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Reactive oxygen species in exercise and insulin resistance: Working towards personalized antioxidant treatment

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ABSTRACT

Reactive oxygen species (ROS) are well known for their role in insulin resistance and the development of cardiometabolic disease including type 2 diabetes mellitus (T2D). Conversely, evidence supports the notion that ROS are a necessary component for glucose cell transport and adaptation to physiological stress including exercise and muscle contraction. Although genetic rodent models and cell culture studies indicate antioxidant treatment to be an effective strategy for targeting ROS to promote health, human findings are largely inconsistent. In this review we discuss human research that has investigated antioxidant treatment and glycemic control in the context of health (healthy individuals and during exercise) and disease (insulin resistance and T2D). We have identified key factors that are likely to influence the effectiveness of antioxidant treatment: 1) the context of treatment including whether oxidative distress or eustress is present (e.g., hyperglycemia/lipidaemia or during exercise and muscle contraction); 2) whether specific endogenous antioxidant deficiencies are identified (redox screening); 3) whether antioxidant treatment is specifically designed to target and restore identified deficiencies (antioxidant specificity); 4) and the bioavailability and bioactivity of the antioxidant which are influenced by treatment dose, duration, and method of administration. The majority of human research has failed to account for these factors, limiting their ability to robustly test the effectiveness of antioxidants for health promotion and disease prevention. We propose that a modern “redox screening” and “personalized antioxidant treatment” approach is required to robustly explore redox regulation of human physiology and to elicit more effective antioxidant treatment in humans.

Key points

- Research-to-date provides a confusing narrative on whether antioxidant treatment has the potential to be an effective strategy for improving health and/or treating and preventing chronic disease.
- Factors influencing effective antioxidant treatment include 1) whether oxidative distress/eustress is present (sedentary behavior, poor diet, disease, or exercise), 2) whether an endogenous antioxidant deficiency exists, 3) whether the antioxidant treatment is specifically designed to target and restore a deficiency (antioxidant specificity), 4) and the bioavailability and bioactivity of the antioxidant which are influenced by treatment dose, duration, and method of administration.
- We propose that a modern “redox screening” and “personalized antioxidant treatment” approach to research and clinical practice,

which considers the context of treatment and the individual, is required to robustly explore redox regulation of human physiology and to elicit more effective antioxidant treatment in humans.

1. Introduction

Oxidation-reduction (redox) reactions occur ubiquitously in biology and are necessary to maintain an environment optimal for cell signaling and function. These redox reactions involve reactive oxygen species (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) which regulate several physiological and pathophysiology processes ranging from cell apoptosis and death, to cell differentiation and growth [1–6]. In humans, some of the most well-known stimuli for inducing ROS production include postprandial substrate oxidation and the mechanical and physiological stress induced through exercise [7–15]. To maintain

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cellular redox homeostasis, numerous enzymatic and non-enzymatic antioxidant defenses have evolved to regulate cellular levels of ROS [1,2,16–21]. Examples of enzymatic antioxidant sources include superoxide dismutase, glutathione peroxidase, and catalase, whereas examples of non-enzymatic sources include glutathione (GSH) and vitamins C and E. An increase in ROS or a decrease in antioxidant activity can lead to a cellular redox environment commonly referred to as “oxidative stress”.

Overwhelming evidence exists supporting the pathological role of ROS in the development of chronic conditions and diseases including insulin resistance and type 2 diabetes (T2D) [9,10,13,22–27]. In stark contrast, accumulating evidence now supports the physiological role of ROS in maintaining cardiometabolic health and the prevention of conditions and diseases including insulin resistance and T2D [22,24,28–34]. Acknowledging this dual role, the simplified concept of oxidative stress as being exclusively “bad” has been vetoed by many in the field of redox biology and physiology in favor of the more recently preferred descriptive terms of oxidative distress and oxidative eustress [35,36].

Antioxidant is a broad all-encompassing term used to describe endogenous and exogenous compounds or biological processes that can directly (e.g., free radical scavenging) or indirectly (e.g., the regulation of ROS-producing and/or antioxidant enzymes) alter the oxidative dis/eustress redox environment [8]. Although the literature on the use of antioxidant treatment for decreasing “oxidative stress” continues to expand [8,22,37,38], a unifying consensus on the effectiveness of treatment for improving health and/or preventing disease has yet to arise. Inconsistent findings and a lack of consensus likely stem from generic antioxidant treatment practices which fail to acknowledge the dynamic and dual role of ROS in both human physiology and pathophysiology. In this narrative review of the literature, we discuss previous and emerging research that has investigated oxidative stress, antioxidants and insulin and glucose regulation under various oxidative eustress and distress conditions, including acute and regular exercise, hyperglycemia, hyperlipidemia, and overt T2D. Our discussion is focused on research in humans, however, where necessary we refer to findings from relevant animal models or *in vitro* studies that may provide a mechanistic basis for the observations in human studies. The redox health paradox is further explored through the discussion of antioxidant treatment under the varying conditions of oxidative distress and eustress, and the subsequent effects on insulin and glucose regulation. Finally, we synthesize and contextualize current antioxidant treatment evidence to help guide a new era of research - one that harnesses the potential of personalized antioxidant treatment to robustly explore redox regulation of human physiology.

2. Oxidative distress: implications for glycemic control and disease

Reactive oxygen species cause oxidative modification to lipids, proteins, and DNA. This can lead to impaired transcription and translational processes, altered protein function, and production of secondary by-products and metabolites which lead to further ROS production and/or cellular damage [39–46]. One of the most well-documented stimuli for producing ROS in biological organisms is through postprandial metabolism of carbohydrates, lipids, and protein [9,22,23,47,48]. The detrimental effects of substrate metabolism on insulin and glucose regulation are largely thought to occur through excess lipid and carbohydrate intake [9,10,13,23–27]. Hyperglycemia and hyperlipidemia at rest (basal) or following meal ingestion (postprandial) can lead to increased cellular oxidative distress through mitochondrial electron leak and/or incomplete fatty acid oxidation, the formation of advanced glycation end products and other oxidation products including lipid hydroperoxides, and increased circulation of free fatty acids (FFA), diacylglycerol, and ceramides [9,10,23,49–51].

Pathways of oxidative distress-induced insulin resistance are

reviewed in detail elsewhere [9,22,28]. In brief, hyperglycemia and hyperlipidemia-induced production of ROS and ROS byproducts can lead to impaired insulin sensitivity through structural and functional changes to the insulin molecule decreasing its bioactivity [52], and through activation of redox-sensitive cell signaling pathways that interfere with insulin signaling and glucose cell transport [22,28]. Redox-sensitive components of signaling pathways identified to contribute to insulin resistance include p38 mitogen-activated protein kinase (p38 MAPK), c-Jun N-terminal kinase (JNK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), extracellular-signal regulated kinase (ERK), and protein kinase C (PKC), and the lipid peroxidation product 4-hydroxy-2-nonenal (4-HNE) [22,24,27,28,52–62]. Activation of these pathways increase serine and threonine phosphorylation of the insulin receptor substrates (IRS) 1 and 2, inhibiting downstream insulin signaling through attenuated tyrosine phosphorylation and IRS proteasomal degradation and subcellular re-localization [22,24,28,53–62]. Additionally, increased activity of the redox-sensitive protein tyrosine phosphatase (PTP) family also leads to inhibition of the insulin signaling pathway, as observed during high-lipid culture conditions in skeletal muscle cells [14]. Insulin resistance and mitochondrial dysfunction promote a vicious cycle of increased hyperglycemia and hyperlipidemia, ROS production, impaired insulin sensitivity, and the eventual development of overt T2D and other cardiometabolic diseases [22,28,51]. Although the authors acknowledge the complex and integrated nature of tissue-specific regulation of glucose homeostasis and ROS production, the current review focuses predominantly on skeletal muscle as it is one of the major sites for insulin dependent and independent glucose disposal [63,64].

Oxidative distress and impaired glycemic control. Elevated biomarkers of systemic oxidative stress and/or decreased antioxidant activity in blood, urine, or muscle, are reported among individuals who are sedentary or obese, have impaired glucose tolerance (IGT), and/or have T2D [10,65–70]. For example, skeletal muscle mitochondrial H₂O₂ emissions in obese individuals at rest (fasting basal conditions) are two-fold higher compared to lean individuals [10]. Furthermore, both the ingestion of a single high-fat meal or a 5-day high-fat diet in healthy individuals leads to increased muscle oxidative stress, as measured by a decrease in the GSH and oxidized glutathione (GSSG) ratio and elevated skeletal muscle mitochondrial H₂O₂ production, to similar levels of that observed in obese insulin-resistant individuals [10]. Additional experiments revealed that comparable high-fat conditions in rodents lead to a similar shift towards a more oxidative environment in muscle alongside the development of insulin resistance [10]. Human studies have also reported greater measures of systemic oxidative stress and lower antioxidant activity in blood from T2D and IGT individuals at rest (fasting) and following high-fat meal ingestion compared to healthy controls [68], supporting the notion of increased oxidative distress in populations characterized by insulin resistance.

However, oxidative distress and insulin resistance can also occur in healthy individuals. For example, independent of health status, markers of systemic oxidative distress can remain elevated for up to 4 h following the ingestion of pure carbohydrate [71,72], fat, or protein [48], and mixed macronutrient meals containing high-fat [73–78] and high-carbohydrate content [79]. Larger meals and meals higher in lipid content generally elicit greater postprandial oxidative stress [80,81], which are proposed to contribute to the development of acute and chronic insulin resistance [9,10,23,51]. Transient hyperlipidemia in healthy humans elicited by intravenous lipid infusion for 6 h leads to a dose-dependent increase in plasma free fatty acids (FFA), glucose, insulin and oxidative distress as measured by increased plasma thiobarbituric acid reactive substances (TBARS) and a decrease in GSH/GSSG ratio [82]. Furthermore, under the same 6 h intravenous lipid infusion conditions, insulin stimulated whole-body glucose uptake measured via euglycemic hyperinsulinemic clamp is impaired [82]. Unfortunately, due to the indirect nature of manipulating the cellular redox environment, these findings in humans are unable to directly

Table 1
Summary of human research investigating oxidative distress and glycemic control.

Author, (year)	Participants	Oxidative Stress Stimuli	Antioxidant Treatment	Oxidative stress/ Antioxidant outcomes	Health Outcomes
Anderson et al., 2009 [10]	8 male adults who were lean and healthy (n = 5) or morbidly obese with no metabolic disease history (n = 3)	Acute high-fat meal (fat > 60% kcal, 35% daily kcal intake). High-fat diet for 5 days (fat > 60%, iso-caloric).	None	Obese compared to lean: ↑ 2-fold higher mtH ₂ O ₂ emissions ↓ 50% cellular GSH ↓ 50% cellular GSH/GSSG ratio Following high fat meal: ↑ 4 folds higher mtH ₂ O ₂ emissions ↓ 50% cellular GSH/GSSG ratio within 4 h ↔ cellular GSH	Obese compared to lean: ↓ HOMA-IR
Chen et al., 2006 [70]	37 adults with T2D with or without essential hypertension and plasma vitamin C <40 μM	T2D	Vitamin C (800 mg/day) for 4 weeks	Vitamin C: ↑ plasma vitamin C	Vitamin C: ↔ no effects on fasting glucose, clamp insulin sensitivity, blood lipids or forearm blood flow in response to acetylcholine, sodium nitroprusside or insulin
Darko et al., 2002 [102]	35 adults with uncomplicated T2D	T2D	Vitamin C (3 × 500 mg/day) for 21 days	Vitamin C: ↔ F ₂ -isoprostanes ↑ plasma vitamin C	Vitamin C: ↔ endothelial function ↔ blood pressure ↔ glycemic control
Gregersen et al., 2012 [103]	15 healthy adult participants with no family history of T2D (FH ⁻ , n = 8) or a family history of T2D (FH ⁺ , n = 7)	high-fat and high-carbohydrate meals (CHO) (1000 kcal)	None	Following high-CHO meal: ↓ plasma TAS ↓ muscle Cu/Zn-SOD ↔ GPX1 Following high-fat meal: ↔ plasma TAS ↔ muscle Cu/Zn-SOD ↔ GPX1 GPX1 positively correlated with BMI	High-CHO Vs high-fat meal: ↑ blood glucose ↑ serum insulin
Konopka et al., 2015 [104]	25 obese insulin-resistant women with PCOS and 14 lean women (baseline control)	Participants consumed a high-fat mixed meal (35% of DCI, avg: 874 kcal)	None	Following high-fat meal: ↔ mtH ₂ O ₂ emissions	High-fat meal post-exercise training: ↓ glucose AUC ↓ insulin AUC
Lu et al., 2005 [95]	17 adults with T2D	T2D	Vitamin C (3 × 1000 mg/day) for 14 days	Vitamin C: ↑ plasma vitamin C ↔ oxLDL, IL-6, IL-1Ra, hs-CRP, and fibrinogen	Vitamin C: ↓ total cholesterol ↔ no effects on glycemic control, triglycerides, HDL cholesterol or microvascular reactivity
Mason et al., 2019 [97]	31 adults with T2D	T2D	Vitamin C (2 × 500 mg/day) for 4 months	Vitamin C vs. placebo: ↓ F ₂ -Isoprostanes (8,12-iso-iPF ₂ α-VI) ↑ plasma vitamin C	Vitamin C: ↓ postprandial glucose AUC ↓ 24-h hyperglycaemia ↓ 24-h average glucose ↓ blood pressure
Mason et al., 2016 [96]	13 adults with T2D	T2D	Vitamin C (2 × 500 mg/day) for 4 months	Vitamin C: ↓ skeletal muscle DCFH oxidation during hyperinsulinaemia ↑ plasma vitamin C ↔ skeletal muscle or blood F ₂ -isoprostanes and GSH/GSSG	Vitamin C: ↑ skeletal muscle insulin sensitivity (Δ Rd) ↔ glycemic control measures ↔ liver insulin sensitivity
Paolisso et al., 1993 [67]	10 healthy controls with normal glucose tolerance and 15 patients with T2D	T2D	Vitamin E (900 mg/day) for 4 months	Vitamin E (both T2D and control): ↓ GSSG/GSH ↑ plasma vitamin E	Vitamin E vs. Placebo: In control patients: ↓ glucose AUC during OGTT ↑ total body glucose disposal and nonoxidative glucose metabolism during clamp In patients with T2D: ↓ glucose AUC during OGTT ↑ Rd, total body glucose disposal and nonoxidative glucose metabolism during clamp ↓ HbA1c, fasting glucose
Paolisso et al., 1996 [82]	30 healthy young adults	Four-part study all conditions performed following a 12-h	GSH infusion (15 mg/min for 6-hr)	Fasting study: Fasting plasma FFA correlated with	Lipid-infusion dose-response:

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Table 1 (continued)

Author, (year)	Participants	Oxidative Stress Stimuli	Antioxidant Treatment	Oxidative stress/ Antioxidant outcomes	Health Outcomes
		overnight fast Fasting study: All participants (n = 30) - relationship between fasting glucose, insulin, FFA, TBARS and GSH/GSSG ratio Time-course study: Participants (n = 10) 24-h infusion of intralipid with heparin (0.4 mL/min). TBARS measured at 0 h, 6 h and 24 h and another 24hr later after wash-out period. This was repeated with IV infusion of 0.9% NaCl Dose-response study: Participants (n = 9) 6-h intralipid/heparin infusion at various doses (0.1, 0.2, 0.3, 0.4 mL/min), on different days and random order. Plasma samples taken at start/end of each dose. Insulin sensitivity/stimulation study: Participants (n = 11) underwent a 6-h euglycemic hyperinsulinemic clamp with co-infusion of: Control: 0.9% NaCl Lipids: 10% intralipid (0.4 mL/min) with heparin Antioxidant only: GSH (15 mg/min for 6-h) Antioxidant + lipids: 10% intralipid + GSH (15 mg/min for 6 h)		fasting plasma TBARS and lipid peroxides Lipid-infusion time-course: ↑ TBARS (peak at 24 h) ↑ 3-fold plasma FFA ↓ GSH/GSSG ratio (at 6 h & 24 h) Lipid-infusion dose-response: progressive ↑ fasting plasma FFA. Varied according to intralipid infusion rate ↑ plasma TBARS until a plateau was reached between the last two infusion rates (0.3 and 0.4 mL/min) ↓ GSH/GSSG ratio (no difference from 0.3 mL/min) Insulin sensitivity/stimulation: Lipid infusion only ↑ plasma TBARS Antioxidant only (GSH) ↓ TBARS Antioxidant (GSH) + lipids ↓ lipid-induced TBARS	↑ plasma insulin ↑ plasma glucose with increasing doses of intralipid Insulin sensitivity/stimulation: Lipid infusion only ↓ insulin sensitivity Antioxidant infusion only ↑ insulin sensitivity Antioxidant + lipid infusion ↑ insulin sensitivity/attenuate lipid-induced insulin resistance
Sanguanwong et al., 2016 [105]	100 adults with T2D	T2D	Vitamin C (1 g/day) for 60 days	Vitamin C: ↓ plasma MDA ↑ plasma total antioxidant capacity	Vitamin C: ↓ fasting glucose ↓ triglycerides ↓ HbA1c ↓ fasting insulin ↓ HOMA-IR ↑ HDL cholesterol ↑ QUICKI
Siavash et al., 2014 [93]	67 adults with T2D	T2D	Vitamin C (1 g/day) or gemfibrozil (600 mg/day) or combined vitamin C (1 g/day) and gemfibrozil (600 mg/day) for 6 weeks	None	Vitamin C + gemfibrozil: ↓ TG and total cholesterol ↑ HDL ↓ fasting blood glucose ↔ HbA1c Vitamin C only: ↔ TG and total cholesterol ↑ HDL ↔ fasting blood glucose ↔ HbA1c Gemfibrozil only: ↓ TG and total cholesterol ↑ HDL ↔ fasting blood glucose ↔ HbA1c
Tousoulis et al., 2007 [94]	41 adults with T2D and no evidence of microangiopathy	T2D	Vitamin C (2 g/day) or atorvastatin (10 mg/day) for 4 weeks	Vitamin C: ↔ serum IL-6, TNF-α, sVCAM-1, CRP and ADMA Atorvastatin: ↓ serum IL-6, TNF-α, sVCAM-1, CRP and ADMA	Atorvastatin: ↑ maximum hyperaemic forearm blood flow (FBF) ↑ reactive hyperemia % ↔ on other blood flow measures Vitamin C: ↔ on blood flow measures
Tripathy et al., 2003 [106]	10 healthy participants (lipid infusion) 7 healthy participants (control infusions)	4-h heparin and lipid infusion at 50 ml/h. IV infusion with saline or 5% dextrose for 4 h (control)	None	Following lipid infusion: ↑ plasma FFA ↑ plasma triglycerides ↑ PMNs at 2 and 4 h ↑ MNCs at 4 h ↑ plasma TBARS at 2 h ↔ plasma TBARS at 4 h and 6 h Saline or low dextrose (control): ↔ in measures	Following lipid infusion: ↔ plasma insulin and glucose
Tushuizen et al., 2006 [75]	17 healthy males	Two consecutive high-fat mixed nutrient meals at 8:30hr and	None	Following consecutive high-fat meals:	Following consecutive high-fat meals compared to <i>(continued on next page)</i>

Table 1 (continued)

Author, (year)	Participants	Oxidative Stress Stimuli	Antioxidant Treatment	Oxidative stress/ Antioxidant outcomes	Health Outcomes
Vincent et al., 2009 [98]	48 healthy young adults who were normal weight (n = 25) or overweight (n = 23)	12:30pm (isocaloric: 50 g fat, 55 g CHO, 30 g protein, 900 kcal). Overweight	Vitamin E (800 IU/d), vitamin C (500 mg/d) and β -carotene (10 mg/d) for 8 weeks	↓ FMD ↑ MDA ↑ oxLDL Antioxidants in normal weight group: ↓ plasma lipid peroxidation ↑ adiponectin Antioxidants in overweight group: ↓ plasma lipid peroxidation ↑ adiponectin ↓ sE-selectin and sICAM-1	baseline: ↑ plasma glucose ↑ triacylglycerol Antioxidants in normal weight group: ↔ HOMA-IR ↔ fasting glucose or insulin ↔ QUICKI Antioxidants in overweight group: ↓ HOMA-IR ↔ fasting glucose or insulin ↔ QUICKI
Xiao et al., 2008 [92]	Study 1: 9 overweight or obese males Study 2: 6 overweight or obese males	Studies 1 & 2: Intravenous infusion of intralipid for 48 h	Study 1: Oral NAC, loading dose 140 mg/kg, followed by 70 mg/kg every 4 h for 48 h during intralipid infusion Study 2: Oral Taurine, 3 g/day for 2 weeks and during 48 intralipid infusion	Intralipid infusion: ↑ plasma MDA ↔ plasma 4-HNE NAC: ↔ lipid-induced MDA ↔ 4-HNE Taurine: ↓ lipid-induced MDA ↓ 4-HNE	Intralipid infusion: ↓ insulin clearance rate (Cl_i) ↓ insulin sensitivity index (S_i) ↓ disposition index (DI) NAC + lipids: ↑ Cl_i ↔ S_i and DI Taurine + lipids: ↑ Cl_i , S_i and DI

Abbreviations: 4-HNE: 4-Hydroxynonenal, CHO: Carbohydrate, DCFH: 2,2-dichlorodihydrofluorescein, FFA: Free fatty acids, FMD: Flow-mediated dilation, GSH: Reduced glutathione, GSH/GSSG: Ratio of reduced (GSH) to oxidized (GSSG) glutathione, MDA: Malondialdehyde, MNCs: Mononuclear cells, NAC: N acetyl cysteine; oxLDL: Oxidized low-density lipoprotein, PCOS: Polycystic ovary syndrome, PMN: Polymorphonuclear leukocytes, RCT: randomized controlled trial, ROS: Reactive oxygen species, TAS: Total antioxidative status, TBARS: Thiobarbituric acid-reactive substance, VCAM-1: Vascular cell adhesion protein-1.

demonstrate a causal relationship between increased oxidative distress and impaired insulin sensitivity. This is especially true given that ROS are necessary for insulin signal transduction and glucose cell transport [30,31,83,84]; prohibiting the conclusion that systemic oxidative stress measured during conditions of excess lipid and carbohydrate intake in humans is exclusively attributed to pathological ROS production. Nevertheless, findings in humans are consistent with findings from rodent and cell culture studies that have directly manipulated the ROS and/or antioxidant redox environment to link increased oxidative distress, including during excess substrate metabolism [13,24], to impaired insulin sensitivity and glucose metabolism [13,24–26,52].

Antioxidant treatment and oxidative distress. Exogenous antioxidant treatment in rodents and cell culture have provided strong evidence supporting the restoration of redox homeostasis and insulin sensitivity during acute and chronic conditions of elevated oxidative distress [10,13,24,26,27,85–88]. In humans, however, the effectiveness of exogenous antioxidant treatment for enhancing or restoring insulin sensitivity and glycemic control is far less consistent [70,89–95]. Nevertheless, acute intravenous GSH infusion (1 and 6 h infusions) or longer-term oral ingestion of vitamins E and C (over 4 months) improves insulin sensitivity in healthy individuals [65,67,82] and patients with T2D [65,67,96]. Other measures of glycemic control and insulin sensitivity including HbA1c, HOMA, and both fasting and postprandial glucose and insulin excursions, have also been reported to improve with various antioxidant treatment regimes in populations spanning the IGT and T2D continuum [67,97–101]. A recent meta-analysis of twenty-eight studies (n = 1574) reported that oral vitamin C supplementation in people with T2D significantly lowers HbA1c [89]. However, the current certainty of evidence is low due to many studies being mostly short term (less than 6 months) and with a small number of participants (n < 100) [89]. High quality, large and long-term randomized controlled trials are still required to establish the efficacy of vitamin C for the treatment of T2D. Furthermore, an equal number of studies have reported null or limited effects with antioxidant treatment on glycemic control [70,90,92–95], questioning the efficacy of antioxidant treatment in humans.

In addition to longer-term models of insulin resistance and T2D, antioxidant treatment has also been reported to attenuate insulin

resistance in humans caused by short-term excess nutrient intake. Research by Paolisso et al., 1996 [82] revealed that impairments in insulin sensitivity after 6 h of lipid infusion in healthy individuals is partially restored with the intravenous co-infusion of GSH [82]. Moreover, lipid-induced impairments in insulin sensitivity correlated with increased plasma TBARS, whereas restoration of insulin sensitivity via GSH co-infusion correlated with decreased plasma TBARS [82]. Similar research investigating oral taurine ingestion in overweight and obese individuals also reported restoration of insulin sensitivity and the prevention of increased plasma malondialdehyde and 4-HNE following 48 h of lipid infusion [92]. Rodent and cell culture models of lipid-induced insulin resistance confirm the potential of antioxidant treatment for preventing high-fat-diet induced insulin resistance [10,13,25,87]. As such, antioxidant treatment may yet prove to be a promising candidate for restoring systemic redox homeostasis and improving insulin sensitivity in a variety of populations under a variety of oxidative distress conditions (Table 1).

3. Oxidative eustress: implications for glycemic control and exercise

Despite evidence for the contribution of oxidative distress to insulin resistance, oxidative eustress, on the other hand, is necessary for physiological insulin signaling and glucose regulation. As a key regulator in the proximal insulin signaling pathway, the protein tyrosine phosphatase (PTP) family which includes PTP1B, phosphatase and tensin homolog (PTEN), and protein phosphatase 2 (PP2A), can be reversibly oxidized to either inhibit or propagate insulin signal transduction [22, 28]. Under basal conditions, endogenous catalase and peroxiredoxin antioxidant activity creates a reduced intracellular redox environment promoting PTP activity which, via dephosphorylation, suppresses activation of the insulin signaling cascade [21,47,107]. Upon insulin stimulation, the binding of insulin to the insulin receptor leads to a localized burst of endogenous O_2^- and H_2O_2 production through increased enzymatic activation of the plasma membrane bound NADPH oxidases that, via reversible oxidation, inactivate peroxiredoxin I in the vicinity of the receptor complex [21,30,31,83,84,108]. This leads to a localized oxidative redox environment which decreases PTP activity and permits

insulin-stimulated propagation of the insulin signaling cascade [9,30,31, 83,84]. As such, insulin-induced ROS production is a requirement for the initiation of insulin signaling and glucose cell transport.

Oxidative eustress and enhanced glycemic control. Skeletal muscle contraction and exercise are stimuli that lead to conditions of oxidative eustress [8,22,109–111]. Skeletal muscle contraction directly increases ROS production [11,12,15,112–116], which contributes to beneficial cardiometabolic responses to exercise including improved vascular health and function [117–122], mitochondrial biogenesis and the upregulation of antioxidant defenses [32,110,111,121–130], skeletal muscle force production [131], and skeletal muscle inflammatory response and repair capabilities [129,132]. Furthermore, exercise-induced ROS is a factor in mediating both contraction-mediated and post-exercise insulin-stimulated glucose metabolism [29,32,33, 133–135]. In rodents, knockout mice lacking a key antioxidant enzyme (Gpx1^{-/-}) required for the reduction of H₂O₂, demonstrate improved insulin sensitivity 1 h after treadmill exercise compared to wild type mice [29]. Henriquez-Olguin et al., 2019 [133] also recently reported that mice lacking the O₂ producing enzyme NADPH oxidase 2 (NOX2) exhibit lower exercise-induced cytosolic ROS production concomitant with impaired glucose metabolism compared to wild type mice [133]. These findings suggest the direct role of increased ROS in both contraction-mediated and post-exercise insulin stimulated glucose metabolism, at least in rodents. However, the dynamic and complex redox reactions elicited by exercise, nutrient intake, and substrate-oxidation, combined with the difficulties in directly and accurately measuring ROS and their functional role in humans, presents challenges for studying their effects. Nevertheless, we previously showed that enhanced insulin sensitivity in middle-aged obese men in the hours after acute high-intensity cycling exercise, was associated with greater exercise-induced phosphorylation of redox-sensitive proteins JNK, p38 MAPK and NF-κB in skeletal muscle [135]. Additionally, plasma SOD and CAT activity increased, plasma TBARS and H₂O₂ decreased, and 4-HNE increased in skeletal muscle after both exercise and insulin stimulation [135], whereas skeletal muscle mitochondrial H₂O₂ production decreased [136]. As such, exercise and insulin stimulation dynamically change the redox environment in humans in a tissue and subcellular organelle specific manner. Combined with numerous other reports of increased oxidative stress in muscle and blood alongside increased redox-sensitive cell signaling during and after exercise in humans [11,32,133,137–140], a time-period in which insulin-dependent and independent glucose uptake are consistently enhanced [22,141], exercise-induced ROS likely leads to a state of oxidative eustress [142]. However, research by our team and others have also discussed and identified the potential contribution of oxidative distress during exercise which may limit exercise capacity and muscle force production [7,131,143]. As such, caution when interpreting systemic markers of oxidative stress in humans is required.

Antioxidant treatment, oxidative eustress, and glycemic control. Directly linking ROS to the regulation of insulin and glucose metabolism in humans remains technically challenging. Nevertheless, exogenous antioxidant compounds have been used to manipulate redox homeostasis *in vivo*. Seminal research by Ristow et al., 2009 [32] suggested a link between exercised-induced oxidative eustress and improved insulin sensitivity in humans. In this study, oral supplementation of vitamin C (1000 mg/day) and vitamin E (400 IU/day) not only blocked the acute exercise-induced increase in muscle TBARS, but also prevented the improvements in insulin sensitivity elicited by 4-weeks of aerobic exercise training in both previously untrained and trained individuals [32]. Furthermore, exercise training led to increased skeletal muscle Cu/Zn-SOD, Mn-SOD and Gpx1 antioxidant gene expression; beneficial training effects that were prevented in the antioxidant treatment groups [32]. Another study showed that intravenously infusing the antioxidant N-acetylcysteine (NAC) in healthy adults during aerobic cycling exercise increased the muscle GSH/GSSG ratio, decreased protein carbonylation, and lead to a small but significant decrease in

post-exercise insulin sensitivity [134]. Trewin et al., 2013 [144] also reported that NAC infusion during high-intensity interval exercise in well-trained cyclists increased blood glucose levels alongside increased fat oxidation, indicating an alteration of substrate oxidation during exercise with antioxidant treatment which coincided with impaired time trial cycling performance. In rodents, administration of NAC prevents the enhancement of post-exercise insulin sensitivity in Gpx1^{-/-} mice [29], and impairs contraction-mediated glucose uptake during *ex vivo* mouse skeletal muscle contraction [145]. Together, findings suggest that exercise-induced ROS play a key role in insulin and glucose regulation and raises doubt over the use of antioxidants as a one-stop-shop treatment for improving health and wellbeing.

Exercise as an antioxidant. The potential benefits of exercise-induced oxidative eustress on insulin and glucose regulation may also extend beyond their direct involvement in mediating insulin-dependent and -independent glucose uptake. It is well-established that acute exercise transiently increases ROS generation, which over-time with regular exercise training, promotes the adaptation and upregulation of endogenous antioxidant defenses and/or decreased measures of systemic oxidative distress [32,124,125,128–130]. Exercise induced changes in redox homeostasis, including decreased markers of systemic oxidative distress, often coincide with improvements in insulin and glucose regulation [104,146,147]. Twelve weeks of moderate-intensity cycling exercise training in obese individuals increased skeletal muscle Cu/ZnSOD and MnSOD protein content, decreased urinary 8-OHdG and 8-isoprostanes, decreased skeletal muscle 4-HNE and protein carbonyls, while improving HOMA-IR and 2 h OGTT glucose and insulin levels [146]. Associations between reductions in urinary 8-OHdG and reduced glycated albumin following 12 months of moderate intensity aerobic training in T2D patients have also been reported [147]. Konopka et al., 2015 [104] reported that 12 weeks of aerobic exercise training in obese females with polycystic ovary syndrome decreased fasting skeletal muscle mitochondrial H₂O₂ production and muscle 8-oxo-dG, increased muscle catalase activity, and prevented the increase in muscle protein carbonyls observed over 12-weeks of sedentary behavior in obese controls [104]. These changes in muscle redox homeostasis coincided with improved insulin sensitivity as measured by hyperinsulinemic-euglycemic clamp, HOMA and postprandial responses to a high-fat mixed nutrient meal [104]. Exercise intensity may also play a role in altering redox homeostasis and glycemic control [50,138,140, 148], with one study revealing that 12 weeks of high-intensity interval-exercise training in adults with T2D led to improved HOMA-IR, decreased TBARS and von Willebrand factor, and increased glutathione peroxidase and nitric oxide, effects that were absent in the continuous training comparison group [149].

Redox homeostasis and measures of glycemic control are, however, not always consistently altered by exercise training [150–153]. For example, despite lower baseline fasting levels of urinary 8-iso PGF2α correlating with lower minimum glucose levels measured by continuous glucose monitoring (CGM) over a 24 h period in T2D patients, urinary 8-iso PGF2α was unchanged after 2 weeks of interval walking training despite improvements in CGM-derived measures of glycemic control [152]. In adults with T2D, neither 1 year of high-intensity interval training or moderate-intensity continuous training significantly altered total antioxidant capacity, F₂-isoprostanes, glutathione peroxidase, or protein carbonyls, although sex-specific sub-analysis revealed MICT decreased protein carbonyls in females and decreased total antioxidant capacity in males [153]. Krause et al., 2014 [150] reported an increase in plasma catalase antioxidant activity and decrease in plasma protein carbonyls in participants with T2D after 16 weeks of moderate-intensity exercise training, however, measures of glycemic control were unaffected. Furthermore, an energy-restricted diet combined with 12 weeks of aerobic exercise training in overweight and obese patients with T2D led to similar changes as the diet alone including decreased plasma MDA, increased total antioxidant status (TAS), and improved measures of body fat, blood pressure, lipids, blood glucose, HbA1c and HOMA2-IR

Table 2
Summary of human research exploring exercise, oxidative eu/distress, and glycemic control.

Author (year)	Participants	Study Design/Intervention	Oxidative stress/Antioxidant outcomes	Health Outcomes
Farinha et al., 2015 [151]	23 women with metabolic syndrome (MS)	Exercise: 12 weeks, 30–60 min treadmill exercise, 3 x p/wk at 50–65% HRR Antioxidant intervention: None	Oxidative stress and antioxidant status, plasma parameters: ↓ TBARS ↓ AOPP ↑ total thiols ↔ NOx ↓ protein carbonyls	↔ BP or resting HR ↓ body weight ↓ BMI ↓ glucose
Henriquez-Olguin et al., 2019 [133]	3 young healthy men	Exercise: 30 min of cycling exercise at 65% peak power output Antioxidant intervention: None	Oxidative stress marker: ↑ muscle dichlorodihydrofluorescein diacetate oxidation	Human component: None measured. Animal component: Mouse model with loss of function NOX2 subunit - > lack of ROS generation - > impaired glucose uptake during exercise
Kasimay et al., 2010 [155]	14 adults with IGT	Diet & Exercise Program: 12 weeks, 40 min treadmill exercise, 3 x p/wk at 35–60% HR reserve, combined with a diet intervention (daily calorie intake reduced by 500 kcal/d based on daily energy expenditures) Antioxidant intervention: None	Diet & Exercise Program: ↓ TBARS ↑ sulfhydryl groups ↔ carbonyl groups ↓ plasma NO	Diet & Exercise Program: ↓ BMI, BF, BP ↓ glucose ↓ postprandial glucose ↓ HbA1c
Krause et al., 2014 [150]	25 sedentary obese men with (n = 13) or without T2D (n = 12)	Exercise: 12 weeks, 30 min treadmill exercise, 3 x p/wk at low (fat-max; 30–40% VO _{2max}) or moderate (T _{vent} ; 55–65% VO _{2max}) intensity Antioxidant intervention: None	Exercise in obese men without T2D: ↑ muscle nNOS and tNOx (T _{vent} only) ↔ tNOx, GSSG, GSH, GSSG/GSH, CAT, protein carbonyls or TAC Exercise in obese men with T2D: ↑ CAT (T _{vent} only) ↓ protein carbonyls ↔ tNOx, GSSG, GSH, GSSG/GSH, or TAC	All groups: ↔ body composition ↔ aerobic fitness ↔ insulin resistance
Karstoft et al., 2017 [152]	14 older adults with T2D	Exercise: 2 weeks crossover, interval walking training (IWT), continuous walking training (CWT), and control (habitual lifestyle). 10 x supervised treadmill sessions, CWT 10 × 60 min walking sessions at ~73% VO _{2peak} . IWT: 10 × 60 min walking sessions alternating 3 min repetitions at slow (~54% VO _{2peak}) and fast (~89% VO _{2peak}) walking speed Antioxidant intervention: None	All interventions: ↔ 8-iso PGF _{2α}	All interventions: ↔ body composition ↔ physical fitness or BP IWT intervention: ↓ glucose levels ↓ fasting glucose ↓ MAGE (mean amplitude of glycemic excursions) ↔ HbA1c CWT intervention: No changes in measures Baseline: 8-iso PGF _{2α} associated with baseline glucose levels Post-interventions: 8-iso PGF _{2α} not associated with glycemic control changes
Konopka et al., 2015 [104]	25 obese IR women with PCOS	Exercise: 12 weeks, 60 min cycling exercise, 5 d/wk, at 65% VO _{2peak} (n = 12). Control (n = 13) no training. High-fat mixed nutrient meal ingested before and after training Antioxidant intervention: None	After exercise training: ↑ muscle catalase activity ↓ muscle mtH ₂ O ₂ production ↓ muscle 8-oxo-dG ↔ muscle protein carbonyl ↓ muscle mtH ₂ O ₂ production 4 h after ingesting meal Control: ↑ protein carbonyl ↔ other measures	Exercise training group: ↓ fasting glucose ↓ fasting insulin Control: ↑ BMI, weight, BF and maintained in AET group.
Mallard et al., 2017 [153]	36 adults with T2D	Two parallel groups: High-intensity interval training (HIIT, n = 20) or moderate-intensity continuous training (MICT, n = 16), 12 weeks of combined supervised/home-based training followed by 40 weeks of home-based training. HIIT: 40 min treadmill exercise, 4 × 4 min intervals at 90–95% HRmax (3 min recovery periods at 70% HRmax), 3 x p/wk. MICT: 120 min p/wk of home-based exercise at ~70% HRmax (exercise bout classified as a minimum of 10 min). Antioxidant intervention: None	Both MICT and HIIT: ↔ F ₂ -isoprostanes ↔ Glutathione peroxidase ↔ TAC ↔ Protein carbonyls ↔ Interleukin-10, -6, -8, or TNF-α Sub-analysis revealed sex-specific differences. Females: ↓ protein carbonyls with MICT Males: ↓ TAC with MICT	None reported.
McNeilly et al., 2012 [156]	11 obese middle-aged adults with IGT	Exercise: 12 weeks, 30 min walking exercise, 5 d/wk at 65% HR _{max} Antioxidant intervention: None	Exercise: ↓ blood 34% lipid hydroperoxides ↔ blood SOD	Exercise: ↓ BMI, %BF, WC, HC significant ↔ adiponectin ↔ mean glucose ↔ insulin

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Table 2 (continued)

Author (year)	Participants	Study Design/Intervention	Oxidative stress/Antioxidant outcomes	Health Outcomes
Medeiros Nda et al., 2015 [157]	25 obese sedentary adults	Exercise: 2 parallel groups underwent 26 sessions of concurrent training, 70 min at 50–75% VO_{2peak} (5 min warmup, 30 min walking, 30 min resistance training, 5 min stretching). Concurrent training (CT1) – frequency: 5 d/wk, n = 12 Concurrent training 2 (CT2) – frequency: 3 d/wk, n = 13 Antioxidant intervention: None	CT1 Only: ↓ GPx activity ↔ CAT ↓ protein carbonyls ↔ TBARS ↔ SOD CT2 group: ↓ GPx activity ↓ CAT ↔ protein carbonyls ↓ TBARS ↔ SOD	CT1 only: ↓ fasting glucose ↑ fasting insulin ↑ HOMA-IR ↓ body weight, BMI, and body fat % ↑ fat free mass ↑ basal metabolic rate CT2 group: ↓ fasting glucose ↔ fasting insulin ↓ HOMA-IR ↓ body weight, BMI, and body fat % ↔ fat free mass ↔ basal metabolic rate
Merry et al., 2010 [158]	9 healthy adult males	Exercise: 80 min cycling exercise at ~62% VO_{2peak} Antioxidant intervention: IV infusion of N-acetylcysteine (NAC) or saline infusion (control)	Exercise – Control and NAC: ↔ GSH or GSSG NAC infusion: ↓ S-glutathionylation	NAC condition: ↔ plasma glucose
Mitranun et al., 2014 [149]	43 adults with T2D	Three parallel groups: Sedentary (Sed, n = 15), continuous exercise (Con, n = 14), and interval exercise (Int, n = 14) training, matched for exercise duration and energy expenditure. Sed: Remained sedentary. Con: Treadmill exercise intervals of 30–40 min alternating between 50–60% and 80–85% VO_{2peak} , 3 d/wk over 12 weeks. Int: Continuous treadmill exercise of 30–40 min at 60–65% VO_{2peak} , 3 d/wk over 12 weeks. Antioxidant intervention: None	Con and Int: ↔ SOD activity Int only: ↓ TBARS ↓ von Willebrand factor ↑ nitric oxide ↑ glutathione peroxidase Sed: No changes in parameters	Con and Int: ↓ fasting glucose ↓ HOMA-IR Int only: ↓ HbA1c Sed: No changes in parameters
Nojima et al., 2008 [147]	103 adults with T2D	Three parallel groups: aerobic training combined with the use of a fitness center (group A, n = 43), aerobic training only (group B, n = 44), or standard care control (group C, n = 16) Exercise: Group A & B 30 min at 50% VO_{2peak} , 3 x p/w over 12 months Antioxidant intervention: None	Group A and B (exercise) ↓ urinary 8-OHdG Control: ↔ urinary 8-OHdG	Group A and B (exercise): ↔ VO_{2peak} ↓ glycated albumin Control: ↔ VO_{2peak} ↓ glycated albumin
Parker et al., 2017 [140] and Parker et al., 2018 [138]	8 healthy adults	Crossover design, cycling exercise: SIE: 4 × 30 s all-out sprints; 4.5 min recovery periods. HIIE: 5 × 4-min cycling bouts at 75% of W_{max} ; 1 min recovery periods. CMIE: 30 min at 50% of W_{max} . Antioxidant intervention: None	All Ex. Modes: ↓ SOD activity ↓ TBARS ↑ CAT activity ↑ hydrogen peroxide SIE versus CMIE: ↑ peak hydrogen peroxide ↑ peak CAT activity HIIE versus CMIE: ↑ peak hydrogen peroxide	Between exercise modes. Exercise-intensity and post-exercise time-course effects on insulin protein signaling in skeletal muscle
Parker et al., 2016 [135]	11 middle-aged obese men	Exercise: 30 min cycling interval exercise, 4 min at 95% HR_{peak} , 2 min passive recovery periods. Two-hour hyperinsulinemic-euglycemic clamp at rest (control day) and 60 min after exercise (separate exercise day) Antioxidant intervention: None	Insulin stimulation both at rest and after exercise, comparable increase in: ↑ SOD activity ↑ CAT activity ↑ skeletal muscle 4-HNE Significant decreases in: ↓ TBARS ↓ hydrogen peroxide	Exercise compared to rest: ↑ insulin sensitivity
Parker et al., 2016 [50]	27 inactive obese or overweight sedentary adults	Standard breakfast ingested on a control day, or 1 h before undergoing either low-volume high-intensity interval exercise (LV-HIIE) or continuous moderate-intensity exercise (CMIE) LV-HIIE Group: 24 min of cycling interval exercise, 8 × 1-min cycling bouts at 100% of W_{max} with a 5 min warm up, 1 min active recovery between sets, and 3 min cool down CMIE Group: ~38 min cycling exercise at 50% W_{max} Antioxidant intervention: None	1 h post-breakfast: ↑ CAT activity ↑ SOD activity ↑ TBARS ↑ hydrogen peroxide 3 h post-breakfast: ↑ CAT activity ↑ SOD activity ↑ hydrogen peroxide LV-HIIE group: ↑ 3 h postprandial TBARS ↑ 3 h postprandial hydrogen peroxide ↔ SOD or CAT CMIE group: ↓ 3 h postprandial TBARS ↓ 3 h postprandial SOD ↔ hydrogen peroxide or CAT	Both exercise groups: ↓ 24-hr average glucose levels ↓ 24-hr hyperglycemic excursions ↓ 24-hr peak glucose ↓ 2-h postprandial glucose response to dinner ↓ postprandial glucose response to breakfast LV-HIIE: ↑ plasma insulin and glucose compared to CMIE
Ristow et al., 2009 [32]	20 healthy young men who were untrained (n	Exercise: 4 weeks, 85 min exercise sessions (warm-up, cycle/running, circuit training, cool-down), 5 x p/wk	Post-Exercise: ↑ adiponectin levels ↑ TBARS	Exercise intervention: ↑ insulin sensitivity Antioxidant treatment and

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Table 2 (continued)

Author (year)	Participants	Study Design/Intervention	Oxidative stress/Antioxidant outcomes	Health Outcomes
	= 20) or trained (n = 20)	Antioxidant intervention: Vitamin C (1000 mg/day) and vitamin E (400 IU/day) oral supplementation (n = 20) or placebo (n = 20)	↑ CuZnSOD ↓ MnSOD ↑ GPx1 Exercise & antioxidant treatment: ↓ in antioxidant enzymes Prevented ↑ in TBARS	exercise: ↓ insulin sensitivity
Samjoo et al., 2013 [146]	9 obese and 9 lean men (matched for age and training status)	Exercise: 12 weeks, 30–60 min cycling exercise, 2–3 x p/wk at 50–70% VO _{2peak} Antioxidant intervention: None	Post-training in obese men: ↓ 4-HNE ↓ protein carbonyls ↓ 8-isoprostane ↑ MnSOD ↔ catalase ↔ Cu/ZnSOD ↔ urine 8-OHdG Post-training in lean men: ↓ 4-HNE ↔ protein carbonyls ↔ 8-isoprostane ↑ MnSOD ↔ catalase ↔ Cu/ZnSOD ↔ urine 8-OHdG	Post-training (obese and lean): ↓ postprandial glucose ↔ HOMA-IR ↑ VO _{2peak} ↓ WC ↓ gynoid fat % ↓ leg fat %
Schaun et al., 2011 [159]	20 middle-aged healthy sedentary men	Exercise: Three parallel groups, 12 weeks of exercise. CT group (n = 10): concurrent (aerobic & strength) training, 20 min of cycling exercise and ~15-min of strength exercises, 3 x p/wk for at 65%–80% HRR AT group (n = 10): 30 min cycling exercise, 3 x p/wk at 65% HRR Antioxidant intervention: None	CT group: ↓ lipoperoxides ↓ exercise-induced lipoperoxides ↓ exercise-induced GSSG/GSH ↓ GSSG ↔ GSH ↔ basal GSSG/GSH AT group: ↓ lipoperoxides ↓ exercise-induced GSSG/GSH ↔ GSSG ↔ GSH ↔ basal GSSG/GSH	CT group: ↓ blood glucose AT group: ↓ blood glucose
Trewin et al., 2015 [134]	7 young healthy adults	Exercise: 55 min cycling exercise at 65% VO _{2peak} and 5 min at 85%VO _{2peak} Antioxidant intervention: IV infusion of N-acetylcysteine (NAC) or saline (control), crossover design	Antioxidant (NAC) Intervention: ↓ post-exercise protein carbonylation ↑ GSH/GSSG	NAC (post-exercise) compared to Control: ↓ insulin sensitivity
Trewin et al., 2017 [136]	9 middle-aged obese men	Exercise: 30 min cycling interval exercise (4 × 4 min intervals at 95% HR _{peak}). Insulin clamp 1 h post-exercise. Antioxidant intervention: None	Post-exercise: ↔ no change in GSSG ↔ no change in GSH/GSSG ↓ JH ₂ O ₂ 1-hr post-exercise	Post-exercise: ↑ insulin sensitivity
Vinetti et al., 2015 [160]	20 older males with T2D	Exercise: Older males (n = 10) underwent 12 months aerobic, resistance and flexibility training for 140–270 min p/wk, 15–35 min per session at gas exchange threshold increasing intensity to ventilatory compensation point Control Group: Older males underwent standard medical care only (n = 10) Antioxidant intervention: None	Training group: ↓ plasma POVC ↓ plasma PGPC ↔ PBMC PGPC or POVC Control group: ↔ plasma PGPC or POVC ↔ PBMC PGPC or POVC	Training group: ↑ insulin sensitivity ↓ WC, total and LDL cholesterol ↓ HOMA-IR ↓ fasting insulinemia ↔ HbA1c and BW Control group: ↔ in above measures
Wycherley et al., 2008 [161]	37 overweight or obese sedentary adults	Diet and exercise (n = 13): 12 weeks, 25–60 min walking/jogging, 4–5 x p/wk at 60–80% HR _{max} . Combined with high-protein, energy-restricted diet (~5500 kJ/day) Diet only (n = 16): 12-week high-protein, energy-restricted diet (~5500 kJ/day) Antioxidant intervention: None	Diet and exercise: ↔ no change in TAS ↓ plasma MDA ↑ 24-h urinary nitrate/nitrite Diet only: ↔ no change in TAS ↓ plasma MDA ↑ 24-h urinary nitrate/nitrite	Diet and exercise: ↓ BW, BF, WC, BP ↓ glucose ↓ HOMA-IR ↓ HbA1c ↓ total and LDL cholesterol Diet only: ↓ BW, BF, WC, BP ↓ glucose ↓ HOMA-IR ↓ HbA1c ↓ total and LDL cholesterol Change in MDA correlated with changes in glucose, HOMA-IR and HbA1c
Yfanti et al., 2011 [162]	21 moderately trained young men	Exercise: 12 weeks, cycling exercise 5 x p/wk, alternating interval sessions (75–91% Power _{max} and duration 60–80 min) and continuous sessions (55–66% Power _{max} and duration 85–155 min) Antioxidant intervention: oral supplementation with vitamin C (ascorbic acid, 500 mg daily) and vitamin E (RRR-α-tocopherol, 400 IU daily) (n = 11) or placebo (n = 10) for 16 weeks	None	Similar in both groups after training: ↑ insulin sensitivity ↑ VO _{2peak} ↔ glucose

Abbreviations: **AOPP**: advanced oxidation protein products; **BF**: body Fat, **BP**: blood Pressure, **BW**: body weight, **CAT**: Catalase, **CuZnSOD**: copper-zinc-superoxide dismutase, **GPx1**: glutathione peroxidase 1, **GSH**: reduced glutathione, **CMIE**: continuous moderate-intensity exercise, **GSSG**: glutathione disulphide, **GSH/GSSG**: ratio, **HIIE**: high-Intensity Interval Exercise, **HbA1C**: Hemoglobin A1C, **HOMA-IR**: homeostatic model assessment of insulin resistance, **HRR**: Heart rate reserve, **IGT**: impaired glucose tolerance, **IR**: insulin resistant, **LV-HIIE**: low-volume high-intensity interval-exercise, **MDA**: malondialdehyde, **mtH₂O₂**: mitochondrial hydrogen peroxide, **NAC**: N-acetylcysteine, **NOx**: nitrogen oxides; **NOX2**: nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2, **oxPAPC**: oxidation of 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine, **PBMC**: peripheral blood mononuclear cells, **POVPC**: 1-palmitoyl-2-[5-oxovaleroyl]-sn-glycero-3-phosphorylcholine, **PGPC**: 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphorylcholine, **SOD**: superoxide dismutase, **T2D**: type 2 diabetes, **TAS**: total antioxidant status, **TBARS**: thiobarbituric acid-reactive substances; **T-SH**: total thiol content, **WC**: waist circumference.

[154]. Thus, improvements in redox homeostasis are possibly also due to improvements in cardiometabolic health that accompany weight loss, in addition to the direct effects of exercise. This creates a unique redox health paradox in which exercise and muscle contraction increase ROS production, yet possess the capacity to decrease ROS production directly by increasing antioxidant activity, and/or indirectly by decreasing ROS that is produced through lifestyle factors such as physical inactivity and excess adiposity [9,22].

The most direct evidence for the role(s) of exercise-induced ROS and oxidative eustress in regulating insulin and glucose homeostasis comes from rodent and cell culture studies. Attempts to link training-induced improvements in glycemic control to altered redox homeostasis in human studies remain inconclusive. The lack of consensus likely stems from methodological limitations in human research which, to-date, lack the ability to use the mechanistic approaches that are possible in genetic rodent models and cell culture experiments. In the absence of more sophisticated study design and mechanistic experiments in humans, it is difficult, if not impossible, to delineate the complex and dynamic interactions between redox biology and insulin and glucose regulation. The vast array of exogenous antioxidants used, variation in doses and duration of treatment, and differing methods of administration, likely also influence the efficacy of the treatment and thus contribute to inconsistent findings (Table 2).

4. Working towards an era of personalized antioxidant treatment

Antioxidant treatment: a one-stop shop solution, snake oil, or a bit of both? Despite convincing evidence from rodent and cell culture research [10,13,24,26,27,85–87], experimental studies exploring antioxidant treatment in humans do not always translate to observable improvements in insulin and glucose regulation [70,90,92–95]. For example, high-dose oral NAC treatment in obese individuals (initial dose of 140 mg/kg followed by 70 mg/kg every 4 h during lipid infusion) fails to mitigate insulin resistance induced by 48 h of lipid infusion [92]. Furthermore, oral vitamin C ingestion (800 mg/day over 4 weeks) in T2D patients was not found to improve fasting measures of insulin, glucose, QUICKI, and HOMA, or insulin sensitivity measured by isoglycemic-hyperinsulinemic clamp [70]. Numerous other studies have also reported minimal to no effect of vitamin C treatment on fasting blood glucose, insulin and/or HbA1c in T2D patients [93–96]. Finally, a meta-analysis of seventy-eight randomized trials involving 296,707 participants also concluded that antioxidant treatment (beta-carotene, selenium, or vitamins A, C, and E) does not lead to significantly better overall health outcomes or survival in healthy participants or patients with various diseases including those with T2D [90].

Similar ineffective antioxidant treatment or null findings have been reported during conditions of oxidative eustress including muscle contraction and exercise. For example, Yfanti et al. [162] reported that antioxidant supplementation (vitamin C: 500 mg daily, vitamin E: 400 IU daily) in young and healthy men did not alter insulin sensitivity after 12-weeks of intensive cycling exercise training, performed five times per week [162]. Likewise, NAC infusion during exercise in active young healthy men did not affect glucose metabolism during 80 min of cycling exercise at ~62% VO_{2peak} despite attenuation of exercise-induced oxidative stress measured by S-glutathionylation [158]. NAC treatment also failed to alter insulin-stimulated glucose uptake in isolated

mouse skeletal muscle [145] or post-exercise insulin sensitivity in wild type mice [29]. A possible contributing factor to the contradictory or null findings is the large redox interindividual variability in humans, or in other words, the large variation in redox phenotypes, [142]. In this unique study, redox screening in healthy individuals revealed markedly varied levels in an array of redox biomarkers at rest (basal), and in response to acute and chronic exercise training [142]. Importantly, this study showed that individuals who exhibited greater systemic oxidative stress in response to acute exercise (urinary F₂-isoprostanes) also exhibited greater training-induced improvements in VO_{2peak}, time trial and Wingate performance, compared to individuals with lower exercise-induced oxidative stress. These findings, and others [143,163,164], indicate that even in apparently healthy individuals, measures of redox homeostasis can be markedly different at the individual level which has important implications for health (e.g., exercise capacity) and the potential effectiveness of antioxidant treatment.

Current evidence is lacking to provide a clear consensus on antioxidant treatment as a one-stop-shop solution for improving insulin and glucose regulation in humans. On the contrary, due to the large variation in antioxidant treatment regimens and populations investigated, and the acknowledged coexistence of oxidative distress and eustress (Tables 1 and 2), it is possible that the effectiveness of antioxidant treatment is influenced largely by the context of treatment.

Context-sensitive treatment. In a meta-analysis of 22 RCT trials involving 937 adults ranging across the healthy and T2D spectrum, it was concluded that vitamin C treatment did not lead to significant improvements in common measures of fasting and postprandial glycemic control [91]. Notably, however, subgroup analysis revealed that despite a lack of an effect of treatment in healthy individuals, improvements in fasting and/or postprandial glucose concentrations were observed in individuals with T2D or coronary heart disease [91]. As such, antioxidant treatment that is designed to restore redox homeostasis may be more effective at improving health outcomes in populations with a pre-existing history of a redox imbalance or antioxidant deficiency.

Recently, nicotinamide riboside supplementation was found to improve muscle isometric peak torque and fatigue only in older individuals whom, compared to younger controls, exhibited lower basal erythrocyte glutathione and NAD(P)H levels, and higher urine F₂-isoprostanes [165]. Similarly, one study found that antioxidant treatment with vitamin C or E for 2 months reduced plasma F₂-isoprostane levels only in individuals that had high baseline levels of F₂-isoprostane [166]. Furthermore, 1 h of intravenous glutathione infusion in T2D patients improves the intraerythrocytic GSH/GSSG ratio (~47%) to that of similar levels measured in healthy control individuals alongside substantially improved insulin sensitivity (~24%), whereas comparatively only marginal improvements in the GSH/GSSG ratio (~18%) and insulin sensitivity (~6%) were observed in healthy controls [65]. Vitamin E treatment (900 mg/day for 4 months) leads to substantially greater improvements in insulin stimulated glucose disposal in T2D patients (~48%) compared to healthy individuals (~22%) [67]. Likewise, vitamin C treatment in individuals with T2D appears to be more effective at reducing HbA1c levels when baseline HbA1c levels are higher [89]. Measures of redox homeostasis can be markedly varied even among apparently healthy individuals (i.e., even healthy individuals can be in a state of high, moderate or low systemic oxidative stress), which importantly has been shown to directly influence the effectiveness of antioxidant treatment [142,143]. The impact of

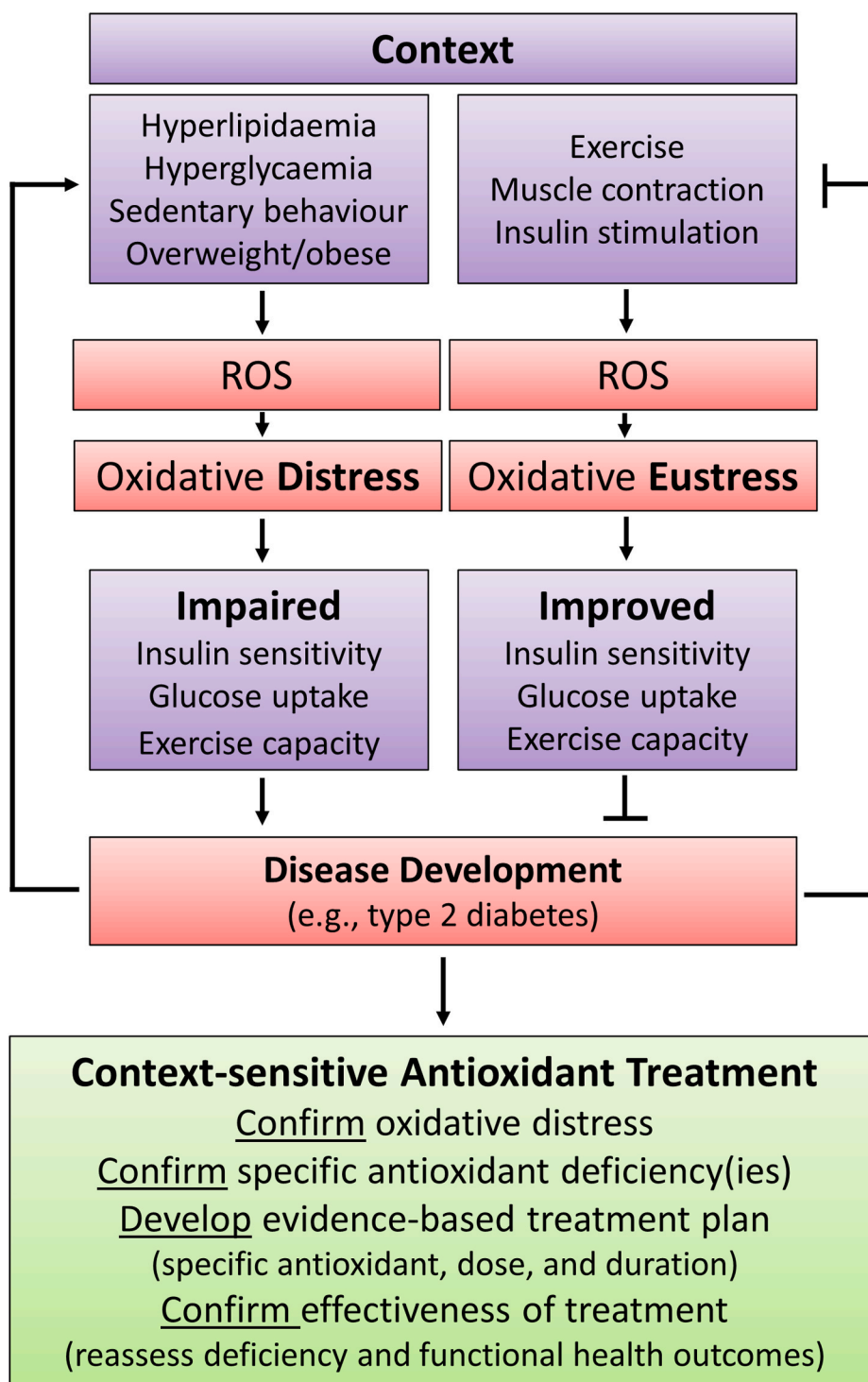


Fig. 1. ROS play both a physiological and pathophysiological role in human health and disease. As such, effective antioxidant treatment will need to consider the dynamic and ever evolving context of oxidative eustress and distress in humans.

different redox phenotypes in healthy individuals on glycemic control warrants future investigation. Overall, findings suggest a greater restorative capacity of antioxidant treatment in populations that are characterized by perturbed redox homeostasis and insulin resistance [10,65–69].

Another necessary factor to consider when prescribing antioxidant treatment for health promotion is the potential diametrical effects during conditions of oxidative eustress or distress. For example, glutathione infusion improves insulin sensitivity in healthy adults [65], yet the

infusion of n-acetylcysteine in healthy adults during exercise impairs the post-exercise insulin sensitizing response [134]. It is possible that antioxidant treatment designed to improve glycemic control and insulin sensitivity in adults with T2D, may also lead to blunting of the beneficial insulin sensitizing response elicited by acute and chronic exercise training, warranting a specific antioxidant treatment plan that considers both oxidative distress and eustress conditions. Although, to our knowledge, this type of research in humans has yet been conducted. We propose that a context-sensitive and considered approach to prescribing

antioxidant treatment for health promotion and disease prevention is required. An approach which has yet to be fully realized due to limitations in current research.

Redox screening and targeted antioxidant treatment. The identification of oxidative distress (i.e., through measuring systemic markers of oxidative stress such as F2-isoprostanes) is an important step for identifying the potential effectiveness of an antioxidant treatment regime [166]. However, recent work by our group has showed that testing for an array of antioxidant deficiencies is likely to be a more sensitive and robust approach to effective redox screening and personalized antioxidant treatment [143,167]. For example, both targeted and non-targeted antioxidant treatment regimes in healthy adults lead to a similar decrease in systemic oxidative stress as assessed by decreased urinary F2-isoprostanes [143]. However, improvements in exercise performance including measures of VO_{2max} , isometric peak torque, time trial, and/or Wingate performance, were only consistently observed when low basal levels of vitamin C or GSH levels were restored by 30 days of treatment with the respective treatments of vitamin C or NAC [143,163,164]. In the context of disease, critically ill patients exhibit similar elevated levels of systemic oxidative stress (F2-isoprostanes) yet deficiencies in specific antioxidant defenses are distinct and varied among patients over a 7-day Intensive Care Unit stay [167]. Taken together, these findings suggest that a similar “redox screening” for antioxidant deficiencies and subsequent “personalized antioxidant treatment” approach may be required to elicit more effective antioxidant treatment for chronic diseases including T2D.

Treatment duration, dose, and antioxidant bioavailability. Increasing doses of oral vitamin C treatment in vitamin C deficient women leads to a dose-dependent increase in protection of plasma samples against *ex vivo* peroxyl radical insult [168]. In vivo, a recent meta-analysis reported that antioxidant treatment with vitamin C in T2D patients is likely to be more effective at improving HbA1c levels when supplementation periods are longer than 12 weeks [89]. Likewise, the effect size of improvements in glucose concentrations (fasting or postprandial) from over 22 randomized control trials in humans were associated with longer treatment periods (>30 days) [91]. In one study plasma vitamin C levels in T2D patients were only modestly improved, and were still well below healthy normative values, after oral vitamin C treatment for 4 weeks at a daily dose 800 mg [70]; a treatment regime which may have contributed to the lack of reported improvements in insulin sensitivity. In the context of exercise, higher dose vitamin C (1000 mg/day) and vitamin E (400 IU/day) treatment in healthy adults attenuates improvements in insulin sensitivity after 4 weeks of endurance exercise training [32], whereas lower dose treatment (500 mg vitamin C and 400 IU vitamin E, per day) has little effect on similar health outcomes after a 12-week training program [162]. As such, it is possible that many studies reporting ineffective antioxidant treatment may be limited by short-term treatment durations and low doses.

Antioxidant bioavailability is also affected by the route of administration. Acute intravenous vitamin C infusion (80 min infusion protocol) substantially increases plasma ascorbic acid levels (>15-fold) in sedentary older men, whereas a more modest increase in plasma ascorbic acid levels (<2-fold) was achieved by oral ingestion (500 mg per day for 30 days) [169]. Notably, brachial flow-mediated dilation in the older sedentary men was improved only with the intravenous vitamin C infusion, suggesting that both bioavailability and bioactivity of the antioxidant are influenced by the method of administration [169]. The effectiveness of other antioxidants such as NAC and mitoQ may also be limited when taken orally due to extensive first pass metabolism and subsequent low bioavailability in the circulation [170,171]. Oral NAC treatment during 48 h of lipid infusion fails to prevent lipid-induced oxidative stress and insulin resistance in obese men [92], whereas intravenous NAC infusion in rodents [172] and glutathione infusion in healthy humans [82] attenuates insulin resistance during similar lipid-induced oxidative distress conditions. In the same cohort of obese individuals where oral NAC treatment was ineffective, it was established

that two-weeks of pre-treatment with the oral antioxidant taurine attenuated both lipid-induced oxidative stress and insulin resistance [92]. Recent evidence in humans indicates that pre-treatment with the mitochondrial-targeted coenzyme Q10 (mitoQ) for 21 days attenuated biomarkers of nuclear and mitochondrial DNA damage in lymphocytes and skeletal muscle following a single bout of high-intensity interval exercise [173]. In contrast, a single dose of mitoQ (20 mg) taken 1 h before exercise had no effect under the same experimental conditions [173]. Adequate treatment duration, dose, and method of administration may therefore be necessary for some oral antioxidants to reach sufficient bioavailability.

Methodological differences in study design likely contribute to contradictory findings among the literature (Tables 1 and 2). Despite this, current evidence supports that antioxidant treatment dose, duration, method of administration, and the subsequent bioavailability, are important factors influencing the effectiveness of treatment in both health and disease. It should be noted however that increasing antioxidant doses does not always lead to a linear increase in bioavailability, such as is the case with NAC and vitamin C [174,175], and in some cases may be linked to greater adverse effects including nausea, flatulence, abdominal discomfort, and diarrhea [175,176]. Furthermore, larger doses can even have the opposite effect and lead to a pro-oxidant redox environment depending on the type of antioxidant [177]. As such, treatment duration and dose need to be carefully considered when investigating the effectiveness of antioxidant treatment for altering redox homeostasis and health outcomes (Fig. 1).

Subcellular redox regulation of insulin resistance and glucose metabolism. Inconsistent reports and lack of clarity around redox mechanisms regulating insulin dependent and independent glucose uptake are not surprising given that redox homeostasis and redox-sensitive cell signaling, especially in humans, are commonly measured at the systemic level (i.e., measured in whole blood or tissue homogenates). Growing evidence now supports that ROS-mediated cellular function, including cell metabolism and many exercise-mediated health adaptations, are likely to be dictated by complex redox reactions and redox cell signaling that occur at the subcellular and intra-organellar level [5,24,34,109,133,178–180].

Elevated mitochondrial ROS production in skeletal muscle is consistently reported during chronic and acute experimental conditions of insulin resistance including high-fat and high-glucose conditions in cell culture, rodents, and humans [9,10,13,25,85]. In contrast, ROS produced during exercise is suggested to be largely cytosolic in nature and occur through non-mitochondrial derived sources, such as NADPH oxidase 2 (NOX2), which reside within the plasma membrane or transverse tubules of skeletal muscle [8,109]. Our team also reported that skeletal muscle mitochondrial ROS production (H_2O_2) is decreased in the hours after exercise [136,181], which in obese middle-aged males occurred alongside improved post-exercise insulin sensitivity [136]. Similar findings in rodents using redox-sensitive fluorescent probes have provided direct evidence that cytosolic ROS production increases in skeletal muscle after acute exercise, whereas mitochondrial ROS is decreased [133]. As such, redox regulation of ROS and antioxidant activity, and the subsequent effects on insulin dependent and independent glucose uptake, are likely to be controlled in a compartmentalized and spatiotemporal manner. However, precise subcellular redox reactions that contribute to divergent outcomes on insulin dependent and independent glucose metabolism remain to be elucidated.

Targeting mitochondrial ROS production with antioxidants that localize within the mitochondria such as SS31, MitoTEMPO, and MitoQ, are reported to be largely effective at improving glycemic control and insulin sensitivity in rodent and cell culture models of insulin resistance [10,13,25]. However, the effectiveness of mitochondrial targeted antioxidants in humans are mixed [173,182–184]. Recently, it was reported that 6 weeks of daily MitoQ (20 mg) treatment in middle-aged men led to a small suppression of mitochondrial H_2O_2 , yet treatment failed to impact mitochondrial function or measures of insulin resistance

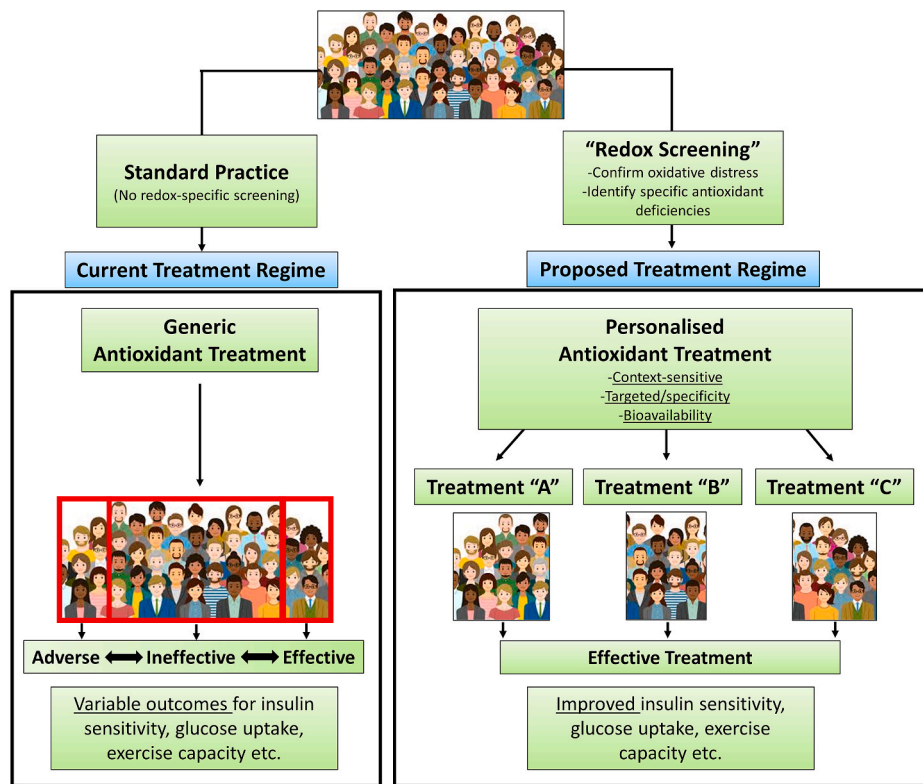


Fig. 2. We propose the effectiveness of antioxidant treatment depends largely on the biological context, involving whether oxidative eustress or distress is present, whether a specific redox deficiency exists, whether the antioxidant is targeted towards restoring a specific deficiency, and the bioavailability and bioactivity of the antioxidant. As such, a “redox screening” and “personalized antioxidant treatment” approach is likely required to elicit the most effective treatment regime in humans.

including HOMA-IR, HbA1c, and fasting insulin and glucose levels [182]. In the context of exercise, markers of mitochondrial damage are decreased during acute exercise with 21 days of daily MitoQ treatment (20 mg) in healthy men [173]. However, research investigating functional outcomes such as exercise capacity are mixed, and like systemic antioxidant treatment (e.g., vitamin C), are likely influenced by the context of treatment including the presence of oxidative eu/distress [183,184].

5. Conclusions and future directions

Research-to-date provides a confusing narrative on whether antioxidant treatment has the potential to be an effective strategy for improving health and/or treating and preventing chronic disease. However, considerable limitations in research methodology, including inadequate study design leading to ineffective antioxidant treatment, preclude accurate interpretation of findings and are likely to be masking the true effects, if any, of antioxidant treatment in humans. Factors influencing effective treatment include 1) whether oxidative distress/eustress is present (sedentary behavior, poor diet, disease, or exercise), 2) whether an endogenous antioxidant deficiency exists, 3) whether the antioxidant treatment is specifically designed to target and restore a deficiency (antioxidant specificity), 4) and the bioavailability and bioactivity of the antioxidant which are influenced by treatment dose, duration and method of administration. However, most studies have failed to account for these factors, leading to ineffective study design and a perpetual cycle of contradictory findings that have not yet been able to fully elucidate the potential of antioxidant treatment for improving health and preventing disease.

Research to-date does not support the consensus that antioxidant treatment is a one-stop-shop solution for improving health and preventing disease. It does, however, provide strong support that, depending on the context of treatment, exogenous antioxidants may be either “good”, “bad”, or “ineffective” at altering health outcomes. For example, pathological conditions characterized by perturbed redox

homeostasis and elevated oxidative distress, such as observed with transient and chronic states of insulin resistance and T2D, may benefit from targeted and personalized antioxidant treatment. Whereas under physiological conditions where redox homeostasis is likely to be retained or in balance (e.g., in healthy individuals with intact antioxidant defenses), antioxidant treatment is less likely to be effective and, in some cases (e.g., exercise), may decrease oxidative eustress leading to diminished health benefits or adverse health outcomes. Surprisingly, research has yet to adequately explore the complex interaction between antioxidant treatment, insulin resistance/sensitivity, and oxidative dis/eustress in humans. Investigating whether antioxidant treatment that is targeted at decreasing oxidative distress in clinical populations (i.e., insulin resistance), may also lead to an inadvertent decrease in oxidative eustress (e.g., exercise and insulin sensitivity) should be investigated to further inform personalized and context-sensitive treatment (Fig. 2).

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Author contributions

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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