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Increased static and decreased capacity oxidation-reduction potentials in plasma are predictive of metabolic syndrome☆,☆☆

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ABSTRACT

Electric conductivity in plasma is the balance between oxidized and reduced molecules (static Oxidation-Reduction Potential, sORP) and the amount of readily oxidizable molecules (capacity ORP, cORP). Adults with metabolic syndrome (MetS) have increased inflammation, dyslipidemia and oxidative stress; therefore, participants with MetS were hypothesized to have higher plasma sORP and lower cORP than those measures in healthy adults. Heparin-anticoagulated plasma from healthy and age- and gender-matched individuals with MetS (BMI: 22.6 ± 0.7 vs. 37.7 ± 3.0 kg/m^2, respectively) was collected in the fasting state at 0, 24, 48, and 72 h during each of four separate interventions in a clinical trial. At baseline, plasma sORP was 12.4% higher (P=0.007), while cORP values were less than half (41.1%, P=0.001) in those with MetS compared with healthy participants. An sORP > 140 mV detected MetS with 90% sensitivity and 80% specificity, while a cORP < 0.50 μC detected MetS with 80% sensitivity and 100% specificity. sORP and cORP values in participants with MetS compared with healthy adults were linked to differences in waist circumference and BMI; in plasma markers of dyslipidemia (triglycerides, HDL-cholesterol, and oxidized LDL-cholesterol) and inflammation (C-reactive protein, IL-10); as well as with urinary markers of lipid peroxidation (e.g., 2,3-dinor-5,6-dihydro-8-iso-PGF$_{2\alpha}$; 2,3-dinor-8-iso-PGF$_{2\alpha}$). Higher sORP values are a robust indicator of metabolic stress, while lower cORP values act as an indicator of decreased metabolic resilience.

1. Introduction

The term "Metabolic Syndrome" (MetS) is a rubric that describes patients with increased risk for cardiovascular diseases, diabetes mellitus, and all-cause mortality. Approximately 25% of the adult US population has MetS, with patients fulfilling at least three out of the following five criteria: 1) waist circumference ≥102 cm (males) or ≥88 cm (females), 2) fasting plasma glucose ≥100 mg/dL, 3) blood pressure ≥130/85 mm Hg, 4) fasting plasma triglyceride ≥150 mg/dL, or 5) high-density lipoprotein-cholesterol (HDL-C) < 40 mg/dL (males) or < 50 mg/dL (females) [1,2]. In addition to cardiovascular diseases and all-cause mortality [3], MetS is linked to increased risk of the major cancers [4–7], chronic kidney disease [8], as well as to a decline in cognitive function [9,10]. MetS patients have double the health-care, pharmaceutical, and short-term disability costs in the workplace [11]. Therefore, early identification of MetS patients is critical so that aggressive therapeutic interventions can begin.

RedoxSYS® is a novel technology that measures in 4 min the static oxidation-reduction potential (sORP), measuring the potential of an electrochemical cell under static conditions; followed by measuring the capacity oxidation-reduction potential (cORP), which is the total amount of readily oxidizable molecules [12]. Previous studies demonstrated that sORP are linked to the proportion of cysteinylated residues on albumin in serum as measured by LC-MS [13] and thus may indicate concentrations of oxidized molecules in blood. Higher plasma sORP values have been observed in patients with traumatic brain injury

Abbreviations: CV, coefficient of variation; cORP, capacity Oxidation Reduction Potential; sORP, static Oxidation Reduction Potential; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein; IL, Interleukin; TNFα, Tumor Necrosis Factor alpha

☆ Reprints will not be made available from the authors.

☆☆ This trial was registered at www.clinicaltrials.gov as NCT01787591.

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[13,14], sepsis [15,16], and in MetS patients with type II diabetes [17]. In addition, patients with sepsis have lower cORP [16].

The objective of this study was to evaluate the utility of measures of plasma sORP and cORP, and to compare them with functional indicators of MetS in non-diabetic patients. We hypothesized that MetS adults would have higher plasma sORP values and lower cORP values than those in healthy subjects. To test our hypothesis, plasma and urine samples that were collected over several months during a randomized, cross-over, double-blind study in healthy and MetS participants taking part in a clinical trial (NCT01787591) to evaluate vitamin E status and pharmacokinetics [18], as well as vitamin E catabolites as biomarkers of status [19], were used to perform measures of oxidation-reduction potentials and to assess these latter outcomes as biomarkers of metabolic health.

2. Materials and methods

2.1. Materials

Disposable 3-electrode sensor strips and the RedoxSYS® Diagnostic System were provided as a gift from Luoxis' RedoxSYS® Diagnostic System (Englewood, CO). Authentic samples of 8-iso-prostaglandin F2α (8-iso-$PGF_{2\alpha}$); PGF2α; 2,3-dinor-8-iso-$PGF_{2\alpha}$; and 2,3-dinor-5,6-dihydro-8-iso-$PGF_{2\alpha}$ and the deuterated internal standard 8-iso-$PGF_{2\alpha}$-d_4 were obtained commercially (Cayman Chemical; Ann Arbor, MI).

2.2. Description of RedoxSYS® measurements

Plasma sORP and cORP values were measured using the RedoxSYS® Diagnostic System according to the manufacturer's instructions (Luoxis Diagnostics, Inc., Englewood, CO, USA). In brief, heparinized plasma (30 μL) was placed on a disposable RedoxSYS® sensor, containing three electrodes (working, counter, reference), which had been inserted into a galvanostat-based reader, the RedoxSYS® analyzer. Manufacturer's literature indicates that plasma sORP values are measured while applying a low oxidizing current (1 nA; does not affect sample integrity) to the sample. After allowing 1 min and 50 s for equilibration, the reader measured twice per second over 10 s the difference in potential between working and reference electrode in mV (the detection limit is 1 mV). Subsequently, plasma cORP values are measured while applying a linearly increasing oxidizing current until the charge rapidly changes between working and reference electrode, which indicates that all readily oxidizable molecules are oxidized. The time until the charge changes is used to calculate the number of electrons needed to cause charge changes and is reported in μCoulomb (μC). Specifically, as explained in an electrochemistry textbook [12]: "Coulometric methods are based on Faraday's law that the total charge or current passed during an electrolysis is proportional to the amount of reactants and products in the redox reaction. If the electrolysis is 100% efficient—meaning that only the analyte is oxidized or reduced—then we can use the total charge or current to determine the amount of analyte in a sample."

2.3. Assessment of measurements of sORP and cORP in vitro

To verify the integrity of the measurements made by the RedoxSYS® system, the potentials of increasing concentrations of ascorbic acid (10–80 μM) in 10 μM phosphate buffered saline (pH 7.2) as the test matrix, were analyzed in triplicate using the RedoxSYS® System compared with the electrode strips attached to the CHI Potentiostat (http://www.chinstruments.com). In order to be used with the potentiostat, each component of the strip was identified and the leads were connected accordingly. The strips were composed of silver working, silver chloride reference (3M KCl), and silver counter electrodes, as well as a ground. Using a stock ascorbic acid solution (100 μM), test concentrations of 10 μM, 20 μM, 50 μM, and 80 μM were prepared in PBS; both the stock and the test concentrations were prepared under nitrogen immediately before use in order to minimize the effects of decomposition due to the presence of oxygen. Separate strips were used for each trial and each ascorbic acid concentration. The RedoxSYS® System was operated according to the manufacturer's specifications. For the CHI Potentiostat, the Multi-Current Steps (ISTEP) program was used with the initial step being set to −1 nA for a duration of 120 s with a sample interval of 0.02 s, based on [14]. A cyclic voltammogram of ascorbic acid was performed and the −1 nA current was found to be near the foot of the wave, thus providing a stable background from which to base measurements. The ascorbic acid test solutions were used to generate standard curves from each machine, then the potential vs concentration data, which were generated from each was used to assess the reliability of the measures of a 40 μM ascorbic acid solution.

To test the reliability of the outputs from the RedoxSYS®, a control experiment using plasma as the test matrix was also performed. The oxidation-reduction potentials of a plasma sample with or without added α-tocopherol was analyzed in triplicate using the RedoxSYS® System because we found the ascorbic acid solution too easily oxidizable for our test conditions. The samples for this test were prepared by adding known amounts of α-tocopherol (Sigma-Aldrich) to human recovered plasma (Valley Biomedical, Winchester, VA, #HP1051K3). Confirmation of the vitamin E concentration in the plasma sample was performed using our standard protocol that uses high-performance liquid chromatography with electrochemical detection [20]. The plasma α-tocopherol concentrations were 10 μM; the added α-tocopherol increased the concentrations to 25 and 40 μM.

2.4. Clinical trial study design and assessment of plasma sORP and cORP

Plasma samples from healthy and MetS participants were from a double blind, randomized, crossover study carried out at The Ohio State University from July 2013 to May 2014. This trial was registered at www.clinicaltrials.gov as NCT01787591. The Oregon State University Institutional Review Board (IRB) and The Ohio State University IRB's gave approval for the study. The original study has been described in detail previously and baseline information published [18]. In brief, the study population consists of 20 subjects, aged 24–40 years old (Table 1). MetS subjects (n=10) fulfilled 3 (n=7) or 4 (n=3) of the diagnostic criteria for MetS at baseline. Healthy and MetS subjects were matched by gender (5 per gender and group) and by age (age-matched within 5 years). None of the 10 healthy subjects fulfilled any MetS diagnostic criteria. Other inclusion criteria were stable body weight (± 2 kg during past 3 mo), no use of dietary supplements during the past 2 mo, no use of medications known to affect lipid or glucose metabolism, nonsmoker, < 3 alcoholic drinks/d, < 5 h of aerobic activity/wk, and no history of gastrointestinal disorders or lactose intolerance.

Using a Latin-square design, participants consumed milk (soy milk; non-fat, reduced-fat, or whole milk) in random order with an encapsulated deuterium-labeled (d_6)-*RRR*-α-tocopherol to assess the role of dairy fat on α-tocopherol bioavailability and pharmacokinetics [18]. The four milk trial periods were 2–4 weeks apart and each lasted 72 h. The sORP and cORP measures were made before and after the administration of the d_6-*RRR*-α-tocopherol to assess the sensitivity of the measurements to minor changes in vitamin E intakes (e.g. 15 mg). Fasting blood samples used in the present study were collected from the antecubital vein at 0, 24, 48, and 72 h into sodium heparin–containing evacuated tubes, then centrifuged and immediately frozen. Thus, for each subject there were 4 trials with 4 fasting time points for a total of 16 samples per subject. Blood was centrifuged to obtain the plasma and then immediately snap frozen in liquid nitrogen for subsequent analysis or storage at −80 °C. Frozen samples were shipped