Emerging Science

Glutathionyl systems and metabolic dysfunction in obesity

Matthew J. Picklo, Eric K. Long, Emilie E. Vomhof-DeKrey

Oxidative stress is associated with obesity. However, glutathione (GSH), one of the body’s most abundant antioxidants, plays dual and seemingly contradictory roles in the development of obesity and its comorbidities. Glutathione has complex metabolic and biochemical fates and is a cofactor for several enzymes that function in modifying obesity-related responses. For example, depletion of GSH increases energy metabolism and reduces adipose accretion, while elevation of GSH peroxidase activity induces insulin resistance. This review summarizes the literature linking GSH and its related enzymes, GSH peroxidase, glutaredoxins, and glutathione S-transferases, to obesity and its pertinent endpoints (e.g., energy metabolism, inflammation, and insulin resistance).

INTRODUCTION

Elevated indices of oxidative damage, namely those of lipid peroxidation, are associated with increased body mass index (BMI).\(^1\)\(^-\)\(^4\) Given the relationship between oxidative stress and obesity, it is not surprising that glutathione (GSH), one of the body’s most abundant antioxidant molecules, and GSH-dependent enzymes are involved mechanistically in obesity development and comorbidities associated with obesity, such as type II diabetes and cardiovascular disease. Glutathione was first reported in 1921 by Frederick Hopkins to describe the isolation and chemical characterization of a small, thiol-containing peptide molecule from yeast.\(^5\) In the following years, the scientific output related to the study of GSH has been enormous: a PubMed search yields 6000 reviews using “glutathione,” and a search with the terms “glutathione” and “obesity” yields over 600 publications (with over half being published within the past 5 years).

It is commonly accepted that higher levels of antioxidants and antioxidant enzymes like GSH and glutathione peroxidases (GPx) are positive indicators of health, whereas the generation of reactive oxygen species (ROS) and reactive nitrogen species, as well as the oxidation of lipids and thiols, is pathological. However, recent studies suggest that this view must be refined. Hydrogen peroxide has multiple signaling functions.\(^6\) Moreover, overexpression of glutathione peroxidase 1 (GPx1) can promote inflammation.\(^7\),\(^8\) Elevated GSH content reduces insulin sensitivity in adipocytes.\(^9\),\(^10\) Thus, signaling through ROS, along with other redox events such as thiol oxidation and lipid peroxidation, is associated with beneficial as well as pathological outcomes.

These data lead to the question, “What are the roles of GSH in obesity?” Several lines of evidence implicate GSH and glutathionyl systems in the development of obesity and the regulation of related processes, including energy metabolism, inflammation, and insulin resistance at multiple points. This review comprises two complementary sections: 1) a summary of evidence linking decreases in GSH content in vivo to elevated energy metabolism and prevention of obesity in humans and animal models, and 2) a summary of the recent data detailing the molecular mechanisms by which GSH-dependent processes regulate obesity-associated events.

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DEPLETION OF GLUTATHIONE AND OBESITY RESISTANCE

Multiple lines of evidence indicate that lowering levels of GSH in vivo leads to obesity resistance (Figure 1). Glutamate cysteine ligase (GCL, also known as γ-glutamylcysteine synthetase) is the rate-limiting enzyme of GSH synthesis and is composed of 2 subunits, the catalytic (GCLc) and the modifier (GCLm) subunits. While mice lacking the GCLc subunit die within 1 month of birth as a result of liver failure, mice lacking the GCLm subunit are viable but have reduced (20% of controls) levels of hepatic GSH.12,13 GCLm mice on a high-fat diet resist fat accretion, resist the development of hyperglycemia, and have a higher basal metabolic rate in comparison with wild-type mice.12 In support of these physiological effects, GCLm mice have elevated rates of complex 1–based respiration in hepatic mitochondria. In contrast, the hepatic expression of several genes regulating fatty acid synthesis, including fatty acid synthase (Fasn), elongase 6 (Elovl6), and stearoyl-CoA dehydrogenase 1 (Scd1), is reduced.12

Pharmacological inhibition of GSH synthesis also inhibits obesity development in animals. Experiments by Findeisen et al.14 inhibited GSH synthesis through use of orally administered buthionine sulfoximine, an inhibitor of GCL activity.15 In these experiments, elevated adiposity and insulin resistance are blocked in mice fed a hypercaloric, high-fat diet. Buthionine sulfoximine–treated mice have a higher metabolic rate than untreated animals and also express higher levels of the uncoupling proteins UCP2 and UCP3 in white adipose tissue.14 This study is limited, however, by the lack of data demonstrating the extent of GSH depletion in the animals. Nonetheless, these data suggest that, GSH, perhaps through regulation of redox-sensitive proteins, regulates energy metabolism. Clearer data are needed that define both the levels of GSH depletion necessary to induce an increase in energy metabolism and the mechanistic pathways altered.

DIETARY METHIONINE RESTRICTION, GLUTATHIONE DEPLETION, AND OBESITY

Methionine (Met) is an essential amino acid that is incorporated into proteins and is used for the synthesis of the carbon cycle intermediate S-adenosylmethionine.16,17 Dietary Met is the primary precursor of cysteine, which is required for the synthesis of GSH, and the enzymatic steps involved in these processes, as well as others related to Met metabolism, are well characterized.16,17 Cross-sectional data from Elshorbagy et al.18,19 indicate a positive association between plasma sulfur amino acid content and dyslipidemia. Other cross-sectional data demonstrate a positive relationship between Met intake and risk of coronary events.20

Limiting Met from 0.86% (wt/wt of diet) to 0.17% wt/wt has been studied extensively in relation to longevity, energy metabolism, and obesity prevention. Met restriction reduces the development of adiposity in animal models of obesity, including mice and rats fed a high-fat, hypercaloric diet as well as leptin-deficient ob/ob mice.21–23 To date, one study in humans indicates that Met restriction in combination with caloric restriction does not increase weight loss in obese/overweight people but does increase fat oxidation.24

The effects of Met restriction are reversed by the addition of N-acetylcysteine or cysteine, but not taurine.25 These findings indicate that the loss of the GSH precursor cysteine is responsible for the effects of Met restriction. Met restriction reduces levels of GSH in liver (50%) and kidney (40%) but nearly doubles levels of GSH in whole blood, suggesting elevated secretion of GSH as a source of cysteine.16,26,27

Met restriction has multiple effects on energy metabolism pathways. Rats fed a Met-restricted diet have higher rates of energy expenditure and less fatpad mass than rats undergoing 40% caloric restriction, similar to rats with the GCLm deletion described earlier.12,22 Metabolomic and transcriptomic analyses indicate that Met restriction increases lipid oxidation pathways while decreasing pathways related to lipid synthesis.28 Met restriction also elevates levels of peroxisome proliferator-activated receptor-γ coactivator 1α and peroxisome proliferator-activated receptor-γ coactivator 1β and

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**Abbreviations**: ARE, antioxidant response element; GCL, glutamate cysteine ligase; GSH, glutathione; ROS, reactive oxygen species; RNS, reactive nitrogen species.

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**Figure 1** Pathways linking GSH with energy metabolism. Heavy arrows indicate inductive pathways, whereas thin cross bars indicate inhibition. Abbreviations: ARE, antioxidant response element; GCL, glutamate cysteine ligase; GSH, glutathione; ROS, reactive oxygen species; RNS, reactive nitrogen species.
increases mitochondrial DNA copy number in liver and skeletal muscle.29

The changes in the metabolic pathways above are similar to those caused by activation of the Nrf2 pathway (see below).30 Data support activation of the Nrf2 pathway by Met restriction in vivo. Met restriction elevates the expression of several Nrf2-inducible genes.28 The fact that these genes are turned on under conditions of GSH depletion strongly suggest that Met restriction in vivo increases Nrf2 activation, thus stimulating a signaling pathway that increases energy metabolism (see below).30 In vitro studies using primary rat hepatocytes confirm activation of the Nrf2 pathway during Met restriction.31

Met restriction, however, has drawbacks, and several questions remain. In most studies, young animals were placed on Met restriction diets and had significant growth restriction, reaching only 50% of the size of the Met-replete rats by 6 months of age.27 Other data indicate that Met restriction reduces bone density.21 Furthermore, GSH is needed for multiple functions, such as metabolism of xenobiotics.

**NRF2/ANTIOXIDANT RESPONSE ELEMENT PATHWAY AND ENERGY METABOLISM**

The Nrf2/antioxidant response element pathway is a redox-sensing mechanism linking changes in cellular redox status to the expression of genes whose resulting proteins regulate energy metabolism and metabolism of ROS and products of ROS damage. As such, recent data connect the Nrf2/antioxidant response element pathway to GSH depletion, obesity, increases in energy metabolism, and decreases in lipid deposition.30,32

The antioxidant response element (also known as the electrophilic response element) is a cis-acting element located in the promoter of multiple genes, such as glutathione S-transferases (GSTs), GCL, and NADPH: quinone oxidoreductase 1, that are upregulated as a result of oxidative stress or GSH depletion.33,34 Under basal conditions, the Nrf2 transcription factor resides in the cytoplasm, tethered to the Keap1 protein, and is then degraded.33,35 Oxidation of thiol residues of Keap1 and phosphorylation of Nrf2 by glycogen synthase kinase-3/β in an insulin-dependent manner allow Nrf2 to translocate to the nucleus and bind to the antioxidant response element.33,35

Several studies have shown that Nrf2 activation alters energy metabolism. Nrf2 stimulation increases the proportioning of glucose to the pentose phosphate pathway through increased expression of glucose-6-phosphate dehydrogenase.36 Furthermore, Nrf2 stimulation increases UCP3 expression, thus potentially increasing energy expenditure.37 Pharmacological activation of the Nrf2 pathway by oltipraz and the oleonic triterpenoid 1-[2-cyano-3,12-dioxooleane-1,9(11)-dien-28-oyl]imidazole prevents obesity development in rodents fed a high-fat, hypercaloric diet, in part by increasing energy expenditure.38,39 Furthermore, 1-[2-cyano-3,12-dioxooleane-1,9(11)-dien-28-oyl]imidazole treatment inhibited the hepatic expression of the lipogenic genes fatty acid synthase, acetyl-CoA carboxylase 1, acetyl-CoA carboxylase 2, and Srebp1c.39

Nutritional activation of the Nrf2 pathway can be accomplished by dietary phytochemicals like sulforaphane, curcumin, and others.40–42 Experiments using antioxidant response element reporter mice show that gavaging of animals with extracts derived from turmeric, broccoli, coffee, rosemary, and red onion elevate activation of the antioxidant response element.15 In this study, highest reporter activation was found in adipose tissue and lung, with minimal activation found in the liver. Furthermore, it was found that carnosol, a phenolic diterpene derived from rosemary, alone elevated hepatic Nrf2 pathway activity 8-fold.43 The activation of the Nrf2 pathway provides a connection between consumption of dietary phytochemicals and prevention of obesity. However, more data are needed to determine how absorption and metabolism abrogate the in vivo potency of these phytochemicals and to establish the extent to which dietary phytochemicals activate the Nrf2 pathway and modify energy expenditure. Furthermore, it is not known whether mutations in Nrf2 signaling modify energy metabolism in humans.

**GLUTATHIONE-DEPENDENT ENZYMES AND OBESITY**

The data summarized above provide an overall picture linking GSH to energy metabolism and obesity. In this section, the molecular events linking GSH-dependent processes to obesity-related events that include insulin resistance, inflammation, and mitochondrial energetics are reviewed, and areas for further research are identified. Three main GSH-dependent enzyme systems (Figure 2) are highlighted: 1) reduction of peroxides by GPx; 2) protein S-glutathionylation and glutaredoxins (Grx); and 3) metabolism of lipid peroxidation products like 4-hydroxy-2-nonenal by GSTs.

**Glutathione peroxidases**

Hydroperoxides (hydrogen peroxide or lipid hydroperoxides) are metabolized by GPx intracellularly and extracellularly to yield either water (in the case of hydrogen peroxide) or hydroxy polyunsaturated fatty acids). Most GPx are selenium-dependent enzymes that have selenium, in the form of selenocysteine, at the active site.44,45 While the GPx family consists of 8
members, data will be discussed for GPx1, GPx3, GPx4, and GPx7.44 There are little or no data regarding an association between obesity and GPx2 (an intestinal GPx), GPx5, GPx6, or GPx8. A 2013 review by Brigelius-Flohe and Maiorino44 provides a discussion of these other members of the GPx family.

GPx1 is one of the most studied members of the GPx family and was first isolated from erythrocytes. This homotetrameric enzyme is found in multiple tissue and cell types and is localized mostly to the cytoplasm of the cell.44 GPx1 reduces hydrogen peroxide and organic hydroperoxides such as cumene hydroperoxide and t-butyl hydroperoxide as well as hydroperoxides of nonesterified, but not esterified, polyunsaturated fatty acids.44

Data generated from GPx1 transgenic mice support a positive role for hydrogen peroxide or other peroxyl moieties in the regulation of insulin sensitivity. Overexpression of GPx1 induces insulin resistance and obesity in mice, in part through increased pancreatic insulin secretion.46–48 On the other hand, GPx1 null mice exhibit elevated insulin sensitivity and are resistant to obesity-induced insulin resistance.49,50

GPX1 polymorphisms exist in the human population and modify GPx1 activity. The most prevalent is the Pro198Leu variant, which results from a cytosine to thymine substitution at codon 198.51 The presence of GPX1 genotypic variants was compared with the presence of obesity and other factors contributing to metabolic syndrome in a Japanese cohort (>2200 people).52 The results indicated that presence of the Pro198Leu allele was associated with small but statistically significant elevations in waist-to-hip ratio, fasting triglycerides, and indices of insulin resistance and hypertension. This study was limited by lack of both GPx1 measurements and analysis of selenium status, a major determinant of GPx1 activity. Furthermore, these data contrast with the GPx1 null mouse data presented above, which show that lack of GPx1 activity increases insulin sensitivity. GPx1 is implicated in the development of other obesity-induced diseases. Among individuals with type II diabetes, those with the Pro198Leu variant have higher indices of atherosclerosis, vascular abnormalities, and diabetic neuropathy than those with the Pro198 allele.53,54

GPx3 is often termed the extracellular or plasma GPx. It is largely expressed and secreted from the proximal tubules of the kidney. GPx3 is distributed in the blood and binds to cellular membranes in multiple tissues.55,56 It is expressed to a lesser extent in lung, brown adipose, and white adipose.57

The K_M (Michaelis constant) of GSH for GPx3 is 4.3 mM, over 200 times greater than the concentration of GSH found in plasma.58–60 Given the mechanism of GPx3 activity described above, this low concentration of GSH in the plasma makes it unlikely that GPx3 is able to be efficiently reactivated following peroxide reduction. However, the binding of GPx3 to cellular membranes, in which higher local levels of GSH can exist, suggests that GPx3 functions to metabolize peroxides prior to their entering the cell.55

GPx3 activity is implicated in the development of obesity and its comorbidities.57,61,62 Cross-sectional clinical data derived from a Mexican population indicates that GPx3 content in serum is elevated in overweight and obese subjects.63 Research using genetically obese ob/ob and db/db mice demonstrates that adipose-derived, but not renal, GPx3 expression is reduced by the onset of obesity.57 Moreover, GPx3-deficient mice have elevated platelet aggregation and larger infarct sizes resulting from cerebral ischemia.62 Given that leptin deficiency is rare in humans, data are needed to determine the extent to which GPx3 expression is altered in human adipose tissue as a result of obesity.

GPx4 was initially characterized as a phospholipid hydroperoxide GPx due to its ability to reduce lipid hydroperoxides while still incorporated into membranes, unlike other GPx members.64,65 Given the potential role of enzymatic and nonenzymatic lipid peroxidation in adipose dysfunction and obesity comorbidities, it is surprising that little is known about the influence of GPx4 activity in obesity. GPx4 is expressed in adipose tissue, and one study performed in 3T3-L1 adipocytes indicates that the proinflammatory cytokine tumor necrosis factor α (TNF-α) reduces GPx4 expression.59,66

GPx7 was originally termed the nonselenocysteine phospholipid GPx.67 GPx7 lacks classical peroxidase activity but promotes disulfide bond formation within proteins located in the endoplasmic reticulum.68,69 GPx7 is a regulator of adipocyte differentiation.70 GPx7 expression is reduced during differentiation of stromal vascular cells.
to adipocytes, and knockout of GPx7 expression leads to increased oil red O staining, suggesting either elevated triglyceride deposition in de novo adipocytes or an increase in the number of resulting adipocytes.\textsuperscript{70} GPx7 null mice on a 60% fat energy diet have a higher body mass with an elevated fat-pad mass compared with wild-type mice on the same diet, but no differences are found when the animals are fed a eucaloric, 13% fat energy diet.\textsuperscript{70} The extent to which GPx7 expression is related to the development of obesity in humans is questionable. While it is claimed that deficiency of GPx7 leads to obesity in humans, the individuals genotyped in one study had BMIs that did not meet the definition of obesity (BMI >29.9).\textsuperscript{70} Subsequent work is needed to confirm whether GPx7 genotypes are related to the development of obesity in humans.

**Serum vs whole-blood vs plasma glutathione peroxidase activity**

Clinical studies often use GPx activity determined in serum or whole blood as an index of antioxidant capacity or oxidative stress. While convenient, it is important for investigators to recognize that this GPx activity in serum and whole blood represents a mixture of the extracellular plasma GPx3 activity derived from kidney, adipose, and, potentially, other cells in addition to GPx activity derived from damaged platelets and erythrocytes. Given that the majority of plasma GPx3 is of renal origin, decreases in GPx3 may indicate changes in renal biochemistry and binding of GPx3 to target cell membranes rather than whole-body responses to oxidative stress.

**Protein S-glutathionylation and glutaredoxins**

Analogous to the regulation of protein function by reversible phosphorylation, protein activity is regulated by S-glutathionylation in which a mixed disulfide forms between a protein-bound cysteinyl thiol and the cysteinyl thiol of GSH. Recent studies have focused on identifying the targets of protein-S-GSH adduct formation, the physiological and cellular conditions under which these adducts occur, and regulation of the deglutathionylation process by Grx. Protein-S-glutathione adducts account for less than 0.2% of the total GSH pool.\textsuperscript{71,72} Comprehensive, in-depth reviews of glutathionylation and Grx have been published.\textsuperscript{73–75}

In mammals, 4 Grx members, Grx1, Grx2, Grx3, and Grx5, are known to exist. Glutaredoxins are divided into 2 main families, dithiol (Grx1 and Grx2) and monothiol (Grx3 and Grx5). There are relatively few data regarding the importance of Grx3 and Grx5 in disease, although data indicate a role for these enzymes in the transport and stabilization of iron-containing proteins.\textsuperscript{76,77} Grx1 is a small-molecular-weight protein (\textasciitilde{}12 kDa) located in the cytosol and mitochondrial intermembrane space.\textsuperscript{78,79} Grx2 is similar in size to Grx1 but only shares 36% amino acid identity.\textsuperscript{80,81} Grx2 is primarily localized to the mitochondrial matrix, although a splicing isoform is localized to the nucleus.\textsuperscript{80–82} There are no reports of associations between Grx gene polymorphisms and obesity.

**Targets of glutathionylation**

Levels of protein-S-glutathione adducts are decreased in liver and adipose tissue of obese rats compared with levels in lean controls, with parallel increases in adipose Grx1.\textsuperscript{83} Several other lines of evidence demonstrate the involvement of protein-S-glutathione adduct formation and obesity. Three main functional areas related to obesity are modified by glutathionylation: inflammation, insulin signaling, and mitochondrial metabolism. The extent to which glutathionylation regulates these areas in obesity is not clear and requires further study.

**Inflammation**

Glutathionylation negatively regulates the proinflammatory nuclear factor-\(\kappa\)B (NF-\(\kappa\)B) signaling pathway at multiple steps, and elevation of Grx1 activity is a proinflammatory event in the development of pulmonary inflammation.\textsuperscript{84–89} Thus, stimulation of Grx1 activity elevates the inflammatory response. Mechanistically, this process occurs through deglutathionylation of inhibitory kappa protein kinase \(\beta\). Removal of the GSH from Cys179 of inhibitory kappa protein kinase \(\beta\) allows for phosphorylation and subsequent degradation of the inhibitory \(\kappa\)B proteins that block NF-\(\kappa\)B proteins from entering the nucleus.\textsuperscript{86} Furthermore, NF-\(\kappa\)B signaling induces Grx1 expression, creating a positive feedback loop.\textsuperscript{85} Other studies show that upregulation of Grx1 with activation of NF-\(\kappa\)B occurs with exposure of retinal cells to high (25 mM) glucose.\textsuperscript{90,91} This pathway is also involved in the inhibition of revascularization following injury.\textsuperscript{92}

**Insulin signaling**

While inflammation is upregulated by removal of protein-S-glutathione adducts, insulin signaling is enhanced by glutathionylation of the phosphatases (which possess active-site cysteines) that inhibit the insulin signaling pathway. In particular, glutathionylation of Cys215 of protein tyrosine phosphatase 1B inhibits protein tyrosine phosphatase 1B activity, thus increasing phosphorylation of the insulin receptor.\textsuperscript{93} The extent to which formation of protein-S-glutathione adducts on
protein tyrosine phosphatase 1B regulates insulin signaling in muscle or adipose is not clear.94 Regulation of insulin signaling by inhibitory oxidation of active-site phosphatase residues does occur in vivo, but more studies are needed to define the conditions under which this process occurs.95–97

Mitochondrial energy metabolism

Glutathionylation is a regulator of mitochondrial energy metabolism at multiple levels. Within the Kreb’s cycle, \( \alpha \)-ketoglutarate dehydrogenase and isocitrate dehydrogenase activities are inhibited by \( S \)-glutathionylation.98–100 Unlike cysteinyl thiols, it is the reduced lipoic acid moiety of \( \alpha \)-ketoglutarate dehydrogenase that is \( S \)-glutathionylated.98,100 Components of the electron transport chain activity are also regulated by formation of protein--\( S \)-glutathione adducts. Complex 1 (the 75-kDa and 51-kDa subunits), the flavin adenine dinucleotide–containing 70-kDa subunit of complex II, and the \( \alpha \)-subunit of the F1 complex of ATP synthase undergo reversible glutathionylation.101–106

A series of papers recently demonstrated that the activities of mitochondrial UCP2 and UCP3 are reduced by glutathionylation.107–109 Uncoupling protein activity increases mitochondrial substrate utilization. In addition, uncoupling protein activity increases proton flux across the mitochondrial inner membrane and is thought to reduce mitochondrial superoxide generation. Interestingly, it was found that glutathionylation of UCP3 is reduced in the presence of hydrogen peroxide, perhaps through stimulation of Grx2 activity.108 These data, along with those demonstrating glutathionylation of electron transport chain proteins, indicate that glutathionylation of mitochondrial proteins is a complex process that modulates mitochondrial energy metabolism in normal and pathological conditions. Since elevated glutathionylation of mitochondrial proteins reduces mitochondrial energy utilization, understanding the molecular bases for regulation of glutathionylation and de-glutathionylation of mitochondrial proteins in obesity may provide potential avenues for preventing obesity development. That glutathionylation reduces mitochondrial metabolism while increasing insulin signaling appears contradictory. These findings demonstrate the need to better understand the regulation of glutathionylation and de-glutathionylation at the tissue, cellular, and subcellular levels.

Glutathione \( S \)-transferases

Glutathione \( S \)-transferases (GSTs) comprise 3 families of enzymes, the most studied being the cytosolic GSTs. Cytosolic GSTs comprise 7 classes (Alpha, Mu, Pi, Sigma, Theta, Omega, and Zeta), with multiple, separate gene products in each class.110,111 These enzymes are typically known for phase 2 drug metabolism by catalyzing the formation of a thioether bond between the thiol moiety of GSH and an electrophilic carbon of the target molecule. In addition to metabolism of xenobiotics, GSTs also detoxify endogenously derived electrophilic molecules, including a number of lipid peroxidation products. This area of research, as it pertains to obesity-related metabolic dysfunction, is reviewed below.

Polyunsaturated fatty acids undergo free radical-mediated oxidation, which yields cytotoxic \( \alpha,\beta \)-unsaturated aldehydes such as \( \text{trans-4-4-hydroxy-2-nonenal} \) (4-HNE) and \( \text{trans-4-4-oxo-2-nonenal} \) (4-ONE).112,113 These chemically reactive aldehydes have an electrophilic center that forms covalent adducts on proteins and DNA.114–115 4-HNE and 4-ONE, derived from n-6 polyunsaturated fatty acids such as linoleic acid (18:2, n-6) and arachidonic acid (20:4, n-6), have drawn particular interest with regard to the role of lipid peroxidation in the onset and progression of several diseases.114,115 Adipose tissue is rich in linoleic acid (18:2, n-6), which constitutes 15–30 mole percent of the fatty acid content of adipose tissue, depending on the diet.116,117 Thus, it is not surprising that 4-HNE and 4-ONE exert effects in adipose tissue (see below).66,118,119

4-HNE and 4-ONE are metabolized via a number of phase I and II mechanisms. The aldehyde moiety is a substrate for oxidation by aldehyde dehydrogenases and for reduction by aldo-keto reductases, resulting in decreased chemical reactivity.120 4-HNE and 4-ONE are substrates for GSH conjugation. This process is catalyzed with high efficiency by GST Alpha (GSTA), such as GSTA4, and with less efficiency by other GST (Pi and Mu classes).121,122 Glutathione adducts are exported from the cell by RLIP76 and proteins associated with multidrug resistance.123,124

Obesity results in a white adipose tissue–specific downregulation of GSTA4, elevated (5-fold to 10-fold) levels of 4-HNE and 4-ONE, and increased protein modification by 4-HNE and 4-ONE.66,125,126 Despite decreased GST enzyme content in adipose tissue of mice fed a high-fat diet, levels of the GSH–HNE adduct are increased in adipose tissue, suggesting that a decrease in GST enzyme content is not rate-limiting to metabolism of 4-HNE and 4-ONE in situ.118

Inflammation and glutathione \( S \)-transferases

The GSH–HNE adduct and its derivative \( S \)-glutathionyl-dihydroxynonane are bioactive and have proinflammatory signaling properties,127,128 and in vitro
experiments with 3T3-L1 adipocytes demonstrate that oxidative stress and high glucose induce the production of these adducts. These GSH adducts, in turn, induce production of TNF-α, leukotriene C4, and the expression of a number of genes involved in inflammation in primary and immortalized macrophage cell cultures.119 These resulting inflammatory cytokines, such as TNF-α, interleukin 6, and interleukin 1β, cause mitochondrial abnormalities in 3T3-L1 adipocytes.129

Macrophage infiltration and the subsequent production and release of inflammatory cytokines are key factors in obesity-related adipose tissue dysfunction.130 The findings above provide a mechanism by which oxidative stress induced by obesity results in increased activation and inflammatory signaling of macrophages, which leads to adipose tissue dysfunction. Given that dietary linoleic acid likely is the primary precursor of 4-HNE and 4-ONE in adipose, the question arises of whether obesity-induced adipose inflammation may be attenuated by consumption of diets with reduced linoleic acid content.

**Glutathione S-transferase polymorphisms**

The most commonly studied GSH-related gene polymorphisms are members of the cytosolic-soluble GSTs. GST gene polymorphisms can impair enzyme activity and are linked to a variety of diseases, including obesity, cancer, type 2 diabetes mellitus, and cardiovascular disease.131,132 Correlation studies of GST-Mu (GSTM1), GST-Theta (GSTT1), and GST-Pi (GSTP1) polymorphisms have been evaluated most extensively.133 At the GSTM1 locus, there are 3 different allele polymorphisms, one of which is a gene deletion (GSTM1 null), and another in which a cytosine to guanine substitution at nucleotide position 534 (GSTM1a and GSTM1b, respectively) encodes a Lys127Asn alteration that does not impair activity.134,135 The GSTT1 gene

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**Table 1 Human GST polymorphisms and obesity-related disease**

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<thead>
<tr>
<th>Reference</th>
<th>GSTM1</th>
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</table>

**Abbreviations**: BMI, body mass index; CVD, cardiovascular disease; Hm, homozygous for polymorphism; Ht, heterozygous for polymorphism; I/V, Ile105Val substitution; I/I, homozygous for Ile105/Ile105 genotype; MI, myocardial infarction; T2DM, type 2 diabetes mellitus.
polymorphism occurs by gene deletion (GSTT1 null), whereas the GSTP1 gene polymorphism is a single-nucleotide change of adenine to guanine at codon 105, resulting in an amino acid change of isoleucine to valine. Some studies on this list conflict in whether a single-gene deletion of GSTM1 or GSTT1 leads to an increased risk of type 2 diabetes mellitus, whereas having a gene deletion of both GSTM1 and GSTT1 consistently correlates with a higher risk of type 2 diabetes mellitus. Moreover, there are even greater inconsistencies about whether a gene polymorphism for GSTM1, GSTT1, or GSTP1 increases the risk of cardiovascular disease or even offers protection against cardiovascular disease. These inconsistencies may be attributable to cross-sectional studies that do not account for interindividual variations in gene expression that result from dietary and environmental differences.

**CONCLUSION**

As presented above, GSH is not simply a beneficial, cellular antioxidant. While elevations in GSH content may be protective in many diseases, decrements in GSH content increase energy expenditure, prevent obesity, and reduce insulin resistance. Existing data suggest that reducing GSH activates the Nrf2 transcription factor and reduce insulin resistance. Clinical correlates of oxidative stress in the Framingham Study, Arterioscler Thromb Vasc Biol. 2003;23:434–439.

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