

REVIEW

Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans

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Objective: Oxidative stress may be the unifying mechanism underlying the development of comorbidities in obesity. Evidence suggests that a clustering of sources of oxidative stress exists in obesity: hyperglycemia, hyperleptinemia, increased tissue lipid levels, inadequate antioxidant defenses, increased rates of free radical formation, enzymatic sources within the endothelium, and chronic inflammation.

Method: This review provides a summary of the available evidence on systemic oxidative stress in humans and specific metabolic pathways by which obesity may elevate systemic oxidant stress. The authors suggest possible methods of reducing oxidative stress such as antioxidant supplementation, caloric restriction and/or physical activity and surgical intervention to combat free radicals and reduce adipose tissue.

Results: Obesity is associated with oxidative stress and can be reduced with weight loss (regardless of exercise or surgery induced weight loss), caloric restriction or antioxidant rich diets.

Conclusion: Oxidative stress levels are elevated in human obesity, and these levels are modifiable with various lifestyle modifications and surgical interventions.

International Journal of Obesity (2006) 30, 400–418. doi:10.1038/sj.ijo.0803177; published online 22 November 2005

Keywords: lipid peroxidation; antioxidant; oxidative stress; free radicals

Introduction

Obesity is a prevalent metabolic disorder in the US and in large parts of the developing world.^{1,2} In 2004, the age-adjusted rates of obesity and overweight reported in the third National Health and Examination Survey (NHANES III) were 65.1% for the adult population and 16.0% for children.³ Both morbidity and mortality increase with excessive body weight.⁴ The relative risk values for developing diabetes, hypertension, dyslipidemia, insulin resistance, dyspnea and apnea for obese individuals are >3. The relative risk for coronary artery disease and osteoarthritis is between 2 and 3.⁴ Obesity is also linked to chronic inflammation, thrombotic tendencies and complications of coronary artery disease.^{4,5}

Oxidative stress is an imbalance between tissue oxidants (free radicals or reactive oxygen species) and antioxidants

and may be a unifying mechanism in the development of major obesity-related comorbidities such as cardiovascular disease (CVD) and diabetes.⁶ This paper presents the evidence on associations of human obesity with high oxidative stress and sources of oxidant stress in obesity. Potential lifestyle interventions to lower oxidative stress are presented.

Oxidative stress

Free radicals are highly reactive molecules with unpaired electrons that quickly bind with nearby molecules. Reactive oxygen species (ROS) are oxygen-containing molecules that either may or may not have unpaired electrons, but are highly reactive in tissues. ROS include superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^{\bullet}), nitric oxide (NO), hypochlorite and peroxynitrite ($ONOO^-$, the result of a reaction from $O_2^{\bullet -}$ and NO). Low concentrations of free radicals, ROS and other nitrogen species are necessary for normal cell redox status, cell function and intracellular signaling.⁷ However, in some disease states, free radicals are produced in excess. High concentrations of ROS and free

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Received 17 October 2004; revised 20 September 2005; accepted 7 October 2005; published online 22 November 2005

radicals damage DNA, proteins, carbohydrates and lipid constituents and compromise cell function.⁷

Direct measures of free radicals and ROS, which include electron resonance or spin trapping, capture free radical reactions in real time and are difficult and costly.⁸ Simpler techniques that measure biomarkers or end products of free radical mediated oxidative processes are therefore used often to estimate oxidative stress. For example, malondialdehyde (MDA), thiobarbituric reactive acid substances (TBARS), lipid hydroperoxides, conjugated dienes, 4-hydroxynenal (4HNE) and F₂-isoprostanes (8-epiPGF_{2α}) are widely used as indicators of lipid peroxidation damage. Hydroperoxides are generated in early stages of lipid peroxidation, while MDA or F₂-isoprostanes are formed at the end of long oxidative processes.⁸ The lag time for oxidation of lipid molecules such as low density lipoprotein (LDL) is also sometimes used to indicate the susceptibility of lipids to free radical attack. Lag time is the time required for exponential formation of lipid peroxidation products after exposure to a free radical system. Thus, short lag times indicate rapid formation of lipid peroxidation products, whereas longer lag times indicate resistance of the lipid to free radical damage. Finally, protein carbonyls, tyrosine crosslinks, advanced glycosylation end products, and DNA oxidation products such as 8-hydroxy 2'-deoxyguanosine (8-OHdG) are used as markers of damaged proteins and DNA.⁷ Oxidized lipids and proteins can be cytotoxic and may cause plasmolemma leakage and dysfunction of membrane bound receptors and enzymes, signaling cascades and intercellular function. The most serious consequence of lipid or protein oxidation is DNA and nucleic acid damage and cell death.^{9,10}

Tissue antioxidants

Tissue antioxidant defenses against ROS and radicals have been reviewed elsewhere.^{7,11,12} These defenses include nonenzymatic (dietary antioxidants, thiol-containing compounds) and antioxidant enzymes. Vitamins E and C and β-carotene are among the major dietary antioxidants.¹² The vitamins have received considerable attention in clinical trials of primary and secondary prevention of CVD and cancer. Vitamin E (α-tocopherol is the best known isomer) is found in lipoproteins, cell membranes and extracellular fluids. It terminates lipid peroxidation processes and converts O₂^{•-} and •OH to less reactive forms. Vitamin C is found in high concentrations in the adrenal and pituitary glands, liver, brain, spleen and pancreas. It is hydrophilic and can directly scavenge ROS and lipid hydroperoxides. Vitamin C can also restore vitamin E that has been oxidized by a free radical to its original antioxidant state, and vitamin C can spare selenium. Vitamin A (β-carotene is the most well known carotenoid) is found in the cellular membranes of tissues.¹² Like vitamin E, β-carotene is lipophilic and is an excellent free radical trapper. β-Carotene protects against both lipid peroxidation and DNA oxidation. Other important antioxidants include α-lipoic acid, coenzyme Q10 and

antioxidant minerals (selenium, zinc, copper and manganese) and other phytochemicals such as phenols, flavonoids, lycopene and hydroxytyrosol. In healthy tissues, these primary antioxidants work cooperatively to maintain the prooxidant-antioxidant balance and prevent tissue damage.

Collective antioxidant capacity can be measured by total antioxidant status (TAS), which represents free radical inhibition by all the antioxidants described above in a tissue sample such as plasma or serum. Antioxidants at high levels suppress oxidative processes and protect tissues. Secondary defenses include stress-inducible genes and proteins, such as heat shock proteins, and oxidative repair enzymes such as DNA glycosylase and 8-OHdGPase¹³ that become active once damage has occurred. These enzymes excise damaged protein bases and restore DNA to its normal state.

Antioxidant enzymes and thiols

The major antioxidant enzymes include superoxide dismutase, glutathione peroxidase and catalase. Superoxide dismutase (SOD) converts O₂^{•-} to H₂O₂. SOD has two isoforms, a copper-zinc isoform present in the cytosol (CuZn-SOD) and a manganese isoform present in the mitochondrion (Mn-SOD). These SOD isoforms work to protect the cell against free radicals and ROS. Glutathione peroxidase (GPX) and catalase (CAT) both reduce H₂O₂ and lipid hydroperoxides.¹⁴ Paraoxonase-1 (PON-1), a serum antioxidant enzyme, has been shown to combat lipid peroxidation of LDL.¹⁵ Thiol-containing molecules with S-H bonds, such as glutathione and dihydrolipoate, are powerful nonenzymatic antioxidants that recycle vitamin E and semihydroascorbic radicals and reduce oxidized molecules such as lipid hydroperoxides.⁷ Thiols and antioxidant enzymes are found throughout the blood, liver, brain, skeletal and myocardial muscle.

Obesity and evidence of oxidant stress in humans

Cross-sectional studies

Evidence of obesity-induced oxidative stress in humans has been accumulating over the past few years. A summary of this evidence is shown in Table 1. The majority of human studies relating obesity and oxidative stress have been cross sectional. In one of the first studies, Van Gaal *et al.*¹⁶ measured the oxidizability of LDL and very low-density lipoproteins (VLDL) in nonobese and obese subjects. These researchers found higher basal levels of MDA in lipoprotein samples in obese than nonobese persons (3.13 vs 1.89 mmol/l). Isolated non-HDL lipoprotein fractions oxidized up to 22% faster in samples from obese than nonobese persons.¹⁶ The lag time of LDL oxidation was shorter in obese women than nonobese (92.5 vs 123.4 min, respectively). In addition, basal plasma TBARS were higher in obese subjects.

In obese diabetic and nonobese healthy men and women (N = 20, 30–58 years), Skrha *et al.*¹⁷ found elevated oxidative stress in obese persons. Oxidative stress was directly related

Table 1 Evidence for obesity-related oxidant stress in humans

Study reference #	Cohort	Biomarker	Tissue sample	Major finding
Van Gaal <i>et al.</i> ¹⁶	Premenopausal women	TBARS	Plasma non-HDL lipoprotein	↑ TBARS in obese than nonobese women; BMI correlated negatively with lag time
Skrha <i>et al.</i> ¹⁷	Obese, diabetic men and women	MDA	Plasma	↑ MDA in obese vs nonobese persons; MDA/SOD ratio was ↓ in obese vs nonobese
Dandona <i>et al.</i> ³⁶	Obese, nonobese men and women	Protein carbonyls, TBARS	Plasma	↑ Protein carbonyl levels and TBARS in obese than nonobese; after caloric restriction biomarkers were ↓ by 87 and 15% of baseline values in the obese group
Block <i>et al.</i> ²⁴	Men and women	F ₂ -isoprostanes	Plasma	↑ Isoprostanes in class II obese than nonobese persons
Davi <i>et al.</i> ²⁶	Obese women obese	8-isoprostanes	Urine	↑ Isoprostane levels in both gynoid and android obese vs nonobese women, with highest levels in the android
Olusi ²³	Children, adults obese, nonobese	MDA	Plasma	↑ MDA in obese than nonobese persons; ↓ Erythrocyte CuZn-SOD and GPX values in morbidly obese persons compared with nonobese
Ozata <i>et al.</i> ²²	Men	TBARS	Plasma, Erythrocyte	↑ TBARS and ↓ erythrocyte CuZnSOD and GPX activity in obese than nonobese men
Stojiljkovic <i>et al.</i> ²⁰	Men and women, hypertensives	F ₂ -isoprostanes	Plasma	Following Intralipid and heparin infusion (to increase NEFAs), plasma F-isoprostane formation ↑ more in the obese than the nonobese group
Keaney <i>et al.</i> ²⁵	Men and women	F ₂ -isoprostanes	Serum	Isoprostane levels ↑ linearly in men and women with BMI > 25–27 kg/m ² (for women and men)
Konukoglu <i>et al.</i> ²⁸	Men and women	TBARS	Plasma	↑ TBARS in obese than nonobese persons
Myara <i>et al.</i> ²¹	Men and women	MDA	LDL	↓ LDL oxidation lag time in obese than nonobese persons
Russell <i>et al.</i> ⁵⁰	Men	4-HNE	Skeletal muscle	↑ 4-HNE in obese than nonobese persons
Urakawa <i>et al.</i> ²⁹	Men	8-epi PGF _{2α}	Plasma	↑ 8-epi PGF _{2α} in obese than nonobese men; 8-epi PGF _{2α} correlated with fat weight, visceral fat area
Vincent <i>et al.</i> ¹²⁰	Men and women	TBARS, PEROX	Plasma	↑ Postexercise TBARS and hydroperoxides in obese than nonobese persons
Uzun <i>et al.</i> ⁴⁸	Men and women, morbidly obese	MDA	ox LDL	↓ MDA in LDL following gastric band surgery
Vincent <i>et al.</i> ³³	Older obese women	PEROX	Plasma	↑ Postexercise TBARS and hydroperoxides in obese than nonobese women
Furukawa <i>et al.</i> ³⁰	Men and women, obese and nonobese	TBARS, 8-epi-PGF _{2α}	Plasma, Urine	↑ TBARS and urinary 8-epi-PGF _{2α} levels were correlated with BMI and waist circumference
Yesilbursa <i>et al.</i> ⁴⁶	Men and women, obese	MDA	Plasma	↓ MDA following 6 months of Orlistat therapy
Ferretti <i>et al.</i> ¹⁰⁸	Obese and nonobese women	PEROX	isolated HDL, LDL	↑ PEROX in HDL and LDL in obese than nonobese women
Vincent <i>et al.</i> (in review, 2005) ³⁴	Men and women, nonobese, obese	PEROX	Plasma	↑ Postexercise TBARS and hydroperoxides in obese than nonobese persons
Ozcelik <i>et al.</i> ⁴³	Men and women, obese	MDA	Serum	Orlistat and exercise training ↓ serum MDA in obese persons compared to Orlistat alone

BMI = body mass index; TBARS = thiobarbituric reactive acid substances; MDA = malondialdehyde; SOD = superoxide dismutase; CuZn SOD = copper zinc isoform of SOD; WHR = waist to hip ratio; LDL = low-density lipoprotein; HDL = high-density lipoprotein; NEFA = nonesterified fatty acid; 4-HNE = 4-hydroxynonal; PEROX = lipid hydroperoxides; 8-epi PGF_{2α} = a specific isoprostane, from a novel group of prostaglandin-like compounds.

to fasting serum insulin concentrations and inversely related to vitamin E concentrations. Plasma MDA values were 65% higher (3.1.3 vs 1.89 mmol/l), and the vitamin E concentration was 53% lower in the obese than in the nonobese group. Plasma MDA was inversely related with the glucose disposal rate. When expressed by SOD activity, MDA/SOD ratios were higher in the obese than in the controls (1.43 vs 2.06). The authors concluded that MDA is involved in systemic oxidative stress and impairment of normal glucose metabolism in obese persons. Chronic hyperglycemia in obesity may lead to continuously elevated MDA formation.¹⁸ In the presence of iron, MDA can generate more ROS, leading to a cycle of ROS formation and oxidative stress that impairs normal glucose metabolism in the obese individual.¹⁹

Using a different approach to characterize oxidative stress responses, Stojilkovic *et al.*²⁰ infused intralipid/heparin to increase non-esterified fatty acid (NEFA) levels in the blood of obese, hypertensive persons and nonobese persons. During the 2-h infusion period, F₂-isoprostanes formed nearly 2.5 times faster in the blood of the obese, hypertensive persons compared with nonobese persons. At 2 h following the infusion, plasma F₂ isoprostane values were 14.9±2.8 and 4.6±2.8 ng/ml in the obese and nonobese groups (*P*<0.05). This difference did not persist after 4 h post-infusion. F₂ isoprostane levels were positively correlated with elevations in NEFA, suggesting that these fatty acids contributed to the oxidative stress response in obesity.²⁰

In healthy nonobese and obese adults (ranging from moderately obese (30–34.9 kg/m²) to very severely obese (>50 kg/m²)), Myara *et al.*²¹ measured isolated LDL oxidizability (lag time) *in vitro*. Other blood measures collected included leptin, vitamin E and cholesterol subfractions. The LDL lag time was significantly shorter in the severely obese group than in the nonobese group (41±10 vs 55±21 min), with intermediate lag times for the intermediate obesity categories. Plasma vitamin E levels were lower with each increase in obesity (ranging from 13.8±3.4 nonobese group to 6.1±1.4 mmol/μmol triglyceride in the severely obese group). An increased rate of LDL oxidation and a lower plasma vitamin E level were positively related with adiposity.²¹

The oxidative stress levels of 100 healthy obese (BMI=36.6 kg/m²) and nonobese (BMI=21.7 kg/m²) men were compared by analyzing fasting blood samples for blood lipids, TBARS and erythrocyte antioxidant capacity.²² Cholesterol and lipoproteins were higher in the obese group. TBARS were 98% higher in the obese than the nonobese group (7.77 vs 3.92 nmol/ml). In addition, erythrocyte antioxidant enzyme activities of GPX and SOD were lower in the obese group, by 75 and 42%. Blood zinc levels were lower in the obese than in the nonobese group (512 vs 831 μg/dl). The lowered antioxidant defense coupled with elevations in cholesterol, LDL and VLDL may have predisposed the obese group to TBARS formation in this study.

In total, 300 subjects were randomly chosen from 7500 screened persons and placed into six BMI brackets: 19–25,

30–34, 35–39, 40–44, 45–49 and ≥50 kg/m²,²³ Persons with BMI greater than 40 kg/m² had higher plasma MDA concentrations than persons in the 19–25 kg/m² bracket (4.75 vs 2.53 μmol/l). MDA values increased with each additional increase in BMI to >50 kg/m². CuZn-SOD and GPX activities were lower in persons with BMI exceeding 40 kg/m² than in those with healthy weight. These data suggest the existence of an 'adiposity threshold' at which the system cannot provide adequate antioxidants for a given production of free radicals, thereby generating MDA as an oxidative by-product.

In healthy adults (ages 19–78 years, *N*=298), the distribution, correlates and causative factors of oxidative biomarkers were examined.²⁴ In addition to plasma antioxidants, MDA and free F₂α isoprostanes were quantified. Plasma isoprostanes ranged from 0.042 ng/ml in the normal weight participants (BMI<25 kg/m²) to 0.075 ng/ml in the class II obese participants (BMI>35 kg/m²) (*P*<.0001). Plasma MDA values ranged from 0.81 to 0.99 mmol/l, but the differences were not significant (*P*=0.17). Both plasma MDA and F₂α isoprostanes were positively associated with gender. Women had higher levels of MDA and isoprostanes than men. Cholesterol, plasma ascorbate and γ-tocopherol were positively and inversely related with MDA and isoprostane concentrations. Obesity was positively associated with plasma isoprostane levels. The nonsignificant differences in MDA among persons with low and high BMI values may be due to the variation within the MDA measures in this group. Standard deviations of MDA were 50–80% of the mean values, markedly higher than those reported by Olusi.²³ Alternatively, dietary intake of micronutrients or fat may have influenced MDA variation in this study.

The Framingham Offspring cohort (*N*=2828, ages 33–88 years) underwent a series of laboratory assessments, obesity measures, oxidative stress measures and cardiovascular risk factor measures.²⁵ There were significant positive correlations between BMI and urinary 8-epi-PGF₂α when adjusted by gender and age. BMI was a strong predictor of creatinine-indexed 8-epi-PGF₂α (regression coefficient of 0.094), second only to smoking. Every 5 kg/m² increase in BMI was associated with a 9.9% increase in 8-epi-PGF₂α. To ensure that obesity was truly a predictor of oxidative stress, BMI was replaced with waist-hip ratio (WHR) in the study's multivariate model. High WHR indicates central abdominal adiposity, whereas low WHR values reflect lower central deposition of fat. The positive association between BMI and 8-epi-PGF₂α remained significant after using the WHR in the regression. These data suggest that obesity, particularly central obesity, is an independent predictor of systemic oxidative stress.

Indeed, the level of oxidant damage may be related to adiposity and the pattern of adiposity as shown in a cross-sectional investigation. Davi *et al.*²⁶ examined the role of gynoid and android fat patterns on oxidative stress in women. All obese women in this study had BMI values of 30 kg/m² or greater. Based on WHRs, women were placed

into a gynoid obese group (peripheral fat distribution, WHR 0.80) or an android obese group (visceral fat deposition, WHR 0.96). Urinary isoprostane excretion rates were assessed in healthy controls and the obese women with gynoid and android obesity.²⁶ Urinary F_{2α} isoprostane levels were higher in the android obese group than in either the nonobese or the gynoid obese group. Thus, the android pattern of fat deposition thus placed greater oxidative stress on the system than the gynoid pattern (523 vs 275 U isoprostanes). The high F_{2α} isoprostane levels in the android group were accompanied by high C-reactive protein, plasma leptin, deoxythromboxane and insulin concentrations. Oxidant stress may be a major modulator of disease progression when it is part of a cluster of inflammatory, prothrombotic and metabolic markers in android obesity.²⁷

Konukoglu *et al.*²⁸ measured plasma concentrations of TBARS and copper in middle-aged nonobese and obese normotensive and hypertensive subjects ($N = 130$). TBARS concentrations were highest in the obese hypertensives ($8.45 \pm 1.09 \mu\text{mol/l}$) and lowest in the nonobese normotensives ($5.55 \pm 0.65 \mu\text{mol/l}$). TBARS values ranged between these values for the hypertensive nonobese and normotensive obese groups (6.85 and $7.20 \mu\text{mol/l}$). A similar pattern was observed with plasma copper levels: copper concentrations were highest in the obese hypertensives and lowest in the nonobese normotensives. The combination of obesity and hypertension probably had a direct effect on the concentration of plasma TBARS. The mechanism for this could be that excessive copper catalyzes superoxide to the hydroxyl radical to initiate lipid peroxidation.

Urakawa *et al.*²⁹ investigated the relationship between plasma 8-epiF_{2α} isoprostanes and insulin resistance and adiposity in nonobese and obese men. Isoprostane levels were higher for the obese than the nonobese subjects (45 vs 10 pg/ml). Isoprostane concentrations were positively correlated with BMI ($r = 0.408$), body fat weight ($r = 0.467$), visceral fat area ($r = 0.387$) and total fat area ($r = 0.359$). Insulin resistance was directly associated with isoprostane levels ($r = -0.668$). The authors interpreted these findings to mean that obesity enhances oxidative stress, and oxidative stress may be involved in the development of insulin resistance.

Most recently, Furukawa *et al.*³⁰ examined the relationship of lipid peroxidation (TBARS and 8-epi-PGF_{2α}) and obesity in persons with the metabolic syndrome. Both BMI and waist circumference were directly correlated with plasma TBARS and urinary 8-epi-PGF_{2α}. Based on these findings and findings using comparable animal models, the authors concluded that excessive accumulation of fat leads to enhanced production of ROS in adipocytes and systemic tissues.

Cross-sectional studies of acute exercise responses

Obese persons are vulnerable to oxidative stress at rest and during acute exercise. Four recent reports of exercise-induced

oxidative stress in obese individuals suggest increased susceptibility to oxidant damage following exercise.^{31,32–34} Oxidative biomarkers were elevated in proportion to exercise intensity. The intensity of the exercise was directly related to the degree of peroxidative damage. Specifically, vigorous or maximal exercise induced high levels of peroxidative damage, while moderate to low intensity exercise caused lower levels of damage.³⁵ Saiki *et al.*³¹ reported that following acute treadmill exercise, obese persons had greater elevations in serum hypoxanthine (a metabolite formed during vigorous exercise that may be involved in superoxide formation) than nonobese counterparts. While there was no direct oxidative biomarker in this study, the evidence suggests that obese persons had greater oxidative metabolic stress than nonobese persons.

Healthy nonobese and obese adults performed acute aerobic treadmill exercise and resistance free weight exercise at equal relative intensities. Following each exercise bout, postexercise lipid hydroperoxide and TBARS levels were higher in the obese than the nonobese group.³² Hydroperoxides and TBARS were 20–35% higher in the obese than in the nonobese following the aerobic and resistance exercise sessions, regardless of plasma TAS. Vitamin C intake was negatively correlated with the exercise-induced change in hydroperoxides in the obese group ($r = -0.707$).

Older obese women (67–69 years) performed an aerobic maximal treadmill exercise test.³³ Postexercise lipid hydroperoxide levels were significantly higher in the obese women compared with the nonobese women (0.13 vs 0.02 (nmol) (ml O₂/kg min) following the exercise. This occurred despite 20% shorter exercise times for the obese than for the nonobese women. Antioxidant thiol profiles (total thiols, protein thiols and nonprotein thiols (glutathione)) measured before and after exercise revealed no differences between the two groups at rest or following exercise. After controlling for body fat percentage and baseline hydroperoxide levels, major contributors to the change in hydroperoxides were age, peak heart rate (reflective of exercise intensity) and exercise duration (exposure to the oxidative stimulus). Obese women were at greater risk for oxidative stress than their nonobese counterparts during an oxidative stimulus.

When exercise loads are matched by relative intensity, oxidative stress responses are higher in obese than nonobese persons.³⁴ For example, young adults ($N = 42$, 18–30 years) completed a constant load power output cycle ergometer exercise test. This test was designed to remove body weight differences that may predispose the obese group to excessive metabolic stress. Lipid hydroperoxides were standardized using several exercise variables (workload, energy expenditure, rate of oxygen consumption) and biochemical variables (triglycerides, cholesterol subfractions, glucose and blood lactate) that change during exercise and may contribute to oxidative stress. Obese subjects had higher hydroperoxides than the nonobese subjects when this biomarker was standardized by total work achieved (0.004 vs 0.019 (nmol/ml)/kJ). Similarly, hydroperoxides were higher in the obese

when expressed by energy expenditure (0.004 vs 0.013 (nmol/ml)/kcal) and by the change in the rate of oxygen consumption during exercise (0.054 vs 0.289 (nmol/ml)/ml O₂/kg min). Obesity exacerbates oxidative stress despite weight support in exercise and identical relative workloads. An explanation may be that obesity accelerates the formation of ROS or excessively taxes the available antioxidant pool, leading to lipid peroxidation.

While the correlational data consistently indicate oxidative stress is present in obesity, many of the studies do not account for multiple influential factors in a single study. Dietary intake, differences in muscle mass sources of ROS or other subject characteristics such as ethnicity or age are often not provided in each study. Also, interaction effects of age, gender, ethnicity or other factors on oxidative stress in obese persons are not clear. Further studies are required to address these points.

Longitudinal studies

Dietary and exercise interventions for weight loss. One approach to investigating the role of obesity in oxidative stress is to determine the effect of weight loss (fat loss) with dietary restriction and/or increased physical activity. A 4-week weight loss study in obese patients ($N=9$) collected several measures of oxidative stress before and after the weight loss program. These measures included TBARS, plasma protein carbonyls and two measures of oxidative damage to linoleic acid (13-hydroxyoctadecadienoic acid (13-HODE), 9-hydroxyoctadecadienoic acid (9-HODE)) and ROS by white blood cells.³⁶ All subjects were restricted to 1000 kcal/day consisting of a 200 kcal Sweet Success (Nestle) for breakfast and lunch and a nutritionally balanced self-cooked 600 kcal dinner. The dietary regimen was conducted for 4 weeks, and a follow-up session was completed 3 months after cessation of the dietary restriction. Weight loss (average 4.5 ± 2.8 kg) was accompanied by a 13% reduction in plasma TBARS and a significant reduction in ROS by leukocytes. Plasma carbonyl levels were reduced following the dietary regimen (from 1.39 ± 0.27 at baseline, to 1.17 ± 0.12 μ mol/mg protein at 4 weeks). The 9-HODE and 13-HODE levels were also reduced by Week four. Reductions in all oxidative stress biomarkers occurred rapidly in obese subjects after only 1 week of the dietary restriction. This benefit persisted until Week four but disappeared after cessation of the treatment. By 3 months post-treatment, oxidative biomarkers all had returned to pretreatment values. Because weight loss was modest for the improvements in oxidative stress, the authors suggested that dietary reduction of fat, sugar and calories may also lower oxidative stress in the obese.³⁶ Caloric and protein restriction reduce free radical and ROS formation and inhibit accumulation of oxidative biomarkers in animal models.³⁷ Controlled food intake also reduces blood insulin and glucose concentrations, thereby suppressing insulin-induced free radical formation³⁸ that may occur in obesity. Hence, caloric restriction may reduce body weight

and free radical formation in obese persons, both of which lower oxidative stress.

A combined diet and exercise intervention was administered to a sample of 80 obese persons at the Pritikin Longevity Research Center. The intervention consisted of 3 weeks of high complex-carbohydrate/low-fat diets (<10% fat, 10–20% protein, 70–80% carbohydrate) and 1.5 h of daily exercise (stretching, resistance exercise and cycle/treadmill exercise).³⁹ Following the program, body weight was reduced by 4–5%. LDL shifted from small to large diameter particles (and from phenotype B to A, a lesser risk for CVD). The basal level of serum lipid oxidation was reduced by 21%. The peak rate conjugated diene formation was reduced, and the LDL lag phase of lipid oxidation increased by 13%. While there was no change in LDL vitamin E content, LDL β -carotene content increased by 46%. The β -carotene elevation was not related to lag time, however. Diet and exercise modifications shifted the LDL to a less atherogenic profile and reduced oxidative stress in obese persons.

Obese men completed a 3-week short-term Pritikin vegetarian regimen and walked for 45–60 min/day.⁴⁰ Subjects reduced body weight by 4 kg (a 3.7% loss in body weight) and reduced cholesterol, LDL, HDL and triglycerides during the regimen. Serum 8-isoprostane PGF_{2 α} levels in these obese men were reduced from 210 to 150 pg/ml from baseline to post-treatment. The rapid reduction of oxidative stress may be associated with reductions in body weight and the improvements in blood lipids that accompany dietary change and weight loss.

Two dietary modification programs were administered to both obese hypertensive and nonobese normotensive subjects using a crossover design.⁴¹ Each subject followed the Dietary Approaches to Stop Hypertension (DASH) and a low antioxidant regimen (low fruit/vegetable intake 1–2 servings/day) for 4 weeks each. Ferric acid reducing/antioxidant power (FRAP) and plasma F₂-isoprostanes were assessed before and after each dietary phase. Interestingly, fasting F₂-isoprostane concentrations did not differ significantly between obese and nonobese (42 vs 39 pg/ml on the regular diet). The FRAP increased by 23% with the DASH diet in the obese group. Intralipid infusions (4h) were performed on each group before and after each dietary regimen. F₂-isoprostane elevations during infusion were suppressed in the obese group following the DASH diet, suggesting that increased dietary antioxidant intake suppresses lipid peroxidation during acute lipid loads.

Chen *et al.*⁴² examined the relationship between dietary patterns and oxidative stress in a cohort from the Nutrition and Breast Health Study. Premenopausal women ($N=122$) were randomized into one of four dietary patterns for a 12-month program: (1) no intervention, (2) low fat (15% of energy from fat), (3) high fruit and vegetable intake (FV), nine daily servings per day, and (4) low fat, FV group (combined low fat, high FV intake). Following the intervention, weight loss occurred only in the low fat group. The

concentrations of 8-isoprostane 2α were reduced only in that low fat group. The change in BMI was directly correlated with the change in 8-isoprostane 2α following the intervention. The authors concluded that those who had reductions in oxidative stress were those who lost weight.⁴² Healthy premenopausal women who did not lose weight while consuming prudent diets did not derive oxidative stress reducing benefit.

Evidence of exercise benefit in humans and two animal studies. Data are scarce on the effectiveness or mechanisms of exercise training in reducing oxidative stress in obese humans. However, one recent study compared the effect of adding exercise to pharmacologic treatment to reduce oxidative stress.⁴³ Participants were placed into either an Orlistat treatment group or an Orlistat-Exercise group. Over 12 weeks, the groups were administered either the drug alone or in combination with cycle ergometer exercise ($3 \times$ week, 45 min per session). Serum MDA and vitamins E and A were assessed at baseline and 12 weeks. Following the intervention, weight loss was comparable between groups (8.5 and 10.2% of body weight in the Orlistat and Orlistat-Exercise groups). MDA levels were 29.9% lower in the Orlistat-Exercise group compared with Orlistat alone. Although both groups showed reductions in serum vitamin A and E levels, these values remained higher in the Orlistat-Exercise than the Orlistat group by 12 weeks. The authors concluded that the combination of the weight loss and exercise-induced upregulation of antioxidant defenses in the combined treatment group were effective in lowering systemic oxidative stress.

Two animal studies provide evidence regarding training and reduction of oxidative stress. In one study, the effects of an eight-week treadmill running program in obese Zucker rats ($7 \times$ week, at $\sim 70\%$ of maximal aerobic capacity) were examined.⁴⁴ Following the intervention, obese trained rats had body weights similar to those of sedentary obese rats and higher than the nonobese rats. The trained obese rats had fasting blood glucose concentrations lower than the sedentary obese animals and comparable to the controls. In obese trained rats, exercise training preserved liver glutathione, GPX and Mn-SOD activities at levels similar to the controls. Sedentary obese animals had substantially lower GSH and Mn-SOD and GPX activities than in the trained obese and nonobese controls.

Obese Zucker rats⁴⁵ were separated into a control group, a lipoic acid (LA) group, an exercise group and a LA-exercise group. Animals were either fed 30 mg/kg body weight LA or ran on the treadmill for 60–75 min/day for 6 weeks. Following the intervention, all treatment groups had lower body weights than the control animals. Oxidative stress levels (protein carbonyl content of liver, heart and skeletal muscle) were significantly lower in all treatment groups. Lower protein carbonyl values were associated with improvements in peripheral glucose uptake in the obese animals. Exercise increases antioxidant defenses and lowers

oxidative stress levels in this animal model. Additional studies are needed to confirm whether these specific exercise adaptations occur in obese humans.

Pharmacologic interventions. The effects of Orlistat treatment on lipid peroxide levels were examined in a sample of nondiabetic obese men and women.⁴⁶ All subjects took 120 mg of Orlistat three times daily; no other lifestyle changes were implemented. After 6 months of treatment, the mean body weight of the obese group was reduced from 91.5 ± 9.8 to 84.7 ± 10.1 kg. Waist circumference was reduced from 105 ± 9.7 to 99 ± 9.8 cm. These changes corresponded with modest reductions in cholesterol, LDL and triglycerides. MDA concentrations were dramatically reduced, from 2.0 ± 0.77 to 0.89 ± 0.41 nmol/ml for the treatment.

Surgical interventions. Two recent studies found that gastroplasty attenuated oxidative stress in obese persons. Kisakol *et al.*⁴⁷ performed vertical banded gastroplasty on 22 obese patients. Blood markers including MDA, cholesterol, triglycerides and antioxidants β -carotene and α -tocopherol were assessed at weeks 0, 12 and 24 postsurgery. At 24 weeks, body weight was reduced by 28%, and this change corresponded with increases in plasma α -tocopherol and reductions in serum cholesterol. Also, weight loss was accompanied by a 50% reduction in MDA from 1.51 ± 0.11 to 0.75 ± 0.06 μ mol/l.

Uzun *et al.*⁴⁸ used either open or laparoscopic gastric banding procedures in obese men and women (BMIs ranged from 53 to 54 kg/m^2). At 6 months following the surgery, weight loss was 11 kg for the open surgery and 12 kg for the laparoscopic surgery ($P < 0.05$ from baseline). Leptin values dropped, while antioxidant paronoxase-1 levels increased in both treatment groups. MDA values were reduced by 22 and 23%, and oxidized LDL was reduced by 9 and 12% in open and laparoscopic techniques, respectively.⁴⁸

In these human studies, weight loss was accompanied by a reduction in blood cholesterol and free fatty acids, increased insulin sensitivity and glucose uptake. Changes in all of these variables could reduce systemic oxidative stress.⁴⁹ Some studies found reductions in oxidative stress with short-term interventions that did not cause dramatic weight loss.³⁶ Thus, it is not clear whether the training or dietary-induced reductions in oxidative stress were due to reduced adipose tissue volume, to attenuation of insulin resistance and dyslipidemia, or both. It is possible that controlling disease processes (lowering insulin resistance and lipids via nutritional modification) and weight loss both contribute to reductions in oxidative stress. Research is required to tease out the effect of stored body fat and circulating fat available for oxidation on oxidative stress and what antioxidant mechanisms may be responsible for changes in oxidative stress (enzymatic, dietary or TAS).

Location of oxidative stress biomarkers

Oxidative stress biomarkers are present in several tissues of obese humans, including skeletal muscle, plasma and erythrocytes.^{17,22,23,32,50} Most of the evidence comes from plasma, serum or erythrocyte tissues because these tissues are easiest to harvest. Erythrocyte lysates from obese persons contain more TBARS than those from nonobese persons (7.77 vs 3.92 nmol/ml). Indirect evidence for oxidative stress also can be found with tissue antioxidant depletion observed in erythrocytes. Olusi,²³ for example, reported that erythrocyte CuZn-SOD and GPX were lower (by 32 and 17%, respectively) in morbidly obese than nonobese persons. Ozata *et al.*²² also reported lower erythrocyte CuZn-SOD and GPX activities (42 and 75%, respectively) in obese than in nonobese persons. Only one human study investigated oxidative stress biomarkers in skeletal muscle of obese persons.⁵⁰ Muscle contained more 4HNE adduct content per unit intramuscular triglyceride in obese than nonobese persons (1.35 vs 0.75 HNE arbitrary units, $P < 0.05$).

Potential mechanisms underlying oxidative stress in obesity

There are several possible contributors to oxidative stress in obesity, including hyperglycemia,⁵¹ increased muscle activity to carry excessive weight,^{32,52} elevated tissue lipid levels,^{53,54} inadequate antioxidant defenses,⁵⁴⁻⁵⁷ chronic

inflammation,^{26,58,59} endothelial ROS production,^{60,61} and hyperleptinemia.⁶² These factors are not mutually exclusive. Rather, obesity may involve some or all of these contributors to systemic oxidative stress. Depending on the status of the obese individual, one contributor may exert a greater oxidative stress effect than the others, but this contribution may change as the metabolic and physical status of the individual changes. The evidence regarding sources of oxidative stress has been compiled from several experimental models, including cell culture, animal or human. Significant relationships between these prooxidant and antioxidant processes in obesity are shown in Figure 1.

Hyperglycemia

Diabetes, one of the major comorbidities of obesity, is directly associated with insulin resistance and hyperglycemia. Several oxidative pathways that are activated by hyperglycemia are shown in Figure 2. Advanced glycosylation end products (AGE) formed from proteins, lipids and nucleic acids are diabetes precursors. AGEs bind to specific cell surface receptors (RAGE) and lead to postreceptor signaling and further generation of ROS. AGEs also activate intracellular transcription factors such as nuclear factor- κ B (NF- κ B), which initiates a cascade of intracellular signaling pathways. NF- κ B activates protein kinase C and sorbitol and transcription of vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1). Activation of these intracellular molecules can produce ROS, as has

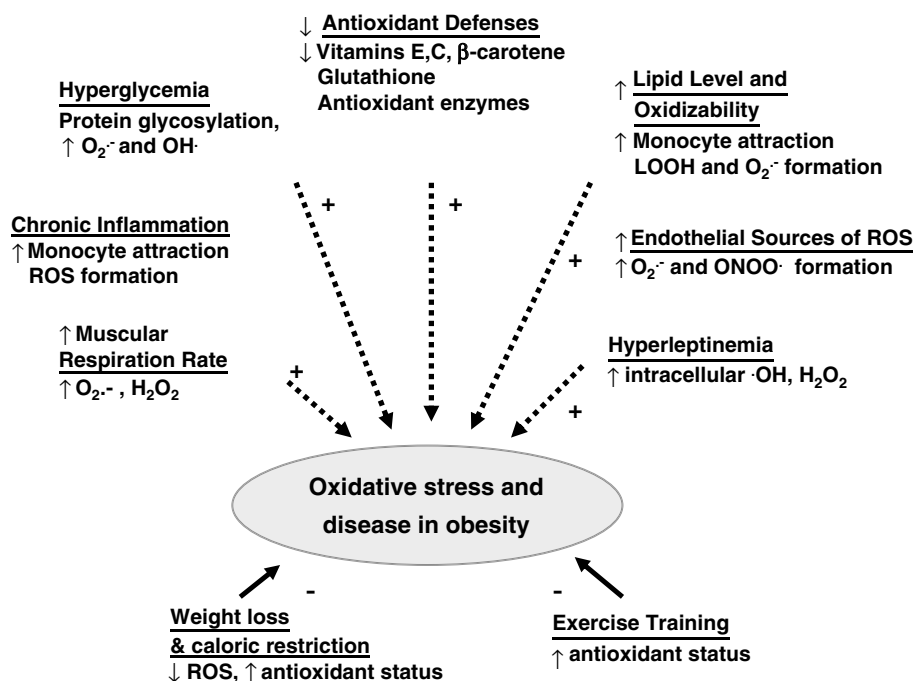


Figure 1 Relationships between pro-oxidant and antioxidant influences in obesity-induced oxidative stress. + symbol represents an increase on oxidative stress, - symbol represents an attenuation effect on oxidative stress. LOOH, lipid hydroperoxides.

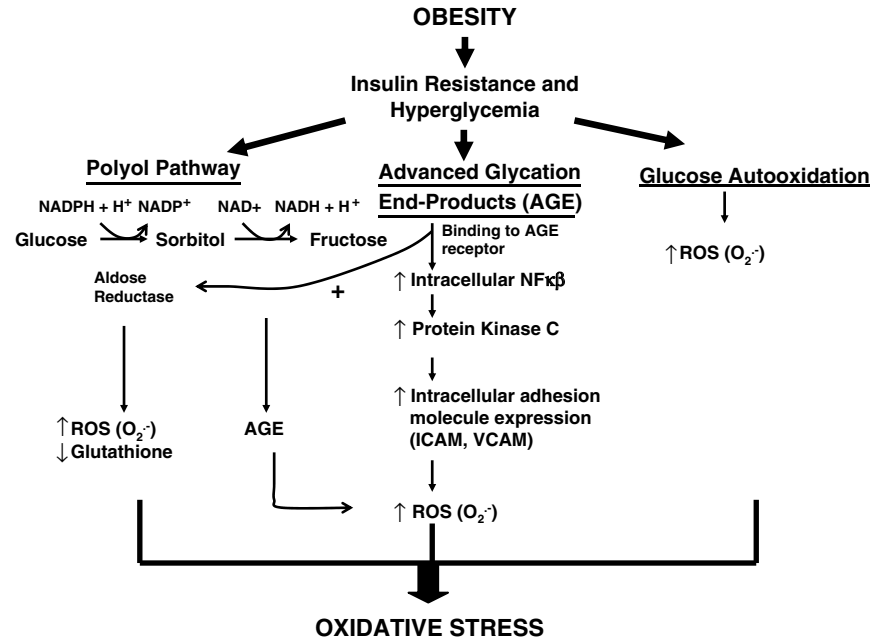


Figure 2 Hyperglycemia-induced pathways of oxidative stress in obesity, including the polyol pathway, the AGE pathway and glucose auto-oxidation. ROS, reactive oxygen species; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule. + symbol = enhanced effect on the pathway.

been shown in rodent vessel tissues.⁶³ Oxidant damage and accelerated monocyte homing to the endothelium are the end results.^{49,51} Intracellular glucose elevations stimulate the polyol pathway in which aldose reductase mediates conversion of glucose to sorbitol. Excess sorbitol causes oxidative damage and activates stress genes, as has been shown in a variety of animal models.⁴⁹ Hyperglycemia also increases nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, and NADPH produces $O_2^{\bullet-}$, especially in the endothelium.⁶⁴ When glucose itself auto-oxidizes, it produces oxidants with reactivity similar to the OH^{\bullet} and $O_2^{\bullet-}$ radicals.⁵¹ Finally, recent evidence has shown that diet-induced elevations in glucose uptake into adipocytes of obese mice increase ROS formation even within adipocytes themselves.⁶⁵ Hallmark molecules of hyperglycemia are glycated hemoglobin (HbA_{1c}) and AGE, both of which are implicated in insulin resistance, β -cell dysfunction, diabetes complications and peripheral neuropathy in obese persons, especially those with concomitant diabetes or insulin resistance.⁴⁹

Increased metabolic ROS production

Increased muscle activity can activate metabolic pathways that form free radicals, including increased electron transport chain activity and conversion of hypoxanthine to urate.³¹ Active skeletal muscle may elicit a 100-fold increase in O_2 flux through the aerobic metabolic pathways.⁶⁶ Rapid electron transfer with increased respiration may cause some electrons to leak from the electron transport chain and

partially reduce oxygen. Partial reduction of oxygen forms $O_2^{\bullet-}$ and subsequently H_2O_2 .^{11,67} Among obese persons, high cell respiration rates and oxygen consumption may be exacerbated in muscle tissue during physical activity due to the additive mechanical load of carrying excessive body weight.^{32,52} For example, during the same absolute load-bearing walking activity, obese persons have 38% higher oxygen consumption values than nonobese persons, and these values were found to be correlated with postexercise lipid hydroperoxide values.³² In addition, obese persons are less mechanically efficient during exercise, and inefficiency contributes to increased energy expenditure for a given exercise load.³⁴ Acceleration of mitochondrial respiration for energy production is associated with increased lipid hydroperoxide production in the obese.³³

Increased concentrations of hypoxanthine during exercise have been documented in obese humans.³¹ When hypoxanthine is converted to urate, $O_2^{\bullet-}$ is formed. Saiki *et al.*³¹ reported higher resting and postexercise hypoxanthine and uric acid levels in obese than in nonobese persons. Thus, when performed sporadically, strenuous muscle activity may actually result in an acute increase in oxidant stress in obese persons.³¹

Inadequate antioxidant defenses

Adequate tissue dietary, enzymatic and nonenzymatic antioxidant defenses are critical to maintain antioxidant-prooxidant balance in tissues. Perturbations to antioxidant defenses occur in obesity. An illustration of the factors

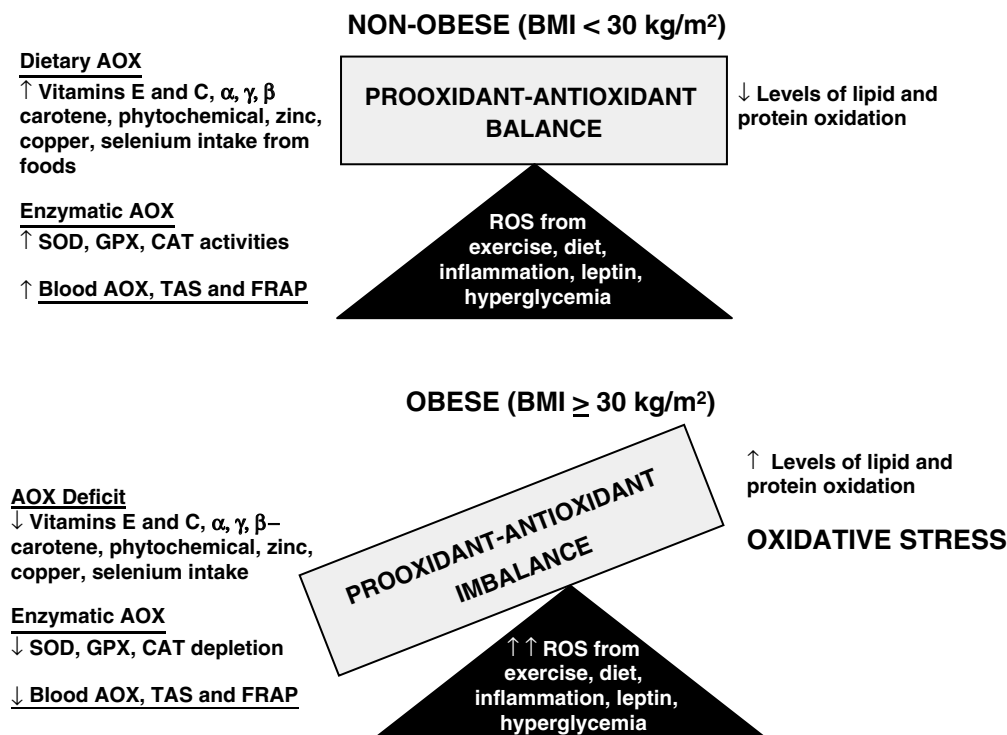


Figure 3 Prooxidant-antioxidant balance with nonobese status (top) and obesity (bottom). Increased dietary intake of antioxidants, and tissue enzymatic and nonenzymatic antioxidants match the pro-oxidant processes with nonobese status. In obesity, an antioxidant deficit exists. Available antioxidants are overpowered by excessive ROS formation, shifting the system toward oxidative stress. ROS, reactive oxygen species; AOX, antioxidant; SOD, superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase; TAS, total antioxidant status; FRAP, ferric acid reducing potential.

affecting oxidative balance is shown in Figure 3. Inadequacy of antioxidant defenses may begin with a low intake of protective antioxidants and phytochemicals in the diet.⁶⁸ Obese individuals have a lower intake of phytochemical-rich foods (fruits, vegetables, whole grains, legumes, wine, olive oil, seeds and nuts) compared with nonobese persons. Phytochemical intake is inversely correlated with higher waist circumference, BMI and plasma lipid peroxidation.⁶⁸ Serum levels of dietary antioxidants (β -carotene, vitamins E and C) and levels of trace minerals (cofactors for antioxidant enzymes zinc, selenium) were found to be lower in obese than nonobese children and adults.^{22,55,56,69,70} Also, dietary antioxidant levels are inversely related to the degree of adiposity.^{57,71} BMI is negatively correlated with plasma vitamin E levels ($r=0.53$).²¹ Hence, obese persons have an 'antioxidant deficit' as a result of poor antioxidant intake, independent of external metabolic stressors on the body. Alarming, plasma vitamin concentrations are progressively lower with increases in BMI, with the obese having the lowest concentrations.^{56,72} While these data do not show cause, these data are strong evidence that incremental increases in BMI are related to increasing imbalance in the antioxidant-prooxidant status.

Despite similar self-reported intakes of fruit and vegetable servings, serum β -carotene concentrations were lower in obese children than in normal weight children (0.22 vs

0.29 $\mu\text{mol/l}$). α -Tocopherol concentrations were also lower in the obese children (2.68 vs 3.17 $\mu\text{mol/l}$).⁷² In adults, blood retinol, tocopherol, vitamin C and carotene concentrations were 18–37% lower in obese women than in lean women, respectively.⁵⁶ Zinc levels were 38% lower in the plasma of obese men than in their lean counterparts.²²

Plasma α -tocopherol or β -carotene levels may be expressed per unit plasma LDL, a method that estimates antioxidant protection within circulating lipids. In a recent study, obese girls had low α -tocopherol/LDL and β -carotene/LDL compared with non-obese girls. Furthermore, these obese girls also had a higher 'peroxidizability index' (lipid peroxidation per amount LDL).⁷³ The authors concluded that there were inadequate antioxidants available within the large LDL lipid pool, and this caused the oxidative stress. Adults also had lower plasma vitamin E concentrations when expressed per unit triglyceride and BMI (13.8 vs 6.1 vitamin E $\mu\text{mol}/\text{mmol}$ triglycerides in nonobese with BMI < 30 kg/m² vs BMI > 50 kg/m², respectively).²¹ Independent of age, dietary antioxidants may be used more rapidly in combating the excessive prooxidant processes in obese persons, leaving the individuals less defended against free radicals.

Activities of the major antioxidant enzymes may also be inadequate in obesity. In rat models of diet-induced obesity, erythrocyte SOD and GPX activities were lower by 29–42% in the high fat, high calorie fed animals compared with the

control animals after the diet period.⁵³ It has been proposed that in the early stages of obesity there may be an initial elevation in antioxidant enzymes to counteract oxidative stress, whereas chronic obesity continually depletes the sources of antioxidant enzymes.^{23,54} The degree of adiposity also affects enzyme activities. In a cross-sectional study, Olusi²³ found that erythrocyte CuZn-SOD activity was lower in the very obese (853 vs 1464 U/g Hb), and GPX activity was lower in the obese (76 vs 98.4 U/g Hb) than in the nonobese controls.²³ Similarly, Ozata *et al.*²² reported 75 and 42% lower erythrocyte GPX and CuZn-SOD activities in obese men than in nonobese men. Erythrocyte zinc levels were 62% lower in the obese group.²²

TAS and ferric reducing antioxidant power (FRAP) have both been used as comprehensive measures of radical-squelching capacity by antioxidants in plasma. Several studies have found lower TAS and FRAP values in obese persons than in nonobese persons^{41,74} and animal models of obesity.⁵³ For example, FRAP values were 22% lower in obese than in nonobese matched controls,⁴¹ and TAS values were moderately lower in obese persons (1.15 vs 1.30 mmol/l). TAS values also were lower in obese than nonobese persons following oxidative challenges such as aerobic exercise.³² Most recently, obese children with the metabolic syndrome had lower plasma TAS levels than their nonobese counterparts (1.22 vs 1.57 mmol/l, respectively).⁷⁵ Lower TAS values

were directly related with lower levels of various forms of plasma carotenoids such as α , β -carotene, α , β and γ -tocopherols.⁷⁵ Figure 3 illustrates the prooxidant-antioxidant imbalance that occurs in obesity. The combination of inadequate dietary, blood and enzymatic antioxidants and increased production of ROS formation creates an imbalance that favors lipid and protein oxidation and oxidative stress in obesity.

Lipids and lipid oxidizability

Obesity is characterized by increased dietary fat intake, increased fat storage, and excessive intracellular triglycerides and dyslipidemia.^{26,32} Lipid-related pathways that may contribute to oxidative stress are shown in Figure 4. Oxidative stress may be due to the metabolic impact of intracellular triglycerides.⁷⁶ For example, by suppressing the mitochondrial adenine nucleotide transporter, excessive triglycerides may increase $O_2^{\bullet-}$ production within the mitochondrial electron transport chain, and this decreases intramitochondrial adenine disphosphate. Electrons then accumulate within the electron transport chain and react with adjacent O_2 to form $O_2^{\bullet-}$.⁷⁶

Abdominal or visceral adiposity is also linked with elevated plasma free fatty acids (FFA). FFA elevate blood glucose and produce nitroxide radicals in smooth vascular

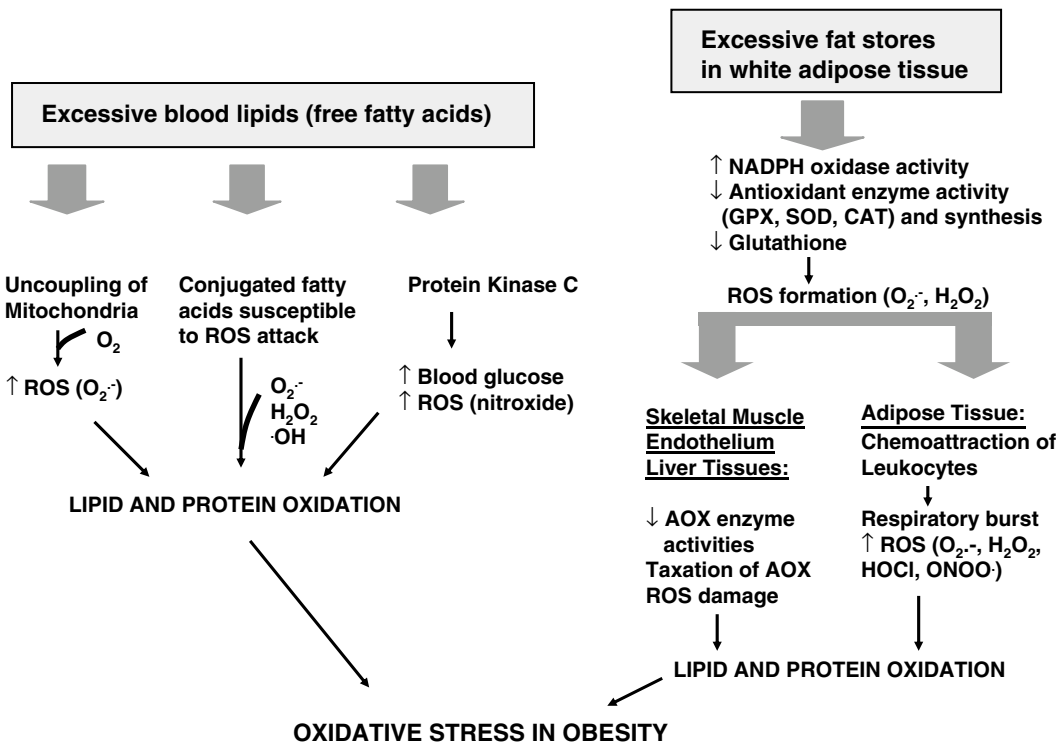


Figure 4 Lipid sources of oxidative stress in obesity. Excessive fatty acids in the blood are involved in three intracellular pathways of ROS formation including mitochondria, specific fatty acid isomers and cell signaling pathways. Excessive fat in adipocytes lowers antioxidants and attracts leukocytes to the adipose tissue; both pathways generate ROS and promote oxidative stress. ROS, reactive oxygen species; SOD, superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase.

and endothelial cells via a protein kinase C mechanism as shown in cultured vascular cells.⁷⁷ FFA can also induce the oxidative respiratory burst in white cells and acutely increase ROS formation ($O_2^{\bullet-}$, hypochlorous acid, ONOO \bullet) in culture.⁷⁷ The lipids serve as a substrate for oxidation, stimulate radical formation, and enhance the accumulation of oxidative by-products, especially in white adipose tissue.³⁰ In excess, oxidation of lipids increases the risk for thrombosis, endothelial dysfunction, and atherosclerosis.^{78,79}

Dyslipidemic profiles in obesity include elevated triglycerides, lowered high-density lipoproteins (HDL), and elevated LDLs.⁸⁰ Hypercholesteremia is associated with enhanced oxidizability of LDL molecules. The lag phase of lipid oxidation is shorter in LDL particles from obese individuals; rapid lipid peroxidation in the polyunsaturated fatty acids of LDL particles subsequently occurs.^{16,22} The susceptibility of lipids to oxidative modification is also shown by higher concentrations of 4-HNE per unit intramuscular triglycerides in obese persons.⁵⁰ Insufficient antioxidant defenses may explain 4-HNE elevations in this study.

Dietary intake of specific lipids also influences systemic oxidative stress. Specifically, consumption of conjugated linolenic acid (an 18 carbon unsaturated fatty acid with two conjugated double bonds derived from dairy products and consumption of meat from ruminant animals) increases urinary concentrations of 8-epiPGF_{2 α} in obese men by up to 420%.⁸¹ Also, Riserus *et al.*⁸² added conjugated linolenic acid (4.2 g/day) or placebo to the normal diet of abdominally obese men for 4 weeks and they found a 50% increase in urinary 8-epiPGF_{2 α} in the treatment group. After resuming a normal diet for 2 weeks, the elevation in oxidative stress disappeared.⁸¹

Alternatively, the increased number of lipid molecules present in obesity may simply be an enlarged target for oxidative modification by ROS.^{23,54} In a comparative study of obese and lean Zucker rats fed either low or high fat diets, myocardial lipid hydroperoxides and TBARS concentrations were elevated in obese animals by 18 and 66%, respectively.⁵⁴ The major contributor to lipid peroxidation in the high fat feeding model was tissue lipid content ($r=0.87$). When lipid peroxidation levels were normalized for tissue lipid, the oxidative stress differences between lean and obese animals largely disappeared.⁵⁴ Furukawa *et al.*³⁰ found that in several obese mice models (KKAy, db/db, diet induced obesity, C57BL/6), accumulation of excessive fat over time in white adipose tissue adipocytes increased TBARS content within the white adipose tissue itself. In addition, obesity in the KKAy mouse increased NADPH oxidase activity and reduced SOD, GPX and CAT activities and mRNA in white adipose tissue. Together, increased excessive fat-induced ROS formation and lowered antioxidant defense promote oxidative damage in adipose tissue. Furukawa *et al.*³⁰ concluded that ROS and lipid peroxidation biomarkers enter systemic circulation and initiate a vicious cycle of systemic oxidative stress in obesity.

Chronic low-grade inflammation

Obesity in humans is considered a state of chronic inflammation,^{59,83,84} and serum adipokines increase with fat mass, especially with visceral fat. Inflammation is characterized by inflammatory cytokine expression, C-reactive protein (CRP) production, and increased white blood cell counts and white cell activity. Figure 5 shows the relationships among adipose tissue, inflammation, sources of ROS and oxidative stress. Adipose tissue is a storage depot for lipid energy and is an active endocrine organ. Fat expresses pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α). In most conditions, the production of CRP by the liver is controlled by cytokines that are elevated with acute infection.⁸³ Expansion of the adipose tissue depot in obesity may increase IL-6 and TNF- α levels. Both IL-6 and TNF- α activate CRP production.^{83,85} Reduction of fat mass is directly associated with reductions in the inflammatory adipokines, as much as 20% depending on the degree of weight loss in both animals and humans.^{79,86}

Elevations of inflammatory molecules stimulate the expression of atherogenic endothelial adhesion molecules and promote the attachment and migration of monocytes into vessel walls, causing conversion of monocytes to macrophages.⁷⁹ Specifically, TNF- α increases the expression of adhesion molecules on the endothelium and smooth muscle cells as has been shown in isolated animal and human vascular cells,⁷⁹ and these molecules also impair the insulin signaling cascade. TNF- α also suppresses insulin signal transduction and expression of the insulin receptor in isolated adipocytes, which leads indirectly to glucose dysregulation and hyperglycemia, and eventual pancreatic β -cell destruction.^{84,87} CRP levels indicate vascular inflammation and also predict LDL levels.⁸⁸ CRP, IL-6 and TNF- α are also associated with CAD, infarction, stroke, thrombosis and peripheral arterial disease.^{59,83,84} Adiponectin, which improves insulin sensitivity and inhibits vascular inflammation,⁷⁹ is present at a low level in the plasma of obese persons but increases with weight loss.⁸⁹ A strong inverse relationship exists between adiponectin mRNA levels and CRP levels.⁹⁰ Thus, the cytokine profile may shift toward a prooxidant state in obesity and toward a balanced prooxidant-antioxidant state with optimal weight.

Plasma TNF- α , IL-6 and CRP concentrations are positively associated with level of adiposity.^{6,23,59,84} In one study, CRP concentrations were 63% higher in persons with android obesity than persons with gynoid obesity²⁶ and nearly four times the values reported in the nonobese group (1.67 vs 0.40 mg/l).²⁶ CRP is positively associated with F₂-isoprostane levels and prothrombotic markers in obese persons,^{24,26} suggesting that obesity is related to a chronic state of oxidant stress and platelet activation. Also, in human studies that induced weight loss or elevated plasma adiponectin concentrations, high plasma concentrations of CRP, TNF- α and IL-6 were attenuated.^{83,91}

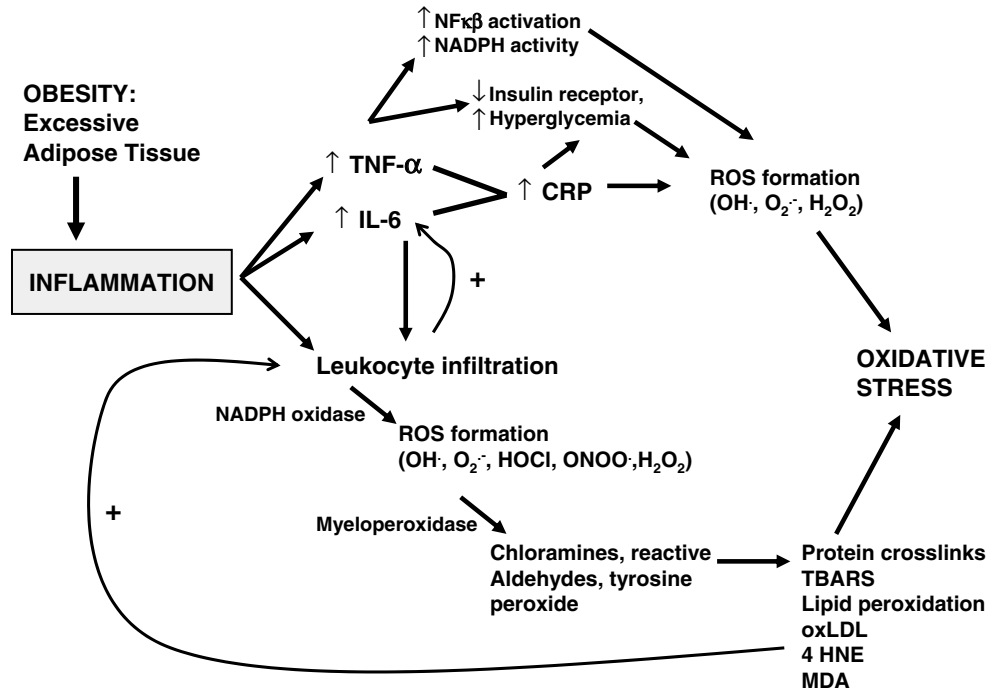


Figure 5 Inflammatory pathways that induce oxidative stress in obesity. Adipokine levels increase, indirectly causing ROS formation via several intracellular signaling pathways and insulin receptor impairment. Leukocyte infiltration causes enzymatic formation of ROS. Both pathways generate ROS and oxidative damage. TNF- α , tumor necrosis factor α ; IL-6, interleukin-6; CRP, C-reactive protein; oxLDL, oxidized low-density lipoprotein; 4 HNE, 4-hydroxynenal; MDA, malondialdehyde. + symbol = enhanced effect on the pathway.

White blood cell counts are higher in obese persons, with elevations occurring in the monocyte subfraction and trends toward elevation in the neutrophil subfraction.⁹² Monocytes produce $O_2^{\bullet-}$, H_2O_2 , OH^{\bullet} , $ONOO^{\bullet-}$, hypochlorous acid and myeloperoxidase, and when developed into macrophages, they produce interleukins and TNF- α . Neutrophils generate $O_2^{\bullet-}$ via NADPH oxidase with reaction intermediates such as H_2O_2 ,⁹³ and neutrophils and monocytes can convert H_2O_2 to hypochlorous acid via myeloperoxidase. The myeloperoxidase- H_2O_2 system can also yield chloramines (long-lived oxidants), reactive aldehydes, and tyrosine peroxide. ROS produced by these immune cells is associated with elevations in tyrosine crosslinks, TBARS, oxidized linolenic acid, and oxidized serum proteins and lipoproteins.⁹³ A high white cell count and myeloperoxidase are consistent, powerful predictors of cardiovascular events.⁹⁴

As obesity develops, there is a progressive infiltration of macrophages into the adipose tissue depots,² in part due to the chemoattraction of leukocytes into adipose tissue by MDA and 4HNE (by-products of fat-induced ROS generation).³⁰ Changes in adipocyte volume and overall fat pad size increase the rate of TNF- α and leptin secretion, and this attracts more macrophages into fat tissue. It has been proposed that with obesity a vicious cycle of adipocyte-initiated macrophage recruitment and cytokine/ROS production by macrophages occurs, which could potentially lead to oxidative damage and disease processes such as atherosclerosis.⁹⁵

The endothelium

Hypertension is a major comorbidity in obesity. Because endothelial dysfunction and oxidant stress have been reviewed extensively elsewhere,^{9,60,61} this paper briefly presents sources of free radicals in the endothelium relevant to obesity-induced hypertension (Figure 6). In human and animal vascular endothelial cells, there are several enzymatic sources of oxidant generation including NADPH oxidase, xanthine oxidoreductase and NO synthase. The major source of endothelial $O_2^{\bullet-}$ appears to be NADPH oxidase.⁹ NADPH can be modified by other cytokines and hormones such as those in the renin-angiotensin system. For example, in angiotensin II-induced hypertension, endothelial $O_2^{\bullet-}$ levels are significantly elevated as a consequence of enhanced NADPH oxidase activity.⁹⁶ A minor enzyme, xanthine oxidoreductase, exists in two forms: xanthine oxidase and dehydrogenase. It is the xanthine oxidase that reacts with oxygen to form superoxide and H_2O_2 , especially under ischemic conditions.⁹⁷ Excessive $O_2^{\bullet-}$ reacts rapidly with NO to produce $ONOO^{\bullet}$, which reduces NO bioavailability and causes nitrosylation of proteins.⁶¹ The last enzyme, NO synthase, is similar to cytochrome P450, which catalyzes electron transport from NADPH to another heme group.⁹ NO synthase enzymes may become uncoupled, resulting in excessive $O_2^{\bullet-}$ or $ONOO^{\bullet}$.⁹⁸ These alterations are implicated in endothelial dysfunction and vascular insensitivity.

Concentrations of the hormones in the renin-angiotensin system are higher in obese persons.⁶⁰ Excessive hormones in

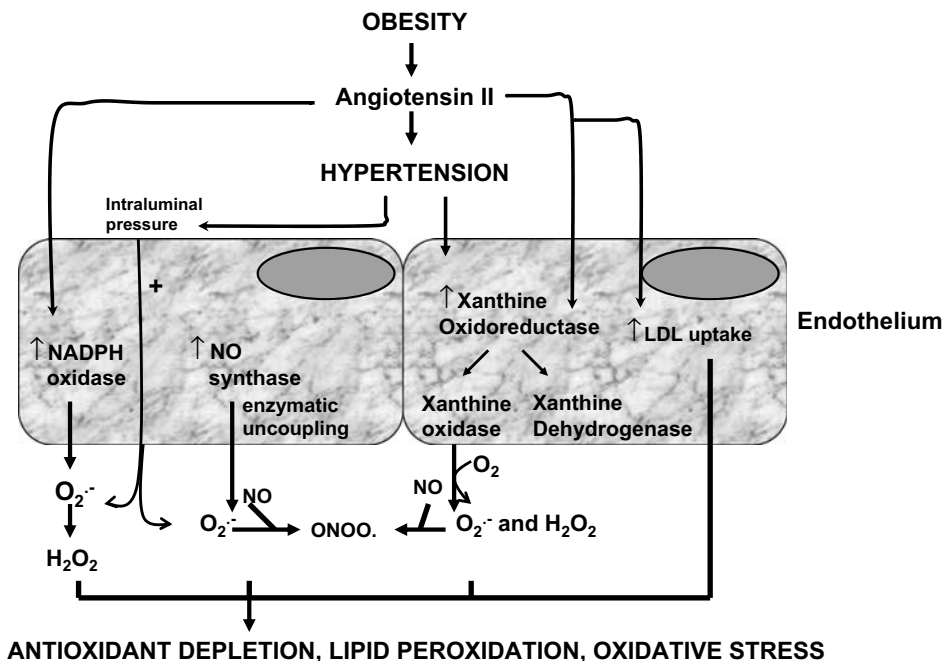


Figure 6 Obesity-induced pathways of oxidative stress in the endothelium. Elevations in the renin–angiotensin system occur in obesity, leading to hypertension. Angiotensin II activates several enzymes (NADPH oxidase, xanthine oxidase) that produce various ROS. NO synthase may uncouple, directly generating superoxide anions. Intraluminal pressure itself generates endothelial ROS. Chronic hypertension in obesity leads to oxidative damage and stress.

this system, especially angiotensin II, may directly affect oxidant stress levels in the vasculature by several mechanisms: (a) dramatic increases in vascular $O_2^{\bullet-}$ formation, in part by activation of NADPH oxidase activity in a dose-dependent manner;^{9,99} (b) increases in the expression of transmembrane proteins for NADPH oxidase; (c) production of H_2O_2 within the endothelial cells;¹⁰⁰ and (d) increases in LDL uptake by macrophages, which increases lipoprotein oxidation and oxidant damage.¹⁰¹

Elevated intraluminal pressure from hypertension may stimulate the production of superoxide anions and $ONOO^{\bullet}$ in the vasculature.¹⁰² The ROS inhibit calcium-activated K^+ channels and reduce vascular sensitivity as has been found in arterial tissues of obese Zucker rats. SOD administration restores vascular sensitivity and lowers oxidant stress in obese rats, suggesting the role of ROS ($O_2^{\bullet-}$) involvement in obesity-hypertension.¹⁰² Hypertension itself may increase oxidant formation, and excessive renin–angiotensin system hormones may exacerbate this process. Both may enhance endothelial dysfunction in obesity.

Hyperleptinemia

Leptin, a polypeptide hormone mediator produced by white adipose tissue, acts on hypothalamic centers to regulate food intake and energy expenditure. Plasma leptin concentrations are proportional to the amount of adipose tissue.¹⁰³ Excessive leptin concentrations are involved in angiogenesis, vascular calcification and thrombosis, and leptin has been

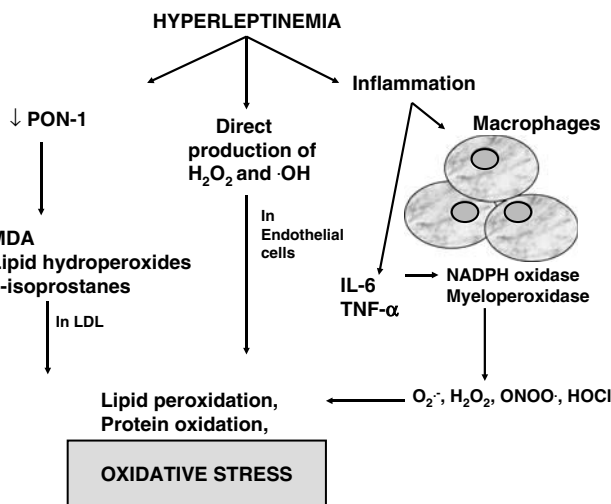


Figure 7 Hyperleptinemia reduces antioxidant capacity (PON-1) and generates oxidative stress by increasing ROS formation and causing low-grade inflammation. Leptin can shift the prooxidant–antioxidant balance to favor ROS formation and oxidative stress. PON-1, paraoxase-1; TNF- α , tumor necrosis factor α ; IL-6, interleukin-6.

postulated to be a cardiovascular risk factor in obese persons.¹⁰⁴ The potential roles of leptin in obesity-induced oxidative stress in obesity are illustrated in Figure 7. Leptin can directly stimulate production of ROS such as H_2O_2 and OH^{\bullet} in cultured endothelial cells and can function as an

atherogenic precursor.⁶² In a rodent model, Wistar rats injected with leptin had higher plasma and urine lipid hydroperoxide, MDA, isoprostane and protein carbonyl content (27–33% higher) than in nontreated controls.¹⁰⁵ Oxidative stress elevations were attributed to lower antioxidant enzyme defenses, such as paraoxase-1.

Leptin and its tissue receptors share similar chemical structures and functions with the IL-6 cytokine family.¹⁰⁶ Leptin is also a proinflammatory substance that stimulates the proliferation of monocytes and macrophages and the production of inflammatory cytokines.¹⁰⁵ Exposure of monocyte-derived macrophages to leptin elevates protein kinase C activity and macrophage lipoprotein lipase activity, which is proatherogenic.¹⁰⁴ Leptin indirectly stimulates production of inflammatory cytokines such as IL-6 and TNF- α , and these cytokines increase NADPH oxidase production. NADPH generates O₂^{•-}. Leptin can also stimulate cholesterol accumulation in a murine macrophage cell line, especially in the presence of high glucose concentrations,¹⁰⁷ a mechanism that may promote lipid peroxidation.

Finally, leptin reduces the activity of another cellular antioxidant, paraoxase-1 (PON-1), and this reduction is related to increased urinary and plasma 8-isoprostane formation and elevated plasma MDA and hydroperoxides.¹⁰⁵ Because PON-1 is involved in preventing the accumulation of peroxides in LDL, reduction in the activity of this enzyme could contribute to CAD development. Ferretti *et al.*¹⁰⁸ recently reported that PON activity in HDL is lower in obese compared with non-obese persons (120 vs 475 IU/mg protein). In the obese group, low PON activity was accompanied by elevations in lipid hydroperoxide content in HDL and LDL, and was inversely correlated with plasma leptin concentrations.¹⁰⁸ Leptin may thus be involved in several mechanisms that promote oxidative stress in obesity.

Interventions to potentially reduce oxidant stress in obesity

Obesity is a major contributor to several metabolic disturbances related to oxidative balance. As noted earlier, physical activity or exercise, dietary restriction and surgical interventions reduce oxidative stress levels. Other options for reducing oxidative stress in obesity include antioxidant therapy and supplementation with lipoic acid.

Antioxidant therapy

Numerous clinical trials have examined the effects of antioxidants on primary and secondary prevention of CAD. These trials, which have been summarized elsewhere, revealed a general lack of benefit.¹⁰⁹ However, the lack of effect may in part be explained by the facts that trial populations were generally older (>40 years) and/or have advanced stages of disease, or had major confounding variables that negated any potential positive supplementa-

tion effect (e.g. cancer tumors). Finally, the timing, type and dosage of antioxidant supplementation may not have been optimal for the specific populations studied.

Data are scarce on the effects of supplementation with various antioxidants on oxidative stress in obese persons. Further, studies of obesity have often used individuals with obesity and comorbidities such as diabetes. Nevertheless, antioxidant administration may be effective in suppressing multiple oxidative pathways to obesity-induced disease. In overweight type II diabetic persons, β -carotene (24 mg), ascorbate (1000 mg) and vitamin E (800 IU) or placebo were administered daily for 12 weeks while subjects were on a weight maintaining diet.¹¹⁰ The lag time for LDL oxidation increased and TBARS formation decreased from 78 to 33 nmol MDA/mg LDL protein. This response indicated protection against formation of oxidative biomarkers with the antioxidant treatment. In another study, zinc supplementation for 6 months (30 mg/day as Zn gluconate) lowered plasma TBARS levels in overweight diabetic men and women up to 15% more than the placebo.¹¹¹

When vitamin E (600 mg α -tocopherol) was administered to obese diabetic patients for 3 months,¹⁷ plasma MDA decreased from 3.13 ± 0.68 to 2.87 ± 0.97 μ mol/l. Plasma vitamin E concentrations increased by 100%. In another study, high dose vitamin E or placebo was administered to obese subjects for 6 months (3 months 800 IU vitamin E/day, 3 months 1200 IU vitamin E/day);¹¹² following the treatment, the vitamin E group had higher plasma vitamin E concentrations and lower plasma lipid hydroperoxide concentrations. These changes directly corresponded with improved insulin sensitivity. In one animal study, 4 weeks of vitamin E supplementation reduced plasma 8 epiprostaglandin F_{2 α} in obese Zucker rats to levels comparable to those of the non-obese controls.¹¹³ The vitamin E treatment reduced glucose-stimulated hyperinsulinemia and glucose area under the curve following a glucose infusion. The authors concluded that vitamin E reduced oxidative stress and improved glucose metabolism.¹¹³

Combinations of dietary antioxidants and phytochemicals may be effective in lowering oxidative stress. Blakely *et al.*¹¹⁴ for example, found lower hepatic MDA in obese Zucker rats following vitamin E, vitamin C and lutein supplementation than in non-treated controls. The enzymatic tissue defenses (e.g. GPX) of the antioxidant-treated animals were higher depending on the combination of the vitamins and lutein administered. In humans, a phytochemical-rich flavonol diet (consisting of English onion, olive oil, tomato ketchup, Italian herbs, and six mugs of Typhoo tea added to the regular diet) increased plasma and urine concentrations of flavonols in obese subjects.¹¹⁵ Despite no change in body weight, oxidative damage to lymphocyte DNA still decreased. Protection against oxidative stress may be conferred by increased dietary consumption of antioxidants or phytochemical-rich foods independent of weight loss.

Infusions of vitamin C reduced ROS production and improved endothelial dependent vasodilation in obese men

and women.¹¹⁶ Vitamin E suppressed DNA oxidation by H₂O₂ in human lymphocytes.¹¹⁷ Further, antioxidant mixes (vitamin E, C and β-carotene) enhanced endogenous plasma and tissue antioxidant defenses (indicated by increased FRAP values) and suppressed DNA oxidation.¹¹⁸ Rigorous, systematic research is imperative to determine whether different antioxidant supplementation regimens or dietary regimens consisting of antioxidant or phytochemical-rich foods impart health benefits and reduce oxidative stress in obese persons.

α Lipoic acid (LA)

LA is a naturally occurring short chain fatty acid antioxidant with sulfhydryl groups.¹¹⁹ LA supplementation (0.5% wt/wt) prevented the elevations in plasma MDA and 8-OHdG that occur in obese Zucker rats. LA also suppressed ad libitum food intake and significantly reduced body weight, lowered plasma FFA and insulin, and reduced pancreatic triglyceride content. The authors concluded that prevention of oxidative stress in obesity suppressed the progression of insulin resistance and diabetes in this animal model.¹¹⁹ Further research is required, however, to understand whether LA can be a useful antioxidant therapy in obese humans.

Summary

In summary, obesity creates the oxidant conditions of a 'breeding ground' for diseases such as diabetes, heart disease, hypertension and CVD. Oxidative stress in obesity is a systemic problem that must be corrected either by improving antioxidant defenses through fat volume reduction, exercise and dietary modification, or a combination of the three.

Acknowledgements

The preparation of this paper was supported, in part, by Grant Numbers T32-AT00052 and K30-AT-00060 from the National Center for Complementary and Alternative Medicine (NCCAM), and from the University of Virginia General Clinical Center Grant Number 5 M01 RR000847. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NCCAM or the National Institutes of Health. We thank Elizabeth Tournquist, MA, and Jewel Holmberg for their editorial expertise in finalizing this paper.

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