumference, total and HDL cholesterol, CRP, TNFα, and γ-tocopheol) could explain 90.9% of the variation in sORP.

DISSCUSSION

This study shows that the RedoxSYS® outputs can be used as a quick indicator of MetS by measuring sORP and metabolic resilience assessed by cORP. MetS subjects compared to healthy adults had 13.4% greater sORP values and 47.5% lower cORP values.

sORP measurements were previously reported for MetS with type 2 diabetes (26). Spanidis et al showed that sORP was increased significantly in MetS patients compared to controls by 13.4%. They also demonstrated that glutathione levels were decreased, but thiobarbituric acid reactive substances, protein carbonyl levels, and total antioxidant capacity showed no statistical differences between groups (26). However, other indicators of inflammation that our study found strongly associated with MetS were not measured by Spanidis et al (i.e. CRP, IL-10).

Further, the present study demonstrated that the measurements have reasonable levels of variability within repeated measures. These results compare favorably to the repeatability and ability to detect sufficient variation among septic, traumatic brain injury

(TBI), and healthy patients to detect any differences present (24, 26, 27, 2). For example, sORP in the sepsis group increased by 9.3% compared to the control, while cORP decreased by 11.8% in the sepsis compared to the control group. Thus, sepsis had higher than normal sORP values, which results in a sample with higher state of oxidative stress (24). Other studies were also conclusive in finding distinct differences between TBI patients and healthy volunteers using similar testing; they found TBI values ranging from 150 mV to 170 mV compared to their control (healthy) samples of 130 mV with a SD of 9 mV (27). Our healthy subject values were also similar to the healthy subjects measured for assessing eccentric exercise-induced oxidative stress, with sORP values averaging 136.1 ± 13.2 mV and cORP values averaging 1.05 ± 0.71 μC compared to our healthy subject values of sORP 136.2±2.8 mV and cORP values of 0.95±0.06 μC (2).

The low coefficients of variation (CV) of periods, trials, gender, milk type consumed, and health status (Figures 2-6) all demonstrate the low variability of determining the oxidative status by the RedoxSYS®. Plasma was collected over 10 months (1), and the low %CV values were a surprising result due to the already tumultuous nature of oxidative stress variability in plasma over large time periods. Individual subject variation between collection increases over months, especially samples over a few months to a year (19). This lack of variation is a prime example why these oxidative stress values are a reliable test demonstrating oxidative stress in MetS patients. However, to further assess variability, future studies with the RedoxSYS® need to evaluate changes in sORP and cORP within a person during a metabolic stress test and also evaluate changes among people that are less different than in our study.

Plasma is a complex mix of various redox lipids and proteins, so measuring individual oxidative stress markers would not provide the complete picture necessary for the oxidative status of the subject. Lipids are prominent molecules for ROS attack, especially since elevated triglyceride levels are associated with obesity and MetS. Thus the lipids present (polyunsaturated fatty acids, etc.) are the most prone to oxidation and are involved in the composite oxidation reduction potential of the sample, with ORP values increasing as the levels of lipid peroxidation increase (22) and oxidized biomolecules contributing to the high sORP values of the plasma sample. Along with lipids, it should also be noted that there is an association with an increase in the amount of oxidized LDL (OxLDL) in MetS patients along with an increase in oxidative stress associated with low high density lipoprotein (HDL), both parameters of MetS (29, 30). Inflammation markers and oxidative stress inducers (isoprostanes, CRP, IL-10, IL-6, TNFα, etc.) are reliable indicators of oxidative stress, with the inflammatory response increased from the accumulation of oxidized biomolecules such as lipid peroxidation products, degraded proteins from protein oxidation, and nucleic acids (22, 23, 25).

Decreased analysis time compared to other methods and the consistent demonstration of low variability in analysis groups even with the natural variability that occurs in blood chemistry is reason for the RedoxSYS[®] to be used as a quick, easy, and accurate oxidative stress measurement system in a clinical setting. Thus, our findings suggest that RedoxSYS[®] can be used as a useful tool in identifying MetS subjects.

CONCLUSION

The RedoxSYS[®] demonstrates a significant difference in oxidative stress parameters (cORP and sORP) between the plasma of MetS subjects compared to healthy

subjects. Capacity ORP is a robust indicator of metabolic resilience, which is closely associated with BMI, lipid metabolism, and inflammation. Higher cORP values indicate greater resilience, as demonstrated by higher cORP values in healthy subjects. Static ORP is a robust indicator of metabolic stress, which is also closely associated with BMI, lipid metabolism, and inflammation, with higher values indicating a greater metabolic stress response. Additionally, α-tocopherol addition to standard plasma decreases sORP and increases cORP values, indicating that vitamin E supplementation increases antioxidant resilience in a biological sample.

FIGURES

Figure 1. Nernst Equation. The concentrations of oxidants and reductants determine the overall redox potential (oxidation-reduction potential) of the plasma when placed on the open circuit single-use electrodes of the RedoxSYS®.

$$E = E° + \frac{RT}{nF} \ln \frac{[Ox]}{[Red]}$$

E = Oxidation-Reduction Potential

E° = Standard Reduction Potential

R = Universal Gas Constant

n = number of electrons transferred

T = Temperature

F= Faraday Constant

[Ox] = Concentration of Oxidized Species

[Red] = Concentration of Reduced Species

Figure 2 a and b. α-Tocopherol (ATOC) addition to plasma to determine the repeatability of the assays. We measured sORP and cORP values of three samples of pooled human plasma, and demonstrated decreasing sORP values as ATOC increases and increasing cORP values as ATOC increases.

a. sORP
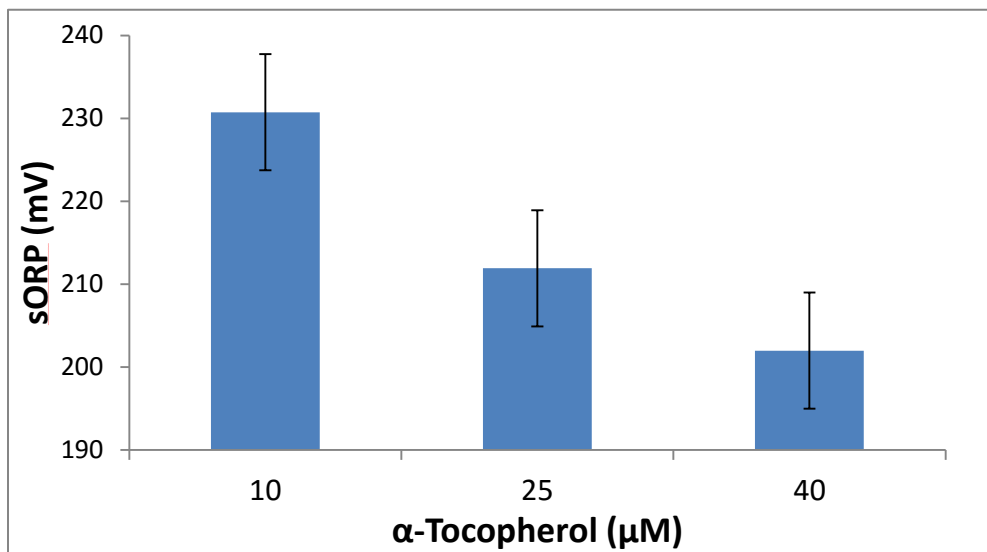
b. cORP
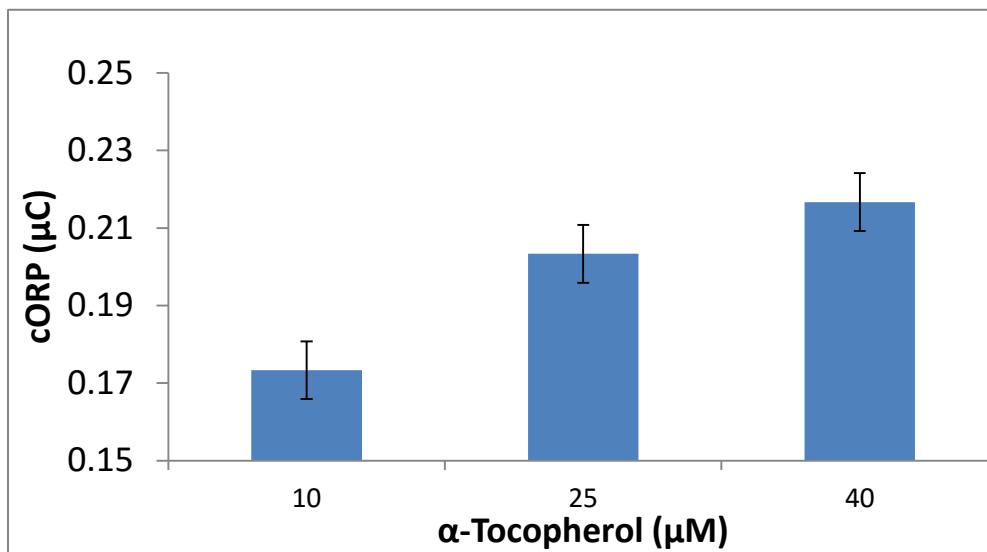
Figure 3 a and b. Scatter plots of the means of the four collection times (0, 24, 48, 72 h) for (a) static ORP (sORP) ($P<0.0001$) and (b) capacity ORP (cORP) ($P<0.0001$) of healthy and MetS subjects, demonstrating the decreased cORP and increased sORP in MetS compared with healthy subjects.
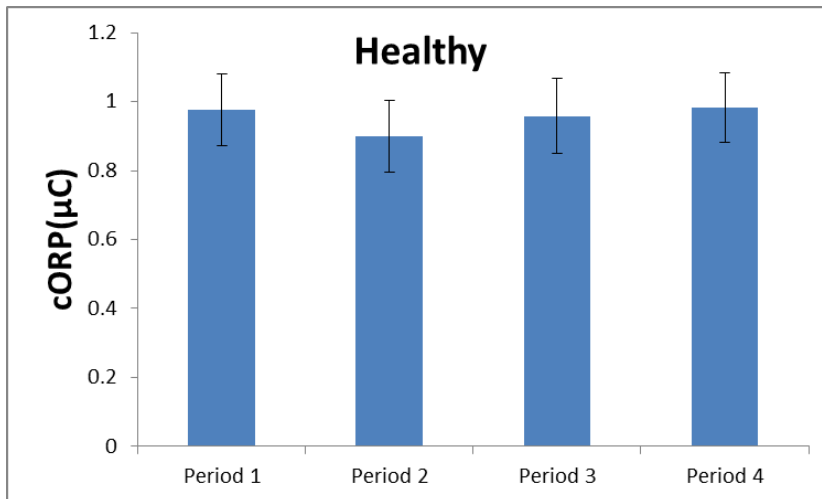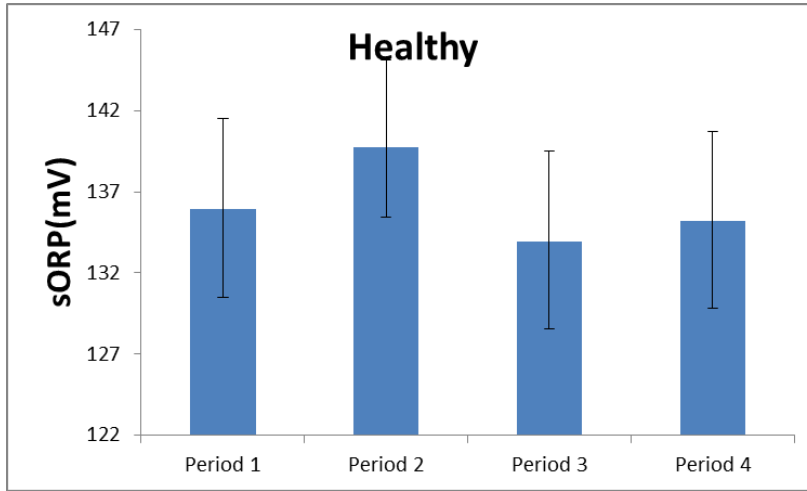
a. sORP
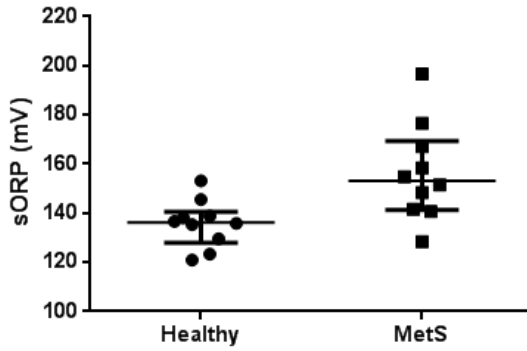
b. cORP
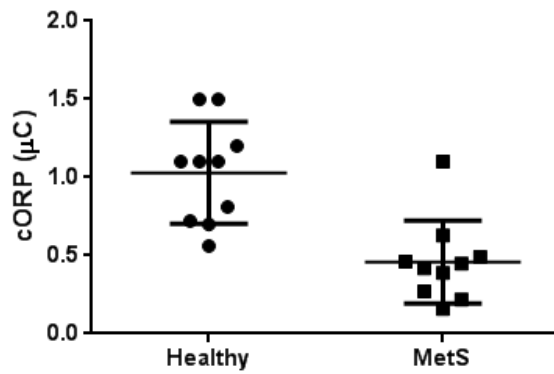
Figure 4. No significant changes within a subject from the effect of trial period on sORP or cORP values for (a) Healthy subject or (b) Metabolic Syndrome subject.
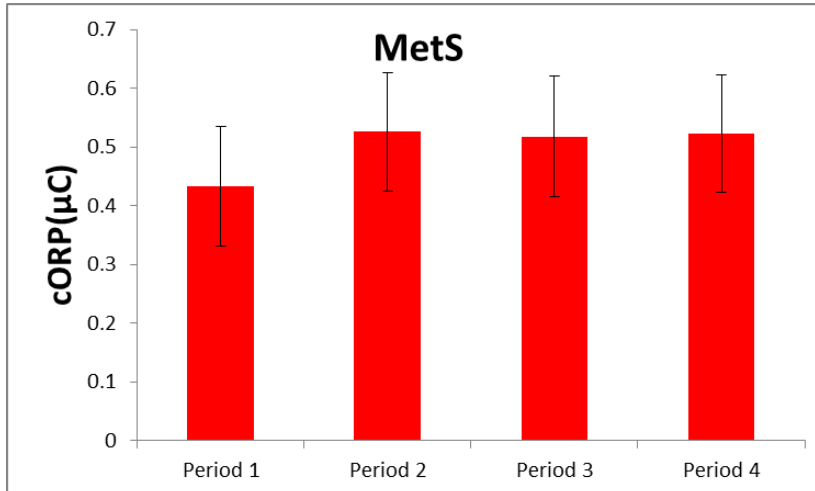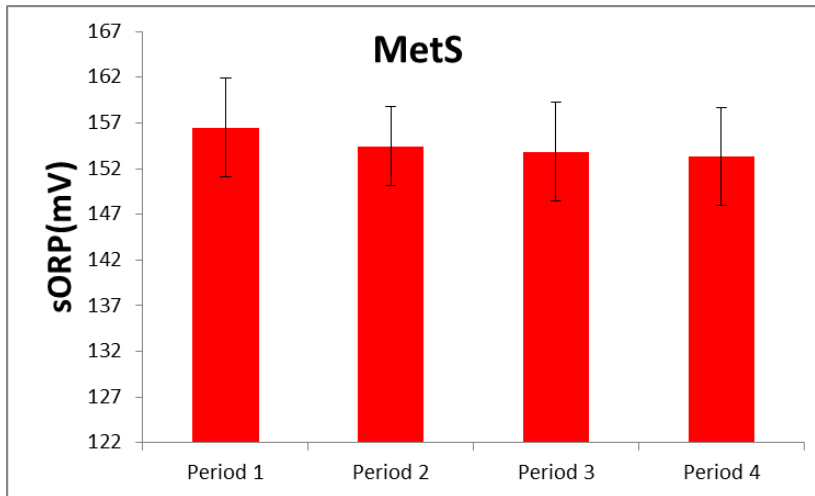
a. Healthy

b. Metabolic Syndrome

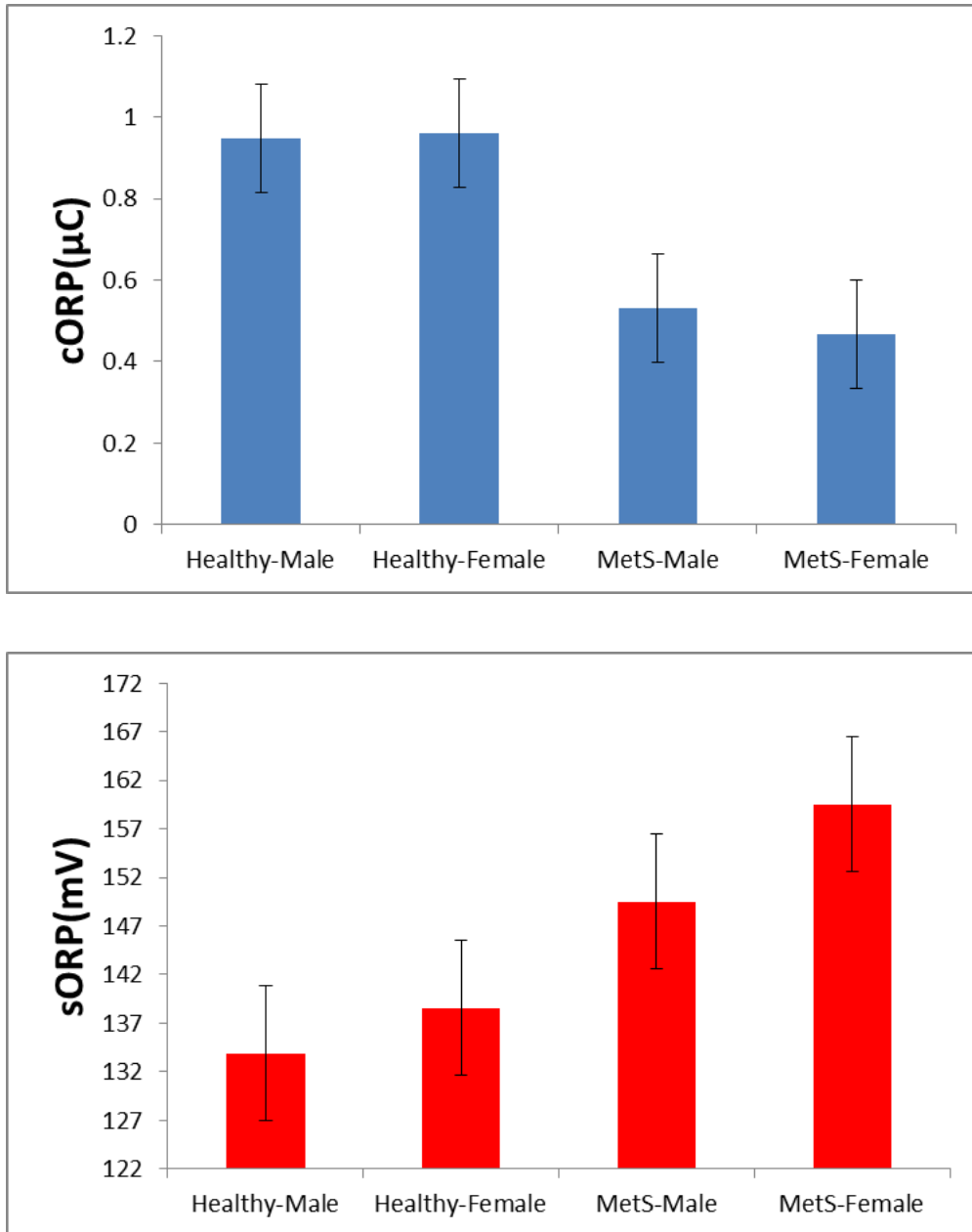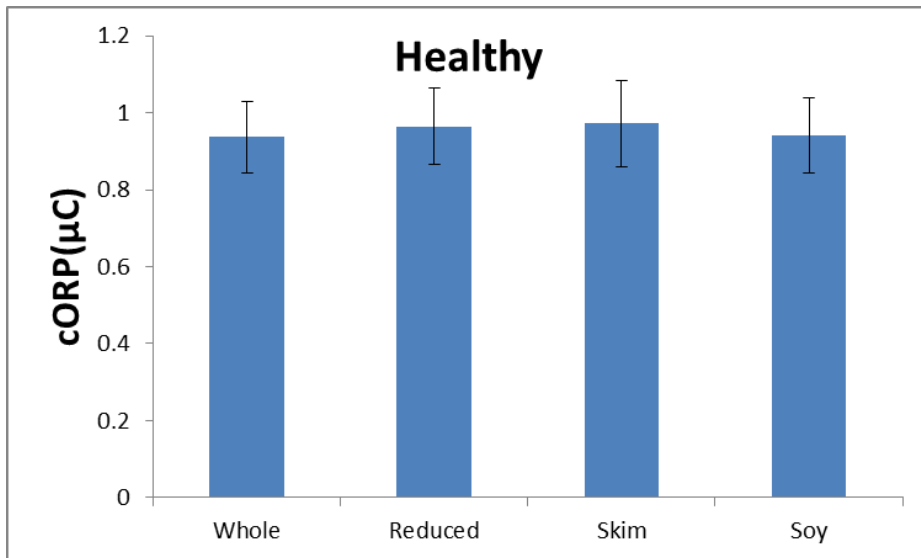Figure 5. Gender within each group had no effect on cORP and sORP.
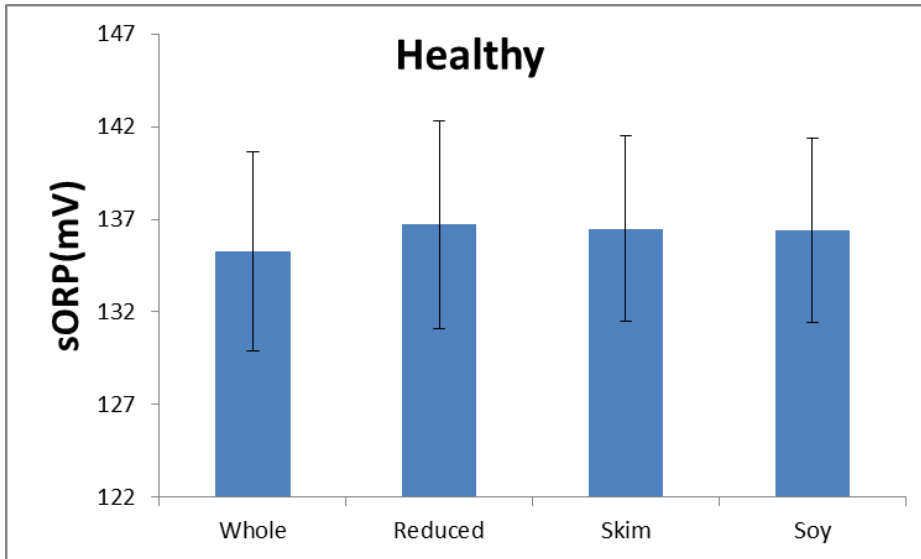
Figure 6. The various milks administered in the four trials had no effect on these parameters in MetS and healthy participants
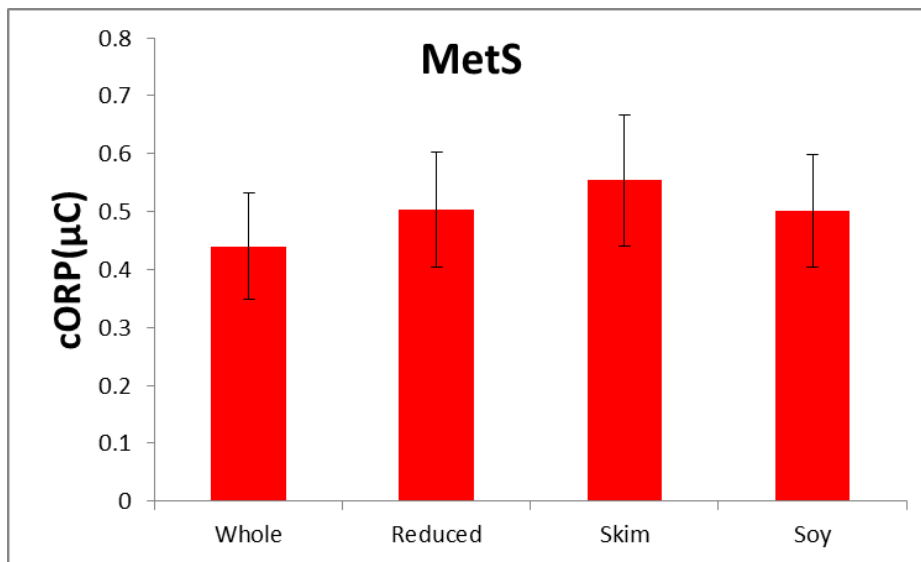
a. Healthy

b. Metabolic Syndrome
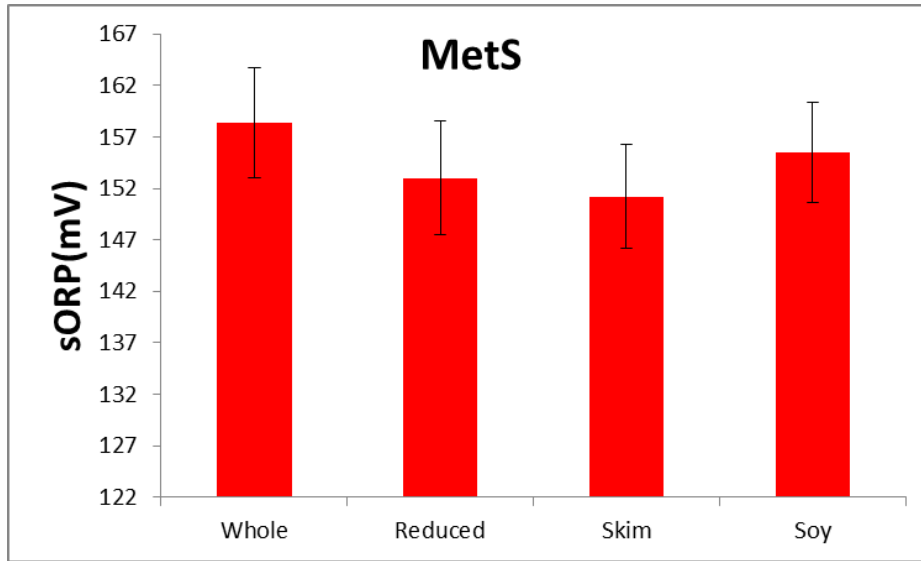
Figure 7. ROC curves for (a) sORP and (b) cORP that show differentiation between Healthy and MetS subjects. A ROC = 1 is perfect differentiation, while a ROC = 0.50 means no relationship.

a. sORP
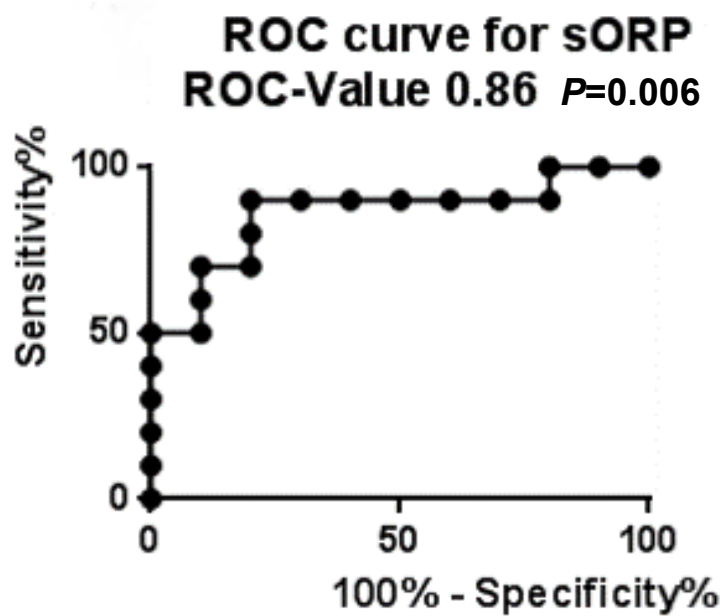
b. cORP
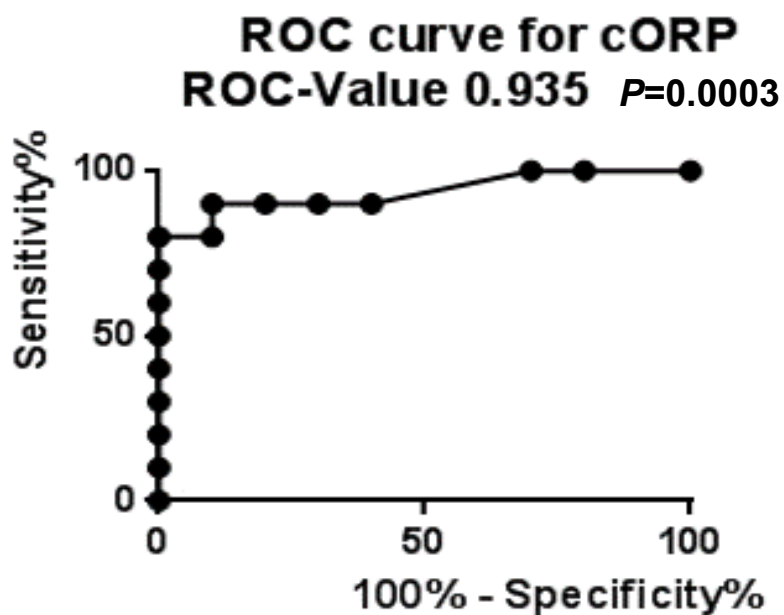
Table 1. Variability in sORP and cORP values (mean ± SD) measured in triplicate using plasma samples with 0, 15, or 30 μM added α-tocopherol (μmol/L plasma).

Treatment (μmol/L):	10	25	40
sORP (mean ± SD)	231 ± 15	212 ± 13	202 ± 7
%CV	6.6%	6.1%	3.4%
cORP (mean ± SD)	0.17 ± 0.02	0.20 ± 0.01	0.22 ± 0.01
%CV	12%	2.8%	2.7%

Table 2. Variability of sORP and cORP values (mean ± SD) measured four times per period and in four separate trials using plasma from a representative healthy subject.

Period	1	2	3	4	Mean Variability of Trials 1-4
sORP	138±7	140±7	132±4	125±4	134±7
%CV Mean	5.2	4.7	2.9	9.3	5.2
cORP	0.81±0.2	0.67±0.2	1.0± 0.2	0.84±0.08	0.83±0.1
%CV Mean	26	22	24	9.3	17

Table 3. Variability of sORP and cORP values (mean ± SD) measured four times per period and in four separate trials using plasma from a subject with metabolic syndrome.

Trial	1	2	3	4	Variability of Trials 1-4
sORP	128±6	135±6	133±3	133±5	132 ± 3
%CV	4.4	4.3	2.1	3.7	2.2
cORP	1.1± 0.3	0.85±0.3	1.1±0.2	1.0±0.2	1.0 ± 0.1
%CV	23	34	21	19	13

Table 4. Mean, %CV, and SEM of male, female, and combined for (a) healthy and (b) MetS subjects.

(a) Healthy (n=5 male, 5 female)

Gender	Male	Female	Combined
sORP Mean±SD	135±10	137±11	136±10
%CV	7	8	7
cORP Mean±SD	1.0±0.4	1.1±0.3	1.0±0.3
%CV	40	30	30

(b) Metabolic Syndrome (n=5 male, 5 female)

Gender	Male	Female	Combined
sORP Mean±SD	153±19	160±20	156±21
%CV	12.7	13.4	7.1
cORP Mean±SD	0.5±0.4	0.4±0.2	0.4±0.3
%CV	73	39	57

Table 5. Mean ± SD and %CV of Soy, Skim, Whole, and Reduced Fat (RF) Milk for (a) healthy and (b) MetS subjects.

(a) Healthy (n=10)

Milk Type	Soy	Skim	Whole	RF	Total
sORP Mean±SD	137±10	136±9	135±10	137±14	136±11
%CV	7.6	6.3	7.2	10	7.7
cORP Mean±SD	0.91±0.3	0.98±0.4	0.96±0.3	0.99±0.4	0.95±0.3
%CV	34	38	34	36	35

(b) Metabolic Syndrome (n=10)

Milk Type	Soy	Skim	Whole	RF	Total
sORP Mean±SD	155±20	151±20	158±21	154±18	155±19
%CV	12.8	13.4	13.1	12.0	12.4
cORP Mean±SD	0.51±0.3	0.55±0.3	0.45±0.2	0.48±0.2	0.50±0.3
%CV	57	51	51	46	50

Table 6. Mean % coefficients of variation between plasma (Primary Identifying Code, or PID) samples, plasma (PID) and time, plasma and milk source, and α-tocopherol addition (ATOC).

	PID	PID x Time	PID x Milk Source	ATOC
sORP (%CV)	5.94	5.71	4.84	5.34
cORP (%CV)	26	23	20	15.8

Table 7. *P*-values of effects of milk type, gender, period, health status, time collected, combined health status and time collected, combined gender and time collected, and combined milk type and time collected.

	sORP	cORP
Effect	**Pr > F**	**Pr > F**
Milk	0.8599	0.7012
Gender	0.0644	0.7448
Period (Trial)	0.9468	0.9165
Health Status	<0.0001	<0.0001
Time	0.492	0.8832
Health Status*Time	0.9201	0.5711
Gender*Time	0.9859	0.8405
Milk*Time	0.393	0.1318

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