Nordihydroguaiaretic Acid, a Lignan from *Larrea tridentata* (Creosote Bush), Protects Against American Lifestyle-Induced Obesity Syndrome Diet–Induced Metabolic Dysfunction in Mice

Jackie K. W. Chan, Stefanie Bittner, Alex Bittner, Suman Atwal, Wen-Jun Shen, Mohammed Inayathullah, Jayakumar Rajada, Mark R. Nicolls, Fredric B. Kraemer, and Salman Azhar

**Geriatrics Research, Education and Clinical Center, Veterans Affairs Palo Alto Health Care System, Palo Alto, California (J.K.W.C., S.B., A.B., S.A., W.-J.S., F.B.K., S.Az.); and Division of Endocrinology, Gerontology, and Metabolism (J.K.W.C., S.B., A.B., S.A., W.-J.S., F.B.K., S.Az.), BioADD Laboratory, and Divisions of Cardiovascular Pharmacology CVI (M.I., J.R.) and Pulmonary and Critical Care Medicine (M.R.N.), Stanford University, Stanford, California**

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**ABSTRACT**

To determine the effects of nordihydroguaiaretic acid (NDGA) on metabolic and molecular changes in response to feeding a typical American fast food or Western diet, mice were fed an American lifestyle-induced obesity syndrome (ALIOS) diet and subjected to metabolic analysis. Male C57BL/6J mice were randomly assigned to the ALIOS diet, the ALIOS diet supplemented with NDGA (NDGA+ALIOS), or a control diet and were maintained on the specific diet for 8 weeks. Mice fed the ALIOS diet showed increased body, liver, and epididymal fat pad weight as well as increased plasma alanine transaminase (ALT) and aspartate aminotransferase (AST) levels (a measure of liver injury) and liver triglyceride content. Coadministration of NDGA normalized body and epididymal fat pad weight, ALT and AST levels, and liver triglycerides. NDGA treatment also improved insulin sensitivity but did not glucose intolerance in mice fed the ALIOS diet. In mice fed the NDGA+ALIOS diet, NDGA supplementation induced peroxisome proliferator-activated receptor α (PPARα; the master regulator of fatty acid oxidation) and mRNA levels of carnitine palmitoyltransferases Cpt1a and Cpt2, fatty acid synthase (Fasn), and diacylglycerol acyltransferase Dgat2. NDGA treatment of ALIOS-fed mice upregulated the hepatic expression of antioxidant enzymes, glutathione peroxidase 4, and peroxiredoxin 3 proteins. In conclusion, we provide evidence that NDGA improves metabolic dysregulation by simultaneously modulating the PPARα transcription factor and key genes involved in fatty acid oxidation, key antioxidant and lipogenic enzymes, and apoptosis and ER stress signaling pathways.

**Introduction**

The prevalence of obesity has reached epidemic proportions (Ng et al., 2014; NCD Risk Factor Collaboration, 2016); recent survey data indicate that currently 37.7% of adults and 17.0% of children in the United States are obese (Seidell and Hawkes, 2016; Heymsfield and Wadden, 2017). Obesity has been implicated as one of the major risk factors for many chronic metabolic diseases such as type 2 diabetes (T2D) (Kramer et al., 2010; Day and Bailey, 2011; American Diabetes Association, 2016), metabolic syndrome (MetS) (Grundy, 2004; Andersen and Fernandez, 2013), nonalcoholic fatty liver disease (NAFLD) (Fabbriani et al., 2010; Patel et al., 2014), certain cancers (Deng et al., 2016), and cardiovascular disease (Kramer et al., 2010; Day and Bailey, 2011; American Diabetes Association, 2016), metabolic syndrome (MetS) (Grundy, 2004; Andersen and Fernandez, 2013), nonalcoholic fatty liver disease (NAFLD) (Fabbriani et al., 2010; Patel et al., 2014), certain cancers (Deng et al., 2016), and cardiovascular disease (Kramer et al., 2010; Day and Bailey, 2011; American Diabetes Association, 2016), metabolic syndrome (MetS) (Grundy, 2004; Andersen and Fernandez, 2013), nonalcoholic fatty liver disease (NAFLD) (Fabbriani et al., 2010; Patel et al., 2014), certain cancers (Deng et al., 2016), and cardiovascular disease (Kramer et al., 2010; Day and Bailey, 2011; American Diabetes Association, 2016), metabolic syndrome (MetS) (Grundy, 2004; Andersen and Fernandez, 2013), nonalcoholic fatty liver disease (NAFLD) (Fabbriani et al., 2010; Patel et al., 2014), certain cancers (Deng et al., 2016), and cardiovascular disease (Kramer et al., 2010; Day and Bailey, 2011; American Diabetes Association, 2016), metabolic syndrome (MetS) (Grundy, 2004; Andersen and Fernandez, 2013), nonalcoholic fatty liver disease (NAFLD) (Fabbriani et al., 2010; Patel et al., 2014), certain cancers (Deng et al., 2016), and cardiovascular disease (Kramer et al., 2010; Day and Bailey, 2011; American Diabetes Association, 2016), metabolic syndrome (MetS) (Grundy, 2004; Andersen and Fernandez, 2013), nonalcoholic fatty liver disease (NAFLD) (Fabbriani et al., 2010; Patel et al., 2014), certain cancers (Deng et al., 2016), and cardiovascular disease (Kramer et al., 2010; Day and Bailey, 2011; American Diabetes Association, 2016), metabolic syndrome (MetS) (Grundy, 2004; Andersen and Fernandez, 2013), nonalcoholic fatty liver disease (NAFLD) (Fabbriani et al., 2010; Patel et al., 2014), certain cancers (Deng et al., 2016), and cardiovascular disease (Kramer et al., 2010; Day and Bailey, 2011; American Diabetes Association, 2016), metabolic syndrome (MetS) (Grundy, 2004; Andersen and Fernandez, 2013), nonalcoholic fatty liver disease (NAFLD) (Fabbriani et al., 2010; Patel et al., 2014), certain cancers (Deng et al., 2016), and cardiovascular
disease (CVD) (Zalesin et al., 2011; Bastien et al., 2014). The prevalence of these clinical conditions is also increasing at an accelerated pace. In addition, both MetS and NAFLD independently increase the risk of T2D and CVD (Grundy, 2006; Haffner, 2006; Smith and Adams, 2011; Anstee et al., 2013). In contrast to T2D and CVD, however, currently no pharmacological agents are specifically approved for the treatment of NAFLD, including nonalcoholic steatohepatitis (Chalasani et al., 2012; Dyson et al., 2014; Barb et al., 2016) and MetS (Swislocki et al., 2012; Lim and Eckel, 2014).

Previous studies from our laboratory have shown that nordihydroguaiaretic acid (NDGA), one of the major constituents of Creosote bush, Larrea tridentata, exerts profound effects on a number of metabolic abnormalities, including improving obesity, dyslipidemia, insulin resistance, hypertension, steatosis, and altered glucose metabolism, in several rodent models (Zhang et al., 2013, 2015, 2016). Although these studies provided important mechanistic information by which NDGA improves metabolic abnormalities, they were carried out mainly using genetically manipulated (e.g., ob/ob mice) animals fed an abnormally high-carbohydrate (high-fructose) or high-fat diet; none of these models fully reproduces the major pathophysiological changes associated with the consumption of typical American fast food or a “Western” diet (Tetri et al., 2008). The goal of our studies was to examine the effect of NDGA administration on metabolic dysfunction in a previously described nutritionally induced mouse model of dyslipidemia and insulin resistance, which was developed using dietary formulation based on commonly consumed fast foods (i.e., mice are fed a high-fat chow containing trans fats and are given the required amount of high-fructose corn syrup equivalents) (Tetri et al., 2008). This model is commonly referred to as the American lifestyle-induced obesity syndrome (ALIOS) model. We focused our studies on hepatic metabolism, considering that the liver is the major site for intermediary metabolism of carbohydrates, fats, and proteins. The liver is also a major storage site of carbohydrate (glycogen) and lipids (cholesterol/cholesterol esters and triglycerides). Here, we provide evidence that dietary administration of NDGA to ALIOS mice improves metabolic dysregulation by upregulation of peroxisome proliferator–activated receptor a (PPARα) protein, increased expression of key genes involved in fatty acid oxidation, and selected antioxidant enzymes and simultaneously downregulation of the mRNA expression of key lipogenic enzymes, apoptosis, and endoplasmic reticulum (ER) stress signaling pathways.

**Materials and Methods**

**Antibodies.** Anti–β-actin, anti–caspase 3, and anti-catalase (CAT) antibodies were purchased from Cell Signaling Technology (Danvers, MA). Anti–glutathione peroxidase 1 (GPX1), anti–PPARα, anti–PPARy, anti–GPX4, and anti–superoxide dismutase 2 (SOD2)/MnSOD antibodies were supplied by Abcam (Cambridge, MA). Anti–C/EBP homologous protein (CHOP) antibody was from Proteintech Group Inc. (Rosemont, IL). IRDye800RD goat anti-rabbit IgG (H + L) secondary antibody was purchased from LI-COR Biosciences (Lincoln, NE). Other antibody details are presented in Supplemental Table 1.

**Chemicals and Reagents.** The Pierce BCA Protein Assay Kit was purchased from Thermo Fisher Scientific (Waltham, MA). NDGA was isolated and purified from Creosote bush (L. tridentata) by Pharmaceuticals International Inc. (Hunt Valley, MD) according to a slight modification (Elakovich and Stevens, 1985) of the procedure of Waller and Gisvold (1945). In brief, dried leaves and small twigs of _L. tridentata_ (collected from the deserts of Arizona) were extracted successively with organic solvents (methyl isobutyl ketone, ethyl ether, acetone, and methanol) followed by water in a Soxhlet extractor. Subsequent purification of the combined concentrated extract by Sephadex column chromatography followed by methanol repurification from the concentrated column eluent containing NDGA yielded beige-cream–colored NDGA powder. The bulk quantity of such a purified preparation of NDGA was supplied by Mr. Glen Kelley (Insmed Inc., Richmond, VA) (Kelley et al., 2004). The purity of NDGA was >97% as determined by nuclear magnetic resonance analysis (Supplemental Fig. 1). Reagents for measuring serum alanine transaminase (ALT), aspartate aminotransferase (AST), glucose, triglycerides, and total cholesterol were purchased from Stanbio Laboratory (Boerne, TX). Free cholesterol and nonesterified fatty acid (NEFA) measurement kits were purchased from Wako Diagnostics (Richmond, VA). Humulin R U-100 (insulin human) was supplied by Lilly USA (Indianapolis, IN). Glucose was purchased from Sigma-Aldrich (St. Louis, MO). All other reagents used were of analytical grade. The custom ALIOS diet (no. TD06303) supplemented with NDGA (NDGA+ALIOS) or without NDGA (2.5 g/kg diet) was prepared by Harlan Teklad/Envigo (Madison, WI).

**Animal Studies.** Six-week-old male C57Bl/6j mice were obtained from the Jackson Laboratory (Bar Harbor, ME). All animal experiments were performed according to procedures approved by the Veterans Affairs Palo Alto Health Care System Animal Care and Use Committee. Animals were housed four per cage in a temperature-controlled facility under a 12-hour/12-hour light/dark cycle. Mice were separated into three treatment groups (chow, ALIOS, and NDGA+ALIOS) normalized by body weight and allowed to acclimate for 2 weeks prior to the study. Mean body weight at the start of the study was 24.8 ± 0.3 g. Mice were housed socially, four per cage, and were fed either standard chow or the ALIOS diet (Tetri et al., 2008). Briefly, the ALIOS diet is a modified high-fat diet in which 45% of the caloric content is derived from fat; 30% of the fat is present in the form of partially hydrogenated vegetable oil, consisting of 28% saturated, 57% monounsaturated, and 13% polyunsaturated fatty acids. In addition, drinking water for the ALIOS groups was supplemented with high-fructose corn syrup equivalents (55% fructose, 45% glucose) at 42 g/l. Mice fed this combination diet become obese and develop glucose intolerance, hyperinsulinemia, and hepatic steatosis associated with a necroinflammatory and profibrogenic response. For the NDGA+ALIOS groups, the ALIOS diet was further augmented with 2.5 g/kg diet NDGA. Animals were maintained on these diets for 8 weeks. Body weights and food and water consumption were measured weekly.

**Glucose Tolerance Test and Insulin Tolerance Test.** The glucose tolerance test (GTT) and the insulin tolerance test (ITT) were performed separately, at least 1 week apart, at weeks 7 and 8 of the study, respectively. For the GTT, animals were fasted for 16 hours overnight, and glucose (1 g/kg body weight) was injected intraperitoneally. Blood was collected from the tail vein prior to injection and at 15, 30, 60, and 120 minutes postinjection to assess glucose clearance. For ITT, animals underwent a 6-hour fast. Insulin (0.75 U/kg) was injected intraperitoneally and blood was collected prior to injection and at 15, 30, 45, and 60 minutes postinjection to assess insulin sensitivity. Blood glucose levels were determined immediately using a glucometer (Precision Xtra; Abbott, Abbott Park, IL). The total area under the curve (AUC) was calculated using the trapezoidal method.

**Quantification of Serum Metabolites.** Blood was collected from the tail vein after a 4-hour fast at the final week of the experimental period. Samples were centrifuged at 4000g for 15 minutes at 4°C and serum was retained and stored at ~8°C. Serum ALT, AST, glucose, triglyceride, total cholesterol, free cholesterol, and NEFA levels were determined with commercial assays according to the manufacturers’ protocols.

**Measurement of Liver Triglyceride Content.** Suitable aliquots (in duplicate) of liver homogenates were extracted with a mixture of chloroform and methanol according to the procedure of...
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To investigate the effects of the ALIOS diet consumption, we measured several physiologic parameters during the 8-week study. Terminal body weights of mice fed chow weighed an average of 31 g. Animals fed the ALIOS diet were significantly heavier, averaging 34 g \((P = 0.0007)\). NDGA supplementation in the ALIOS diet attenuated weight gain and was comparable to chow at 32 g (Fig. 1A). Liver and epididymal fat weights were also significantly increased in the ALIOS group; however, when normalized by body weight, only epididymal fat was significant (Fig. 1B). Mice fed a chow diet had livers weighing 1.05 g compared with ALIOS-fed mice \((1.31 \text{ g}; P = 0.0001)\), whereas liver weights were 1.13 g for NDGA+ALIOS-fed mice \((P = 0.0330 \text{ compared with ALIOS})\). There was no significant difference between the chow and NDGA+ALIOS groups \((P = 0.8252)\). Linear regression on growth curves demonstrated a similar trend comparing the three groups. Although the chow and NDGA+ALIOS groups, on average, gained approximately 0.10 and 0.11 g daily, respectively, ALIOS weight gain was substantially greater at 0.14 g/day (Fig. 1C). However, the increased weight gain in the ALIOS group was not attributable to increased diet consumption. Although chow-fed animals ate the most diet in terms of mass, the ALIOS and NDGA+ALIOS groups had similar intake (Fig. 1D). When diet consumption was converted to total caloric intake, the ALIOS and NDGA+ALIOS groups ingested the most daily (approximately 27% and 20% more than chow-fed animals, respectively) (Fig. 1E).

Next, because we observed significant weight gain in the ALIOS-fed group, we assessed the impact of the diet on glucose tolerance and insulin sensitivity by performing the GTT and ITT, respectively. After an overnight fast, basal glucose levels were similar among the three groups, at 70, 78, and 78 mg/dl for the chow, ALIOS, and NDGA+ALIOS groups, respectively. Tail vein glucose readings reached maximal levels at 30 minutes after a 1-g/kg dose of glucose; chow-fed animals averaged a peak of 271 mg/dl. In comparison, the ALIOS and NDGA+ALIOS groups spiked significantly higher, at 324 \((P < 0.0001)\) and 309 mg/dl \((P = 0.014)\), respectively. There were no differences between the ALIOS and NDGA+ALIOS groups at any time point (Fig. 2A). Calculated total AUCs demonstrated a similar trend; the ALIOS and NDGA+ALIOS groups had significantly higher AUCs compared with chow-fed animals (Fig. 2B). Insulin tolerance was measured after a week of recovery. Six-hour fasted glucose levels indicated significant differences between the three dietary treatment groups. Chow-fed animals had the lowest fasting glucose at 141 mg/dl. Whereas the NDGA+ALIOS group averaged serum glucose levels at 172 mg/dl, serum levels of animals solely fed the ALIOS diet were further elevated to 210 mg/dl \((P = 0.0006)\). At a 0.75-U/kg insulin dose, serum glucose levels were decreased to 47, 84, and 56 mg/dl at 60 minutes postinjection in the chow, ALIOS, and NDGA+ALIOS groups, respectively. Serum glucose was significantly elevated in the ALIOS group at every time point, whereas the insulin tolerance profile in the NDGA+ALIOS

<table>
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<tr>
<th>Gene Symbol</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tbody>
<tr>
<td>CPT1a (Cpt1a)</td>
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<td>5′-TTGAGGCGTCATGCGTCAC-3′</td>
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<tr>
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<td>5′-TTGAGGCGTCATGCGTCAC-3′</td>
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<td>CPT2 (Cpt2)</td>
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Rplp0, ribosomal protein lateral stalk subunit P0.
group was similar compared with the chow group (Fig. 2C). Although calculated total AUC values were higher in the NDGA+ALIOS group compared with the chow group, NDGA supplementation significantly decreased the AUC compared with the ALIOS group only ($P < 0.0023$) (Fig. 2D). Four-hour fasted serum glucose and insulin levels were measured at 4 weeks. Glucose levels were increased significantly in ALIOS-fed mice (225 mg/dl) compared with chow-fed mice (186 mg/dl) ($P < 0.04$), whereas NDGA+ALIOS-fed mice had decreased glucose levels (189 mg/dl) ($P < 0.06$). The ALIOS diet did not significantly change insulin levels (0.586 ng/dl vs. 0.610 ng/dl), but insulin decreased with NDGA (0.330 ng/ml, $P < 0.0001$). Likewise, the homeostatic model assessment–estimated insulin resistance (HOMA-IR) was not changed by the ALIOS diet (8.338), but NDGA-treated mice had significantly lower HOMA-IR (3.738, $P < 0.0008$). NDGA-treated mice had significantly lower HOMA-$\beta$ (24.63) compared with chow-fed mice (44.14) ($P < 0.0001$), although there was no significant difference compared with ALIOS-fed mice (35.63).

To assess liver injury, we measured serum ALT and AST activity levels in response to feeding mice the ALIOS diet. As shown in Fig. 3, A and B, serum ALT and AST activity levels were significantly elevated after ALIOS diet consumption compared with chow ($P < 0.0078$ and $P < 0.0199$, respectively). NDGA supplementation lowered ALT and AST levels to values statistically insignificantly different from chow ($P < 0.1276$ and $P < 0.1417$, respectively). Serum triglyceride, glucose, cholesterol, free cholesterol, and NEFA levels were also quantified. Animals fed the ALIOS diet had significantly reduced triglyceride levels ($P < 0.0001$) that were further reduced after NDGA supplementation (Fig. 3C). Triglyceride content was also measured in the liver. As expected, ALIOS-fed animals had significantly elevated liver triglycerides ($P < 0.0196$). Whereas NDGA supplementation ameliorated this
effect, levels between animals fed chow or the NDGA+ALIOS diet, however, were comparable (Fig. 3D). Furthermore, other serum tests demonstrated increased glucose and cholesterol but decreased NEFAs (Table 2) in ALIOS-fed mice. NDGA supplementation did not affect these values.

Next, key proteins of several pathways were measured to explore potential molecular mechanisms responsible for the altered liver triglyceride content after NDGA supplementation. Since NDGA had been shown previously to induce the PPARα pathway, we measured Ppara expression in the liver.
(Fig. 4A). Compared with chow, Pparα mRNA was unaffected by the ALIOS diet. NDGA supplementation induced Pparα > 4-fold; however, due to the large variance within the group, this association was statistically insignificant (P = 0.078). NDGA significantly induced PPARα protein >6-fold compared with the chow (P = 0.0012) and ALIOS (P = 0.0113) groups. ER stress response and apoptosis signaling proteins CHOP and caspase-3 were measured. NDGA significantly

<table>
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<tr>
<th>Metabolite</th>
<th>Chow (n = 15)</th>
<th>ALIOS (n = 16)</th>
<th>NDGA+ALIOS (n = 8)</th>
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<tr>
<td>Glucose (mg/dl)</td>
<td>186.59 ± 8.25</td>
<td>230.52 ± 8.35*</td>
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<td>Total cholesterol (mg/dl)</td>
<td>110.01 ± 3.26</td>
<td>134.82 ± 7.01*</td>
<td>144.40 ± 7.82*</td>
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<tr>
<td>Free cholesterol (mg/dl)</td>
<td>40.19 ± 2.24</td>
<td>43.82 ± 2.48</td>
<td>42.73 ± 1.50</td>
</tr>
<tr>
<td>NEFA (mEq/l)</td>
<td>1.16 ± 0.19</td>
<td>0.66 ± 0.05**</td>
<td>0.49 ± 0.02*</td>
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*p < 0.01 vs. chow; **p < 0.05 vs. chow (analysis of variance).
reduced liver CHOP protein, compared with chow (P = 0.0495) (Fig. 4B), and also ameliorated ALIOS-induced CASP3 elevation (Fig. 4A).

Next, markers from the carnitine palmitoyltransferase (CPT) family, responsible for the β-oxidation of fatty acids, were analyzed. Cpt1a mRNA level was unaffected by the ALIOS diet and/or NDGA supplementation. However, Cpt1c was significantly induced > 10-fold with NDGA treatment, compared with both the chow (P = 0.0005) and ALIOS (P = 0.0008) groups. Cpt2 expression was also moderately elevated with NDGA, but not significantly (Fig. 5A). Since the CPT family of genes was significantly altered with NDGA, we wanted to examine the effect on lipid uptake and adipogenic PPARγ. In line with expectations, chow-fed animals exhibited very low basal levels of PPARγ protein. However, those fed the ALIOS diet had variable induction (1.9- to 7.3-fold), averaging an approximately 4-fold increase compared with chow; due to the variance within the data, it did not reach statistical significance (P = 0.125). NDGA supplementation reduced PPARγ levels to those similar to chow (P < 0.0001) (Fig. 5B). In view of the upregulation seen in PPARγ protein levels and elevated triglyceride levels, we measured the mRNA expression of diacylglycerol acyltransferase Dgat2 and fatty acid synthase Fasn. The ALIOS diet induced Dgat2 expression significantly (6-fold, P = 0.0359); the addition of NDGA reduced it to basal levels. A similar trend was seen for Fasn, but ALIOS-induced elevation was more modest.

Finally, we measured protein level of genes involved in the oxidative stress response, mainly focusing on antioxidant enzymes related to superoxide and hydrogen peroxide metabolism. Expression of antioxidant enzymes did not display a consistent pattern. CAT and GPX1 remained unchanged, whereas GPX4 was selectively induced by NDGA (P < 0.0001) (Fig. 6). SOD2/MnSOD tended to be reduced in the ALIOS group (P = 0.1522), but expression levels were not rescued by NDGA supplementation. Finally, the mRNA expression of peroxiredoxin 3, a mitochondrion-specific H2O2-scavenging antioxidant enzyme, was reduced by feeding the ALIOS diet and was returned to basal levels in response to dietary administration of the ALIOS diet supplemented with NDGA.

Discussion

In this work, we provide information about the impact of dietary administration of NDGA on ALIOS diet–induced metabolic dysregulation in mice. This diet was developed by Tetri et al. (2008) using a dietary formulation based on commonly consumed fast food; the diet consists of high fat containing trans fats and the required amount of high-fructose corn syrup equivalents given in drinking water. Mice consuming the ALIOS diet developed obesity and metabolic derangements, including hepatic steatosis with liver injury, dyslipidemia, insulin resistance, and glucose intolerance, and exhibited increased expression of key enzyme genes involved in hepatic lipogenesis. Feeding mice an ALIOS diet supplemented with NDGA ameliorated metabolic dysregulation by robustly upregulating PPARα protein expression, the master regulator of hepatic fatty acid oxidation. Thus, NDGA increased key PPARα-regulated genes involved in hepatic fatty acid oxidation, secondarily downregulated the expression of lipogenic genes, markers of ER stress and apoptosis, as well as increased expression of major antioxidant enzymes.

By feeding the ALIOS diet alone for 8 weeks, mice became insulin resistant and glucose intolerant. However, feeding mice the ALIOS diet supplemented with NDGA improved insulin sensitivity, but mice remained glucose intolerant. Previously, we demonstrated that feeding Sprague-Dawley...
rains with a high-fructose diet (in which 60% of total calories were provided as fructose) significantly raised plasma insulin levels (Zhang et al., 2015). A short-term (4 days) NDGA treatment attenuated hyperinsulinemia and fasting plasma glucose and NEFA levels. Analysis of HOMA-IR further confirmed that NDGA treatment ameliorated insulin resistance induced by feeding a high-fructose diet (Zhang et al., 2015). In contrast, we reported previously that NDGA treatment improved both glucose intolerance and insulin sensitivity in ob/ob mice (Zhang et al., 2013). At present, we are unable to provide any explanation as to why NDGA treatment did not improve glucose intolerance in mice maintained on the ALIOS diet. It is possible that the lack of effect of NDGA on ALIOS diet–induced glucose intolerance may be related to the presence of high fructose in the diet. Indeed, high fructose content of the diet is known to induce a wide range of genes along with alterations in both hepatic lipid and carbohydrate metabolism (Koo et al., 2008, 2009; Zhang et al., 2016) and, as such, could impact glucose intolerance in a very complex manner. Further studies are needed to elucidate the complex interactions between high-fructose diet–induced gene changes and alterations in hepatic lipid and glucose metabolism and their relevance to fructose-induced glucose intolerance.

Although the ALIOS diet had no effect on Ppara gene expression, a master regulator of mitochondrial/peroxisomal fatty acid β-oxidation genes (Rakhshandehroo et al., 2010), supplementation of the ALIOS diet with NDGA caused a robust induction of PPARα protein. Consistent with the increased expression of PPARα protein, NDGA upregulated the mRNA expression of fatty acid oxidation proteins, Cpt1c and Cpt2, which are target genes of PPARs. Cpt1 and Cpt2, together with carnitine/acylcarnitine translocase, facilitate the transport of long-chain fatty acids into the mitochondrial matrix for their β-oxidation (Bonnefont et al., 2004). The increased expression of Ppara, along with Cpt1 and Cpt2, suggests an increase in mitochondrial fatty acid degradation and energy consumption. In addition, increased expression of PPARα observed in the NDGA+ALIOS-treated mice strongly points out the possibility that enhanced fatty acid oxidation via activation of PPARα and induction of fatty acid oxidation genes is the major mechanism by which NDGA attenuates hepatic steatosis and improves metabolic dysregulation. Although the exact underlying mechanisms are not apparent, the steady-state mRNA levels of Ppara were not significantly impacted by NDGA treatment, but its protein level was robustly induced. One potential mechanism might be that since NDGA serves as a ligand for PPARα (Zhang et al., 2015), its binding to PPARα protein stabilizes the secondary protein structure and shields it from degradation, thus resulting in increased nuclear protein accumulation. Another possibility is that NDGA promotes posttranslational modification(s) of PPARα protein (Burns and Vanden Heuvel, 2007; Berrabah et al., 2011; Wadosky and Willis, 2012), leading to its stabilization and enhanced accumulation in the nucleus.

We saw no overall changes in the protein expression levels of adipogenic transcription factor, PPARγ, between mice fed the chow diet and mice fed the ALIOS diet due to large experimental variation. However, there was a trend showing that ALIOS diet treatment upregulated the expression of PPARγ protein, which is known to promote hepatic lipogenesis under altered pathophysiological conditions (Rogue et al., 2010). Such a potential increase is supported by previous studies showing enhanced expression of PPARγ in the fatty livers of several animal models of obesity and diabetes, including ob/ob and db/db (Memon et al., 2000), A-Zip (Gavriloa et al., 2003), and KKAy mice (Bedoucha et al., 2001), as well as in the livers of obese patients (Pettinelli and Videla, 2011). Similar to PPARγ, the ALIOS diet also induced mRNA expression of key lipogenic genes such as Fasn and Dgat2. Interestingly, coadministration of the ALIOS diet and NDGA significantly reduced the expression of Fasn and two lipogenesis genes. These data suggest that NDGA also ameliorates dyslipidemia by inhibiting PPARγ-induced hepatic lipogenesis. Interestingly, we also found that the ALIOS diet elevated apoptosis signaling protein, caspase 3, without impacting ER stress response protein CHOP levels in the liver. NDGA suppressed liver CHOP protein and also attenuated ALIOS-induced caspase-3 elevation. Speculatively, NDGA suppression of CHOP and caspase-3 proteins suggests that both apoptosis and ER stress signaling pathways may potentially contribute to ALIOS-induced metabolic dysfunction.

Previous studies have shown that feeding a high-fat diet (Tanaka et al., 2008; Feng et al., 2016) or a high-fructose diet (Chess et al., 2008; Francini et al., 2010) to mice is associated with increased oxidative stress and alteration in the levels of antioxidant enzymes. In this study, we observed variable effects of the ALIOS and NDGA+ALIOS diets on the expression of key antioxidant enzymes and proteins. No changes were noted in the expression of CAT and GPX1, although

Fig. 6. Impact of NDGA and the ALIOS diet on antioxidative response. (A) A moderate response was observed across multiple antioxidant enzyme proteins. (B) Levels of Gpx4 were significantly elevated in the NDGA + ALIOS group. Levels of SOD2/MnSOD were significantly downregulated after consumption of the ALIOS diet and did not recover in response to simultaneous administration of the ALIOS diet and NDGA. NDGA restored PRDX3 expression to similar levels seen in the chow group. Chow, n = 4, ALIOS, n = 4, and NDGA + ALIOS, n = 4 (two-way analysis of variance). ***P < 0.0001 vs. chow; ****P < 0.0001 vs. ALIOS. PRDX3, peroxiredoxin 3.
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**References**


Address correspondence to: Dr. Wen-Jun Shen, Division of Endocrinology, Gerontology, and Metabolism, Stanford University, 3801 Miranda Avenue, Building 4, Room C902, Palo Alto, CA 94304: E-mail: wenjun@stanford.edu; Dr. Salman Azhar, GRECC-182B, VA Palo Alto Health Care System, 3801 Miranda Avenue, Palo Alto, CA 94304; E-mail: salman.azhar@va.gov