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Reduction of glucose intolerance with high fat feeding is associated with anti-inflammatory effects of thioredoxin 1 overexpression in mice

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Aging is associated with reduced ability to maintain normal glucose homeostasis. It has been suggested that an age-associated increase in chronic pro-inflammatory state could drive this reduction in glucoregulatory function. Thioredoxins (Trx) are oxido-reductase enzymes that play an important role in the regulation of oxidative stress and inflammation. In this study, we tested whether overexpression of Trx1 in mice [Tg(*TRX1*)$^{+/0}$] could protect from glucose metabolism dysfunction caused by high fat diet feeding. Body weight and fat mass gains with high fat feeding were similar in Tg(*TRX1*)$^{+/0}$ and wild-type mice; however, high fat diet induced glucose intolerance was reduced in Tg(*TRX1*)$^{+/0}$ mice relative to wild-type mice. In addition, expression of the pro-inflammatory cytokine TNF-α was reduced in adipose tissue of Tg(*TRX1*)$^{+/0}$ mice compared to wild-type mice. These findings suggest that activation of thioredoxins may be a potential therapeutic target for maintenance of glucose metabolism with obesity or aging.

Keywords: *oxidative stress*; *diabetes*; *obesity*; *glucose homeostasis*; *aging*

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Aging is a significant factor in the progressive decline in homeostatic regulation of glucose metabolism (1). The primary dysfunction that causes this decline is the development of peripheral insulin resistance (1). Several factors, including increased fat accumulation, decreased lean mass and elevated circulating fatty acids, can contribute to this phenotype. However, aging alone has been shown to have distinct effects on insulin response in skeletal muscle and other tissues independent of these other factors (1–4). Diminished sensitivity to insulin is the greatest risk factor for the development of metabolic diseases like Type 2 diabetes mellitus, the most common metabolic defect in the elderly (5).

The direct cause of the decline in glucose regulation with age is not clear but recent findings have suggested that an important factor may be chronic inflammation. In humans and rodents, the circulating and tissue levels of several pro-inflammatory cytokines increase with age (6,7). Chronic inflammation has also been proposed to be a significant cause of several age-associated diseases including diabetes (8). For more than a century, it has been known that anti-inflammatory treatments can reduce some effects of diabetes and insulin resistance (9). More recently, the molecular mechanisms linking inflammation with insulin resistance have been clarified using cell culture and mouse models. For example, the pro-inflammatory cytokine TNF-α (tumor necrosis factor α) causes insulin resistance in cell cultures; conversely, mice lacking TNF-α or its receptors are protected from insulin resistance induced by obesity or high fat diet (10,11). Similarly, the pro-inflammatory cytokine IL-6 (Interleukin-6) also reduces insulin signaling and glucose utilization in cell culture (12). These cytokines are

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thought to cause insulin resistance by activating stress-signaling pathways like the JNK (c-Jun N-terminal kinases) pathway (13). In support of this, ablation of JNK in mice prevents obesity-induced insulin resistance (14).

Thioredoxins (Trx) are important oxido-reductase enzymes that function as antioxidants, as redox-regulating enzymes, and as anti-inflammatories. In an NADPH-dependent reaction, disulfide bonds in multiple substrate proteins can by reduced by Trx with subsequent reduction of oxidized Trx by thioredoxin reductases. There are two primary forms of thioredoxins: thioredoxin 1 (Trx1) which is localized to the cellular cytosol and thioredoxin 2 (Trx2) which is localized to the mitochondria (15). Trx itself does have some chemokine-like attributes (16), but the anti-inflammatory effects of Trx1 are thought to be mostly due to its antioxidant actions. Mice that over-express Trx1 show reduced inflammation, immune cell infiltration, oxidative damage and cellular apoptosis when exposed to pro-inflammatory stimuli like cigarette smoke or diesel exhaust particles (17,18). Trx1 also plays a significant role in the reduction of inflammation in murine myocarditis and colitis models and in response to lipopolysaccharide-induced inflammation (19–21). Trx1 may additionally regulate the effects of inflammation by modulating downstream stress-signaling pathways. Reduced Trx1 binds to and inhibits the activity of apoptosis signal-regulating kinase 1 (ASK1) (15). Activated ASK1 promotes the stress-responsive JNK pathway, among others; thus, Trx1 indirectly controls JNK signaling (22).

Because Trx1 plays a central role in the regulation of inflammation, it might be predicted that over-expression of Trx1 can slow aging or age-related diseases. Previous studies have shown that the median lifespan of mice over-expressing human Trx1 [Tg(*TRX1*)$^{+/0}$] is greater than wild-type mice (23,24). Further, Tg(*TRX1*)$^{+/0}$ mice showed a significant reduction in the total incidence of acidophilic macrophage pneumonia as a probable cause of death (24). In this study, we tested whether over-expression of Trx1 could prevent defects in glucose metabolism. Because laboratory mice under normal husbandry conditions do not generally develop diabetes-like phenotypes even with age, we used a dietary intervention to induce glucose intolerance and chronic inflammation. High fat diet feeding and obesity in mice have been clearly shown to stimulate a pro-inflammatory state, particularly in the growing adipose tissue (25). In this study, we used high fat feeding to test whether Tg(*TRX1*)$^{+/0}$ mice were protected from diet-induced obesity. In addition, we tested whether Tg(*TRX1*)$^{+/0}$ mice were protected from the pro-inflammatory state previously shown to be associated with increased fat accumulation.

Methods

Animals and feeding studies

Expression of Trx1 in the strain of Tg(*TRX1*)$^{+/0}$ used in previous studies was shown to decline with age (24). Because these changes in Trx1 over-expression could add a significant confound to data collected using these mice, we generated additional lines of Tg(*TRX1*)$^{+/0}$ mice that use the endogenous TRX1 promoter to regulate expression. These mice were generated using a fragment of the human genome containing the TRX1 gene [a BAC clone (RP11-427L11) from Children's Hospital Oakland Research Institute's (CHORI) BACPAC Resources Center (BPRC), Oakland, CA.] and 8.3 and 12.3 kb of the 5′- and 3′-flanking sequences, respectively. Mice were genotyped using PCR primer pairs designed to screen for the presence of the hTRX1 gene in mice (primer pairs to the 5′ untranslated region, exon 1 and a sequence within exon 5). We have confirmed transmission, stable integration, and successful passing to progeny of the TRX1 transgene in three founder lines. The first line of transgenic mice were used in this study; prior to this study, these mice had been successfully backcrossed into the C57BL/6J background for >7 generations (The Jackson Laboratories, Bar Harbor ME). Further details on the line of mice will be presented in an additional manuscript (*in preparation*).

Male Tg(*TRX1*)$^{+/0}$ and littermate wild-type control mice were maintained on standard NIH-31 chow from weaning at a density of 2–4 mice per barrier cage in a specific pathogen free facility. When mice reached 4.5–6 months of age, cages were randomly assigned to be fed either of two defined diets (Purina Test Diets, Richmond IN): low fat (10% kCal from lard, Purina Test Diet 58Y2) or high fat (45% kCal from lard, Purina Test Diet 58V8). The energy composition of each diet in terms of kCal/gram food is: 18.3% protein:10.2% fat: 71.5% carbohydrate for low fat 58Y2 and 18.3% protein:45.2% fat:36.5% carbohydrate for 58V8. In both cases, the primary source of protein was casein, the primary source of fat was lard and the primary sources of carbohydrates were sucrose, dextrin and maltodextrin.

Body weight was measured prior to feeding studies and biweekly during study. Food consumption per cage was monitored over 2-week periods. At the end of 16 weeks of feeding, fat mass and lean mass were measured by quantitative magnetic resonance (QMR) imaging (Echo QMR, Houston TX). At sacrifice, mice were euthanized by CO_2 with cervical dislocation and tissues were dissected, weighed and stored at −80°C until analysis. For tissue composition, frozen tissues were brought to 4°C on ice and analyzed by QMR. Percentage of fat was calculated by the formula [fat mass/(fat mass+lean mass)]. All procedures involving the mice were approved by the Subcommittee for Animal Studies at the Audie L. Murphy

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Veterans Administration Hospital at San Antonio and the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio.

Glucose tolerance tests and insulin measurements

After 8 weeks of feeding either diet, glucose metabolism was measured by glucose tolerance tests. Mice were fasted for 6 hours beginning at 9:00 AM and fasting blood glucose was determined by One Touch hand-held glucometer (LifeScan Inc., Milpitas CA). Mice were then given intra-peritoneal injection of glucose (Sigma-Aldrich, St. Louis MO) in 0.7% saline at a dose of 1.5 mg glucose/kg body weight. Blood glucose was measured at given intervals over 2 hours. Insulin levels were measured by ELISA (CrystalChem, Downers Grove IL) in whole blood collected from mice after 6-hours fast. HOMA-IR measurements were determined by the formula HOMA-IR = [fasting glucose (mg/dL) × fasting insulin (mU/L)]/405.

Measurement of gene expression by RT-PCR

Total RNA from 50 to 100 mg adipose tissue was prepared using Tri-Reagent (Sigma) treated with DNase I (Life Technologies, Grand Island NY). cDNA was produced using Retroscript for RT-PCR (Life Technologies). Real-time PCR was performed in Applied Biosystems 7900 Real-Time PCR system (Life Technologies, with default PCR program using primers for TNF-α, IL-6, Actin-γ, and Microglubin-β designed using ABI Primer Express software 3.1. Actin-γ and Microglubin-β were used as housekeeping reference genes. Samples were placed in 384-well real time PCR plate using a final volume of 10ul. Optimum reaction condition were obtained with 16.26 μl SYBR Green PCR Master Mix (Life Technologies), 160 nM forward primer, 160 nM reverse primer, and 8 ng template cDNA in each well. Data analysis was performed using an absolute quantification standard curve method.

Statistics

Data for weight gain were measured by 2-Way Repeated Measures ANOVA with 'diet' and 'genotype' as variables. Data for tissue weight and body composition were analyzed by 2-Way ANOVA with post-hoc analysis by Holm-Sidak when applicable. Gene expression were analyzed by Student's *t*-test to determine the difference between genotype. Similarly, data for glucose tolerance tests were analyzed by Student's *t*-test for differences between genotypes in blood glucose values at each time point indicated. Method used to determine Area Under Curve for glucose tolerance tests was the Trapezoid Rule; these data were analyzed for genotype difference by Student's *t*-test. All data were analyzed using SigmaStat (Aspire Software, Ashburn VA).

Results

It was previously reported that the body weight of Tg(*TRX1*)$^{+/0}$ mice maintained on standard mouse chow diet does not differ from wild-type mice, nor is there a difference between mice in food consumption (24). In this study, we fed young adult (4.5–6 months) male Tg(*TRX1*)$^{+/0}$ and wild-type mice defined diets containing either 10% total kCal from fat (low fat) or 45% total kCal from fat (high fat) and tested whether over-expression of Trx1 could protect mice from obesity and/or defects in glucose metabolism. Through the 16 weeks on these diets, mice fed a high fat diet gained significantly more weight in this study than did mice fed a low fat diet (Fig. 1A; Variable 'diet': $F = 53.4$, $p < 0.001$). However, over-expression of Trx1 had no significant effect on weight gain in mice fed either low fat or high fat diet (Fig. 1A; variable 'diet' X 'genotype' interaction: $F = 2.5$, $p = 0.358$). We then assayed body composition of mice on both diets by QMR; as expected, mice fed a high fat diet had significantly more fat mass than mice fed a low fat diet (variable 'diet': $F = 321.1$, $p < 0.001$), but again we found no significant difference between Tg(*TRX1*)$^{+/0}$ and wild-type mice in fat mass on either diet (Fig. 1B; variable 'diet' X 'genotype' interaction: $F = 3.8$, $p = 0.062$). High fat feeding had no effect on overall lean mass (variable 'diet': $F = 0.7$, $p = 0.416$) and over-expression of Trx1 also had no effect on lean mass of mice on either diet (Fig. 1B; variable 'diet' X 'genotype' interaction: $F = 0.192$, $p = 0.665$). Throughout the course of the experiment, Tg(*TRX1*)$^{+/0}$ and wild-type mice did not differ in their food consumption on either diet (Fig. 1C).

We next tested whether over-expression of Trx1 protected mice from defects in glucose metabolism associated with high fat diet and obesity. Glucose tolerance tests were performed after 8 weeks of feeding each diet. In mice fed the low fat diet, we found no difference between Tg(*TRX1*)$^{+/0}$ and wild-type mice in these tests (Fig. 2A). As expected, we found that wild-type mice fed the high fat diet were significantly glucose intolerant relative to wild-type mice fed low fat diet (Fig. 2A,B). Surprisingly, high fat-fed Tg(*TRX1*)$^{+/0}$ mice showed a significant improvement in glucose tolerance relative to high fat-fed wild-type mice (Fig. 2B). That is, Tg(*TRX1*)$^{+/0}$ mice became less glucose intolerant when fed a high fat diet than did wild-type mice, suggesting over-expression of Trx1 prevents obesity-induced glucose dysfunction. These differences remained significant when glucose tolerance tests were analyzed by Area Under the Curve (Fig. 2C). While fasting blood glucose and insulin levels significantly increased with high fat feeding, these measures did not differ between Tg(*TRX1*)$^{+/0}$ and wild-type mice on either diet (Table 1). We used these data points to calculate HOMA-IR, a measurement of whole animal insulin sensitivity. As a group, mice fed high fat diet had significantly increased HOMA-IR suggesting insulin

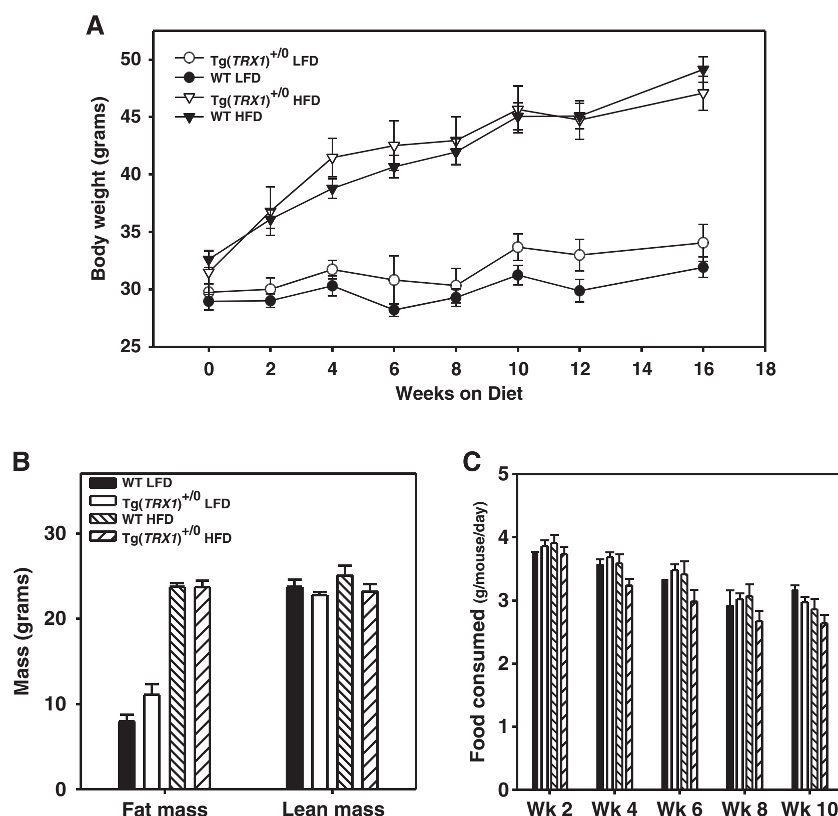

Fig. 1. A. Body weight of wild-type (WT) and Tg(*TRX1*)$^{+/0}$ mice fed either low fat (10% kCal) or high fat (45% kCal) over the course of this experiment. B. Fat mass and lean mass for WT and Tg(*TRX1*)$^{+/0}$ mice after low fat and high fat feeding. C. Average food consumption over 2 week periods for WT and Tg(*TRX1*)$^{+/0}$ mice fed low fat and high fat diets. For A, symbols represent average weight ($\pm$SEM) for indicated group at each time point. For B and C, bars represent the average ($\pm$SEM) of 5–9 mice within that group.

resistance; however, we found no significant difference between wild-type and Tg(*TRX1*)$^{+/0}$ mice fed either diet (Table 1).

The regulation of glucose homeostasis in mice is regulated largely by the skeletal muscle and adipose tissue. Others have shown that defects in glucose metabolism with obesity can be caused by overall fat accumulation, increased accumulation of visceral adipose tissue relative to subcutaneous adipose tissue, and by ectopic fat accumulation in tissues like skeletal muscle (26,27). High fat-fed *Tg(TRX1)*$^{+/0}$ mice showed no reduction in overall fat accumulation (Fig. 1B), so we next measured whether over-expression of Trx1 altered fat distribution among the adipose tissue depots (i.e. away from visceral adipose and towards subcutaneous adipose). The weights of all adipose depots in high fat fed mice were significantly greater than the weights of adipose depots from low fat diet fed mice when analyzed by 2-Way ANOVA (Table 1). However, the mass of depots in Tg(*TRX1*)$^{+/0}$ mice did not differ from those of wild-type mice on either diet (variable 'diet' X 'genotype' interaction: $p > 0.25$ for each adipose depot). Furthermore, the weights of organs made up of primarily lean mass (i.e. heart, liver, kidney, and brain) also did not differ between Tg(*TRX1*)$^{+/0}$ and wild-type mice (Table 1). To test for differences in ectopic lipid accumulation, we quantified the fat content of heart, liver, and hind-limb skeletal muscles by QMR. High fat feeding caused a significant increase in the fat content of liver and skeletal muscle (and a trend for an increase in heart), but there was no suggestion that Tg(*TRX1*)$^{+/0}$ mice had reduced fat content in these tissues (Fig. 3). In fact, the hind-limb muscles of high fat-fed Tg(*TRX1*)$^{+/0}$ mice had significantly elevated fat content compared to wild-type mice.

We next addressed whether the induction of inflammation by high fat feeding was prevented in tissues of Tg(*TRX1*)$^{+/0}$ mice. Preventing pro-inflammatory cytokine production has previously been shown to prevent high fat diet induced insulin resistance (11,14). Pro-inflammatory cytokine production by intra-abdominal adipose tissue depots has been shown to be increased in obesity (28). Perez et al. previously showed that Tg(*TRX1*)$^{+/0}$ mice tended to have reduced activation of JNK signaling and reduction of IL-1β (though not TNF-α) in the liver of young mice on normal chow (24). Because peri-renal adipose depots were available after sacrifice, we used these as representative of intra-abdominal adipose depots. In this depot, we found in high fat animals that

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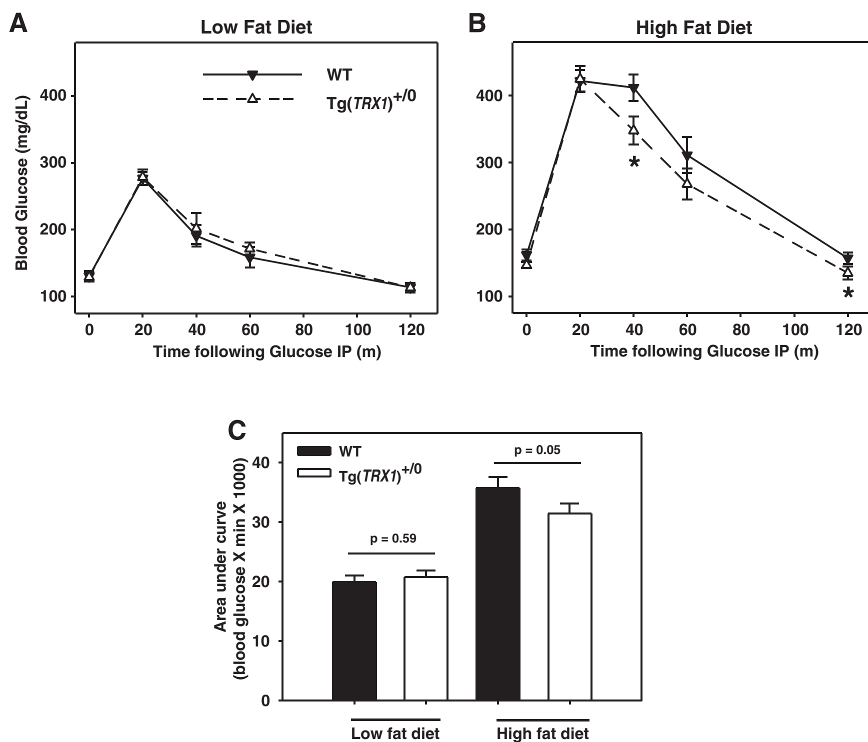

Fig. 2. A. Glucose tolerance tests for mice fed low fat diet. B. Glucose tolerance tests for mice fed high fat diet. For both A and B, average values (±SEM) at each time point are given for WT (closed triangles) and Tg(*TRX1*)$^{+/0}$ (open triangles) for 5–9 mice per group. Asterisks represent difference between genotype at indicated point reached $p < 0.05$ by Student *t*-test. C. Area under curve (calculated by trapezoid method) for WT and Tg(*TRX1*)$^{+/0}$ mice fed low fat or high fat diet. Bars represent average value (±SEM) for 5–9 mice in each group. Given *p*-values are for Student *t*-tests between groups indicated with horizontal line.

TNF-α mRNA expression was significantly lower in Tg(*TRX1*)$^{+/0}$ mice relative to wild-type mice (Fig. 4). However, we found no significant difference in IL-6 expression in peri-renal adipose suggesting that the protection of glucose homeostasis in Tg(*TRX1*)$^{+/0}$ mice may be TNF-α dependent.

Table 1. Mean Tissue parameters (± SEM) of wild-type (WT) and Tg(*TRX1*)$^{+/0}$ mice in this study

	Low fat diet		High fat diet	
	WT	Tg(*TRX1*)$^{+/0}$	WT	Tg(*TRX1*)$^{+/0}$
Blood metabolites				
Fasting glucose (mg/dL)	130±8	129±7	162±8	147±5
Fasting insulin (mU/L)	3.2±1.8	3.3±0.7	14.2±5.8	7.9±1.4
HOMA-IR	0.9±0.6	1.1±0.2	5.9±2.6	3.0±0.5
Adipose depot weights (g)				
Epidydimal	0.99±0.13	1.29±0.18	2.29±0.19	2.17±0.12
Peri-renal	0.56±0.07	0.78±0.10	1.92±0.09	1.97±0.17
Mesenteric	0.48±0.07	0.54±0.08	1.59±0.08	1.45±0.13
Inguinal	0.15±0.02	0.21±0.05	0.31±0.03	0.34±0.04
Subscapular	0.11±0.01	0.11±0.01	0.21±0.04	0.21±0.02
Brown	0.18±0.01	0.24±0.02	0.40±0.03	0.38±0.05
Lean tissue weights (g)				
Heart	0.15±0.00	0.14±0.01	0.15±0.01	0.14±0.01
Liver	1.54±0.08	1.76±0.18	2.45±0.25	2.31±0.21
Kidney	0.42±0.01	0.41±0.01	0.46±0.02	0.42±0.03
Brain	0.45±0.00	0.46±0.00	0.46±0.01	0.44±0.01

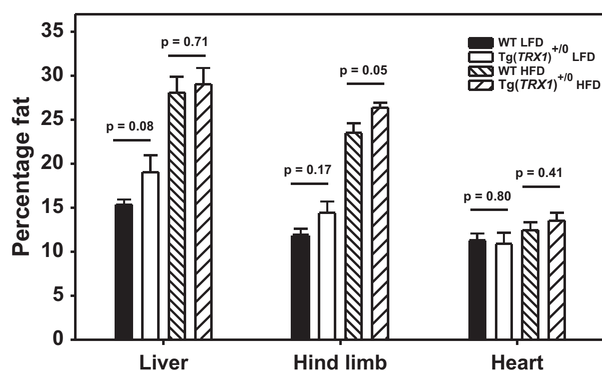

Fig. 3. Average fat content of tissues from WT and Tg(*TRX1*)$^{+/0}$ fed low fat or high fat diets. Bars represent average value (±SEM) for 5–9 mice in each group. Given *p*-values are for Student *t*-tests between groups indicated with horizontal line.

Discussion

In this study, we show that over-expression of Trx1 prevents glucose intolerance caused by high fat feeding without affecting fat accumulation or distribution. Interestingly, Miyamoto et al. found that diabetes and glucose intolerance in humans were associated with elevated levels of plasma thioredoxin (29). Kakisaka et al. also found that plasma thioredoxin levels were significantly higher in patients with Type 2 diabetes than in control patients (30). Because hyperglycemia is associated with increased oxidative stress, a compensatory antioxidant response might be the reason for elevated thioredoxin with glucose metabolism dysfunction (31). While expression of thioredoxin is elevated with diabetes, it has been shown that the *activity* of thioredoxin is actually reduced with hyperglycemia both in vivo and in cell culture (32,33). This reduction in thioredoxin activity has been shown to be caused by hyperglycemia-induced expression of thioredoxin-interacting protein (TxnIP), a negative regulator of thioredoxin activity (32,33). It is possible that Tg(*TRX1*)$^{+/0}$ mice express Trx1 at levels high enough to counter this inhibitory action of hyperglycemia and thus minimize oxidative stress with high fat feeding. It is also possible that the human Trx1 (~93% sequence identity to mouse Trx1) expressed in Tg(*TRX1*)$^{+/0}$ mice is not recognized by mouse TxnIP, thereby maintaining high levels of activity with hyperglycemia.

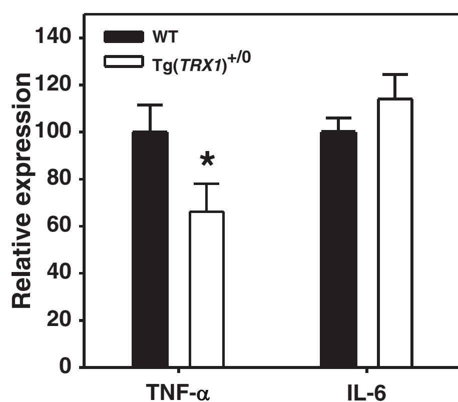

Fig. 4. Relative mRNA expression of TNF-α and IL-6 in peri-renal adipose tissue from high fat-fed WT and Tg(*TRX1*)$^{+/0}$ mice. Bars represent average expression value (±SEM) of samples from 5–8 mice in each group for each cytokine. Asterisk represents difference between genotype for indicated cytokine reached $p < 0.05$ by Student *t*-test.

Obesity is correlated with a significant increase in oxidative stress and damage in several tissues including skeletal muscle and adipose (34,35). Others have shown that transgenic mice that over-express antioxidants like Mn superoxide dismutase (Sod2) or peroxiredoxin 3 are protected from insulin resistance caused by high fat feeding (36,37). Similarly, over-expression of catalase specifically to the mitochondria (MCAT) can prevent defects in glucose homeostasis caused by either high fat feeding or by old age (38,39). Tg(*TRX1*)$^{+/0}$ mice are resistant to agents that generate oxidative stress by redox-cycling, like diquat and paraquat (23,24). Furthermore, Tg(*TRX1*)$^{+/0}$ mice have lower lipid and protein oxidation levels than wild-type mice both under normal husbandry conditions and in response to diquat (24). Thus, one explanation for the maintenance of glucose homeostasis in high fat-fed Tg(*TRX1*)$^{+/0}$ mice could be by reduction of oxidative stress associated with obesity.

Thioredoxins are also anti-inflammatory and our data suggest that over-expression of Trx1 prevents obesity-induced glucose intolerance in part through this action. It has been thought that increased expression of pro-inflammatory cytokines like TNF-α and IL-6 may be the primary cause of insulin resistance with obesity and high fat feeding (10–12). Thioredoxin has been shown to inhibit the production of these cytokines through regulation of nuclear factor kappa B (NFκB) (40,41). Tg(*TRX1*)$^{+/0}$ mice fed a normal chow diet have been shown to have no change in TNF-α expression in the liver (24). We show here that expression of TNF-α is significantly reduced in peri-renal adipose tissue of high fat-fed Tg(*TRX1*)$^{+/0}$ mice. This discrepancy might simply be due to characteristics inherent to these different tissues. It must be mentioned that mRNA expression of these pro-inflammatory cytokines may not directly correlate with their protein levels. Alternatively, it may be that pro-inflammatory stimuli are relatively low under normal dietary conditions and, thus, the activity of Trx1 on TNF-α (and other cytokines) is limited. Conversely, chronic stress like high fat feeding may stimulate pro-inflammatory expression to a greater extent at which time the protective effect of Trx1 is much more apparent. This might also suggest that the effects of Trx1 over-expression on lifespan may be much more dramatic if conducted under chronic stress such as that which occurs with obesity (24,42).

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Previous studies have suggested that Trx1 may have several roles in the prevention of metabolic diseases. For example, mice with targeted over-expression of Trx1 exclusively in pancreatic β-cells can prevent both autoimmune-induced and streptozotocin-induced diabetes (43). In both cases, the prevention of diabetes could be explained by protection of the β-cells and preservation of insulin secretion. Similarly, Hamada et al. found that mice that over-express Trx1 ubiquitously show protection from DNA oxidation caused by streptozotocin treatment (44). Yamamoto et al. showed that over-expression of Trx1 in the diabetic *db/db* mouse could reduce the progression of hyperglycemia and reduce plasma insulin levels (45). These previous studies all suggest that Trx1 may prevent diabetes by maintaining β-cell survival and preserving insulin secretion. We add to these previous findings by showing that Trx1 may also help to preserve peripheral glucose metabolism with high fat feeding. Together, these data suggest that thioredoxins may be an attractive target in treatment of Type 2 diabetes which is characterized by both peripheral insulin resistance and reduced pancreatic insulin production (46).

While this study shows that Tg($TRX1$)$^{+/0}$ mice are protected from high fat diet-induced glucose intolerance, we don't yet know whether age-induced declines in glucose metabolism are prevented in these mice. Work in humans and rodents has suggested that chronic inflammation with age may be a primary cause of many age-related diseases, including insulin resistance and diabetes (8). Lee et al. found that over-expression of catalase targeted to the mitochondria (MCAT) in mice prevented insulin resistance associated with aging (38). The median lifespan of Tg($TRX1$)$^{+/0}$ mice has been shown to be greater than that of wild-type mice (24); thus, it will be of great interest to determine whether the extended lifespan of these mice is also associated with the delayed occurrence of age-related pathologies such as reduced glucose homeostasis.

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