JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

AGE-Induced Interference of Glucose Uptake and Transport as a Possible Cause of Insulin Resistance in Adipocytes

Chi-Hao Wu,[†] Hsiao-Wen Huang,[†] Shang-Ming Huang,[†] Jer-An Lin,[†] Chi-Tai Yeh,[‡] and Gow-Chin Yen*[,†]

[†]Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan

[‡]Graduate Institute of Clinical Medicine, Taipei Medical University, No. 250, Wu-Hsing Street, Sinyi District, Taipei 11031,Taiwan

ABSTRACT: The purpose of this study was to investigate the distinct roles of advanced glycation end products (AGEs) on insulin-mediated glucose disposal in 3T3-L1 adipocytes and C2C12 skeletal muscle cells. AGE-modified proteins, namely, GO-AGEs, were prepared by incubating bovine serum albumin (BSA) with glyoxal (GO) for 7 days. Glucose utilization rates and the expression of insulin signaling-associated proteins, including Akt, insulin receptor substrate-1, and glucose transporter 4, were determined. GO-AGEs caused insulin resistance (IR) by suppressing insulin-stimulated glucose uptake both in 3T3-L1 adipocytes and C2C12 muscle cells. Interestingly, an unexpected finding was that insulin-stimulated glucose transport in adipocytes was affected by GO-AGEs in a biphasic manner, with an initial steep increase (168%) during the first 8 h of incubation followed by a significantly impaired uptake after extended culture times (24−48 h, $p < 0.05$). Treatment with GO-AGEs for 24 h markedly accelerated lipid droplet formation compared to the BSA control; however, it was blocked by incubation with an anti-RAGE antibody. Our study suggests that GO-AGEs induce an early dramatic elevation of glucose transport in adipocytes that may be related to the activation of insulin signaling; however, subsequent IR may result from increased oxidative stress and proinflammatory TNF-α production.

KEYWORDS: adipocytes, advanced glycation end products, insulin resistance, inflammation, oxidative stress, receptor for AGEs

INTRODUCTION

Protein glycation is the process of Schiff base formation through the condensation of a reducing sugar's carbonyl group with a protein's amine group, which loses one molecule of water. This is followed by the formation of relatively stable intermediates through Amadori rearrangement (Amadori products). If these glycated proteins continue to rearrange, they eventually form irreversible advanced glycation end products (AGEs) through dehydration, cyclization, oxidization, or polymerization. The known AGEs are usually produced by proteins with longer half-lives. Some of the proteins are fluorescent and brown in color. The inter- or intramolecular amine groups of a protein can cross-link through covalent bonds so that the cross-linked protein is resistant to proteinases and substantially protected from being metabolized. These results suggest a potential role for AGE-modification in normal aging as well as in age-enhanced disease processes.[1,2]

Glycation and glucose auto-oxidation can produce active carbonyl products with dicarbonyl groups such as glyoxal (GO), methylglyoxal, and 3-deoxyglucosone.[3] Reactive carbonyl species (RCS) have high reactivities, similar to reactive oxygen species (ROS), and they have much higher protein glycation rates than glucose. Their accumulation is toxic to cells and tissues due to physiological carbonyl stress. Therefore, RCS and ROS are commonly considered the main factors that cause aging, diabetes mellitus, and chronic inflammation.[1] The dicarbonyl groups easily interact with Arg or Lys amino acids at the N-termini of proteins, causing inter- or intramolecular protein cross-linking and polymerization to form AGEs,[4] including *N*-ε-carboxymethyllysine,[5] *N*-ε-carboxyethyllysine,[6] glyoxal-lysine dimer (GOLD), and methylglyoxal-lysine dimer (MOLD). In cataract patients, the presence of GOLD and MOLD is direct evidence of crystallin glycation,[3] while CML and CEL can accumulate in skin collagen during aging.[7]

The long-term dysregulation of blood sugar levels in diabetic patients provides the best conditions for studying protein glycation.[8] Many studies have shown that AGEs are the major cause of diabetes complications such as atherosclerotic coronary heart disease, neuropathy, kidney diseases, and cataracts.[1,2,8,9] Recent studies have delineated the possible relationship between AGEs and the development of insulin resistance (IR);[10] moreover, AGE-restricted diets may be helpful for improving glucose and insulin tolerance.[11] Among the major tissues on which insulin acts, skeletal muscle accounts for 40−50% of body weight and 75% of glucose metabolism regulated by insulin.[12] Insulin acts on skeletal muscle to promote glycolysis, which accelerates glucose usage, helps metabolize glucose to produce energy, and promotes conversion of glucose into glycogen, which is preserved in muscles as an energy reserve. The major function of adipose tissue is to store fat, which is stimulated by insulin to suppress lipolysis or increase triglyceride synthesis. Adipose tissue is also involved in many physiological and pathological processes. Recent studies have investigated the secretion of hormones from adipose tissue, and they have shown that these hormones further enhance the responsiveness of other tissues to insulin. Miele et al.[13] and Cassese et al.[14] pointed out that AGEs can induce insulin resistance in muscle cells by interfering with insulin signaling pathways. Furthermore, oxidative stress and inflammation caused by the AGE−RAGE (receptors for AGEs) interaction can also trigger insulin resistance.[15] Additionally, the AGE precursor, methylglyoxal, can glycate insulin to form methylglyoxal−insulin adducts and

Received: March 31, 2011
Accepted: June 8, 2011
Revised: June 4, 2011
Published: June 08, 2011

in turn change the insulin structure, thereby abrogating its physiological function.[16,17]

Clinical studies have shown that patients with type 2 diabetes (T2D) usually consume excessive amounts of fat from food, resulting in elevated levels of plasma free fatty acids. These high free fatty acid levels can increase physiological oxidative stress, inflammation, or macrophage infiltration into adipose tissues and further promote insulin resistance. Studies on lipoatrophic diabetic (A-ZIP/F-1) rats, which have a severe deficit of adipocytes, have shown that the amelioration of insulin resistance by the glucose-lowering thiazolidinediones requires the involvement of adipocytes, thereby suggesting an important role for adipocytes in the process of insulin resistance.[18]

Insulin resistance is one of the main contributory causes of T2D, which is usually accompanied by obesity. Adults lose their lean tissues gradually, while adipose tissues increase with aging; however, few reports are available on the relationship among AGEs, obesity, and insulin resistance. To understand the potential mechanisms by which the AGE−RAGE interaction leads to insulin resistance, we used cultured muscle cells and adipocytes to probe the effects of AGEs on glucose uptake.

MATERIALS AND METHODS

Materials. Glyoxal (GO), fatty acid-free bovine serum albumin (BSA), insulin, and Oil Red O were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Penicillin−streptomycin (PS) solution, fetal bovine serum (FBS), and Dulbecco's modified Eagle's medium (DMEM) were purchased from Invitrogen (Carlsbad, CA, USA). 2-^{14}C-Deoxy-D-glucose (2-DG) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA). 6D12 antibody was purchased from Wako Bioproducts (Clone No. 6D12; Richmond, VA, USA). All the chemicals and solvents used were of analytical grade.

Preparation of Glyoxal-Derived AGEs (GO-AGEs). AGE-modified serum albumins (GO-AGEs) were prepared by incubation of bovine serum albumin (BSA) with GO, according to the method described in Takeuchi et al.[19] Briefly, BSA (0.4 mg/mL) was incubated with 0.1 M glyoxal in 0.1 M sodium phosphate buffer (pH 7.4) at 37 °C for 7 days. At the end of the incubation period, the glycation products were extensively dialyzed by Cellu Sep T-series dialysis membranes (Membrane Filtration Products, Inc., Seguin, TX, USA). Free, unbound substances were removed by dialysis, and endotoxins were removed using a PD-10 column (Pierce, Rockford, IL, USA), according to the manufacturer's instructions. Finally, the reaction mixture was passed through a 0.22 μm sterile filter to obtain the glycated products.

Cell Culture. The murine preadipocyte cell line (3T3-L1) was obtained from the Bioresource Collection and Research Center (BCRC 60159, Food Industry Research and Development Institute, Hsin Chu, Taiwan) and cultured in DMEM supplemented with 10% FBS and 1% streptomycin/penicillin (100 units/mL and 100 μg/mL, respectively) at 37 °C in 5% CO_2. Cells were induced to differentiate into adipocytes 24 h after reaching confluency by changing the medium to DMEM supplemented with adipogenic agents (0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone and 1 μM insulin), 10% FBS and 1% streptomycin/penicillin (100 units/mL and 100 μg/mL, respectively) for 4 days. Thereafter, the cells were maintained in DMEM medium supplemented with 1 nM insulin, 10% FBS and 1% streptomycin/penicillin (100 units/mL and 100 μg/mL, respectively) for 4 days. The medium was changed every 2 days until the cells were used. The cells were harvested on the eighth day after initiation of differentiation.

The mouse myoblast cell line (C2C12) was obtained from the Bioresource Collection and Research Center and cultured in DMEM supplemented with 10% FBS and 1% streptomycin/penicillin (100 units/mL and 100 μg/mL, respectively) at 37 °C in 5% CO_2. Cells were induced to differentiate 24 h after reaching confluency by changing the medium to DMEM supplemented with 2% horse serum and 1% streptomycin/penicillin (100 units/mL and 100 μg/mL, respectively). Cells were harvested 4−5 days after differentiation.

Glucose Uptake. Differentiated 3T3-L1 adipocytes were treated with GO-AGEs and subsequently incubated with 100 nM insulin for 30 min. After incubation, the cells were washed with PBS and kept in prewarmed HBSS solution. 2-^{14}C-Deoxyglucose (2-DG) was dispensed into each well for a final concentration of 0.1 μCi/mL HBSS. The cells were incubated for 20 min, and the reaction was terminated by three washes with ice-cold PBS. Cell-associated radioactivity was determined by lysing the cells with 1 N NaOH followed by liquid scintillation counting (Tricarb 2800TR Liquid Scintillation Analyzer, PerkinElmer Life and Analytical Sciences, Waltham, MA, USA).

Oil Red O Staining of 3T3-L1 Adipocytes. Intracellular lipid accumulation was measured using Oil Red O. The Oil Red O working solution was prepared as described previously.[20] Differentiated 3T3-L1 cells were treated with GO-AGEs followed by incubation with 100 nM insulin for 30 min. After this period, cells were washed with PBS and then fixed with 10% neutral formalin for at least 20 min at room temperature. After the 10% neutral formalin was removed, 100% propylene glycol was added to each well for 3 min. Cells were decolorized with 60% propylene glycol after 1 h staining with the Oil Red O working solution and exhaustive washing with PBS. Stained cells were observed using an IX71 microscope (Olympus, Osaka, Japan), and images were captured by ImagePro (Media Cybernetics, Bethesda, MD, USA). After observation, the Oil Red O-stained material (OROSM) was extracted from cells by RIPA lysis buffer (Upstate, Billerica, MA, USA), and then measured by spectrophotometer at 510 nm. The extent of OROSM was expressed as multiples of BSA control.

Intracellular ROS Content Measurement. Intracellular ROS levels were estimated using 2,7-dichlorofluorescin diacetate (DCF-DA). Differentiated 3T3-L1 cells were treated with GO-AGEs followed by incubation with 100 nM insulin for 30 min. At the end of the incubation period, cells were collected, resuspended in PBS containing 10 μM DCF-DA, and incubated for 30 min. Cells were washed twice with PBS and observed using an IX71 microscope (Olympus, Osaka, Japan). Images were captured using ImagePro (Media Cybernetics, Bethesda, MD, USA).

Western Blot Analysis. Differentiated 3T3-L1 cells were treated with GO-AGEs followed by incubation with 100 nM insulin for 30 min. After incubation, the cells were dissolved in RIPA lysis buffer (Upstate, Billerica, MA, USA) and boiled at 100 °C for 5 min with 5× protein loading dye (2% SDS, 0.1% bromophenol blue, 2.5% glycerol, 60 mM Tris-HCl (pH 6.8) and 14 mM 2-mercaptoethanol). The samples were then subjected to SDS−polyacrylamide gel electrophoresis. Proteins in the gel were transferred onto an Immobilon-P PVDF membrane (Millipore, Billerica, MA, USA) and incubated with a primary antibody (GLUT4, IRS, p-IRS1^{Ser307}, Akt and p-Akt; Cell Signaling Technology, Beverly, MA, USA) at a 1:1000 dilution. Finally, the samples were incubated with a horseradish peroxide-linked secondary antibody (1:8000 dilution), visualized using the ChemiLucent ECL Detection System (Millipore, Billerica, MA, USA), and detected by the Biospectrum AC Imaging System (UVP, Upland, CA, USA). Expression of the proteins of interest was normalized to expression of β-actin. The intensity of the chemiluminescence signal was quantified using UVP VisionWorksLS Image Acquisition and Analysis Software (UVP, Upland, CA, USA).

Measurement of NF-κB Activity. Differentiated 3T3-L1 cells were treated with GO-AGEs followed by incubation with 100 nM insulin for 30 min. After incubation, the cells were dissolved in ice-cold lysis buffer. The NFκB activity was determined with AlphaScreen SureFire NFκB p65 (S536) assay kit according to the manufacturer's instructions

(PerkinElmer, Waltham, MA). Finally, all data were analyzed on an AlphaScreen compatible plate reader.

Cytokine ELISA Assays. 3T3-L1 cells were incubated with or without GO-AGEs (0.1 mg/mL) in six-well tissue culture plates for 24 h. The supernatant (with conditioned medium) was then harvested and assayed for TNF-α secretion using a specific ELISA kit, according to the manufacturer's instructions.

Statistical Analysis. All data are expressed as mean ± SD. ANOVA was used to evaluate the differences between multiple groups. If significance was observed between the groups, Duncan's test was used to compare the means of two specific groups. A $p < 0.05$ was considered to be significant.

RESULTS

Bovine serum albumin (BSA) was used as a target protein, and the reactive carbonyl species, glyoxal, was used to glycate BSA. Glyoxal-derived AGEs (GO-AGEs) were prepared using continuous dialysis, and endotoxins were removed using a PD-10 column. Immunoblot analysis indicated that the GO-AGEs were recognized by the anti-AGE antibody, 6D12 (Figure 1A). Several studies have used the 6D12 antibody to identify AGE-modified proteins in human tissues, indicating its potential usefulness for immunohistochemical identification and biochemical quantification of AGE-modified proteins.[21]

Using an excitation wavelength of 370 nm and an emission wavelength of 440 nm, we found that GO-AGEs displayed the characteristic AGE-specific fluorescence (Figure 1B, $p < 0.05$). Furthermore, GO-AGEs promoted the expression of RAGE in 3T3-L1 adipocytes (Figure 1C) and increased nuclear translocation of transcription factor NF-κB p-p65 (Figure 1D). The addition of the anti-RAGE antibody (70 μg/mL) repressed the activation of RAGE and NF-κB. This demonstrates that GO-AGEs exhibit the features of AGEs and can activate canonical AGE−RAGE signaling through interaction with the membrane receptor RAGE.

3T3-L1 adipocytes and C2C12 muscle cells were cultured with GO-AGEs (0.4 mg/mL) for different amounts of time, and their effects on insulin-stimulated glucose uptake were analyzed by 2-deoxyglucose (2-DG) assays. Compared with the BSA-stimulated control adipocytes, GO-AGEs induced a 23−62% increase in insulin-stimulated glucose uptake after 12 h of culture ($p < 0.05$); however, after 48 h, GO-AGEs repressed glucose uptake by 38% (Figure 2A, upper panel). In addition, after 48 h of culture, GO-AGEs significantly suppressed 2-DG uptake in C2C12 muscle cells (Figure 2A, bottom panel, $p < 0.05$). Pretreatment of adipocytes with anti-RAGE antibodies for 0.5 h suppressed GO-AGE−promoted glucose uptake within 8 h (Figure 2B). Figure 2C shows that the GO-AGEs induced adipogenesis in 3T3-L1 cells after 24 h in culture; however, the addition of anti-RAGE antibodies suppressed this adipogenic effect (Figure 2D). Immunoblotting analysis demonstrated that GO-AGEs suppressed insulin receptor substrate (IRS) phosphorylation on Ser 307 (Figure 3, upper panel) and increased the levels of downstream phospho-Akt (Figure 3, middle panel) and glucose transporter 4 (GLUT4) in 3T3-L1 cells (Figure 3, bottom panel). This indicates that GO-AGEs can activate downstream insulin signaling pathways and promote the phosphorylation of Akt and expression of GLUT4 by suppressing p-IRS^{Ser307}, further promoting glucose uptake in 3T3-L1 adipocytes; moreover, DCF staining and ELISAs specific for proinflammatory cytokines showed that AGEs significantly induced the generation of ROS in 3T3-L1 adipocytes (Figure 4A) and the secretion of TNF-α (Figure 4B) after 12 h of culture, both of which can lead to insulin resistance.[15]

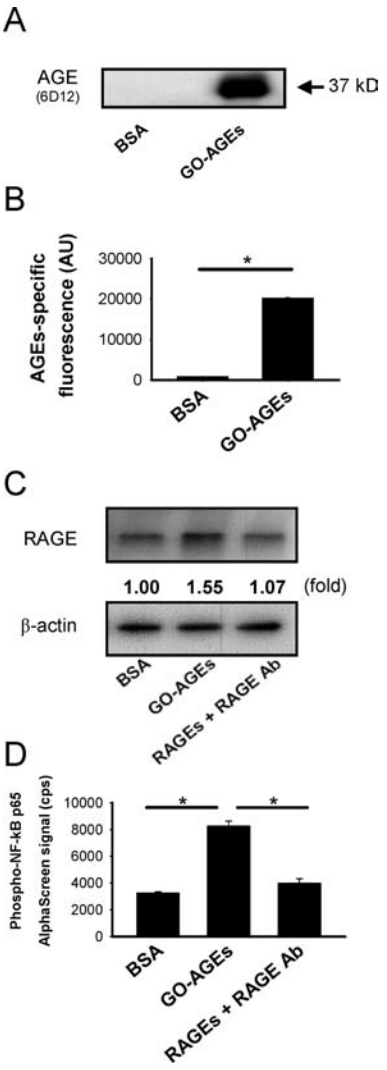

Figure 1. AGE-related characteristics of GO-AGEs. The bovine glycated and nonglycated albumin preparations were tested for (A) AGE formation, as determined by immunoblot analysis using an anti-AGE monoclonal antibody (6D12); (B) fluorescent AGEs, which were measured by fluorescence assays (from 360 to 600 nm) with an excitation wavelength of 370 nm; (C) the activation of RAGE; and (D) phosphorylation of NF-κB p65 in GO-AGE-treated 3T3-L1 adipocytes, which was determined by immunoblot analysis. A neutralizing RAGE antibody (70 μg/mL) was used as positive control. Data shown are mean ± SD from three independent experiments. *$p < 0.05$. Ab, antibody; AU, arbitrary unit; BSA, bovine serum albumin; GO-AGEs, glyoxal-glycated BSA.

DISCUSSION

The reaction of the amino groups in proteins with glucose leads to the formation of advanced glycation end products (AGEs) via products such as Schiff base and Amadori rearrangement products. Clinical studies have demonstrated that AGE-modified proteins are present in several types of human tissue, including atherosclerotic lesions of arterial walls, diabetic neuropathy, diabetic retinopathy, peripheral nerves of β2-microglobulin-forming amyloid fibrils in patients with hemodialysis-related

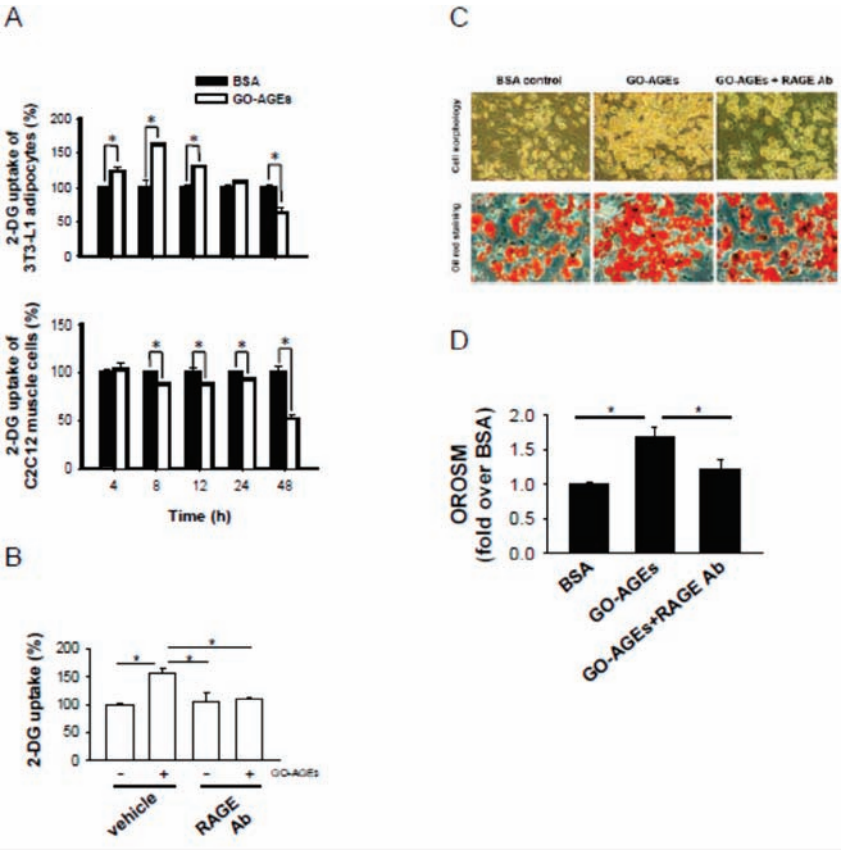

Figure 2. Effects of GO-AGE treatment on insulin-stimulated glucose uptake and intracellular lipid accumulation. (A) 3T3-L1 adipocytes and C2C12 muscle cells were incubated with GO-AGEs or nonglycated BSA at 0.1 mg/mL for indicated time, followed by measurement of ^{14}C-labeled 2-deoxyglucose (2-DG) uptake. (B) GO-AGE (0.1 mg/mL)-stimulated 2-DG uptake in 3T3-L1 adipocytes was decreased by anti-RAGE Ab treatment. (C) 3T3-L1 adipocytes were subjected to treatment with GO-AGEs for 24 h with or without pretreatment with anti-RAGE Ab (70 μg/mL), as described above; cells were stained with Oil Red O. Images in the upper panel show cell morphology without staining, while images in the lower panel show cells stained with Oil Red O. (D) The bar graph shows Oil Red O-stained material (OROSM) extracted from cells, as measured by spectrophotometer at 510 nm. The extent of OROSM was expressed as multiples of BSA control. Data shown are the mean ± SD from three independent experiments. $^*p < 0.05$.

amyloidosis, senile plaques of patients with Alzheimer's disease, skin elastin in actinic elastosis, and ceriod/lipofuscin deposits.[22−24] Bian et al.[25] pointed out that the concentration of glycated human serum albumin ranges from 0.4 mg/mL (healthy individuals) to 1 mg/mL (T2D patients). This study revealed that GO-AGEs at a concentration of 0.4 mg/mL could suppress insulin-stimulated glucose uptake in 3T3-L1 adipocytes and C2C12 muscle cells, thereby causing insulin resistance (Figure 2). Interestingly, AGEs may induce different effects on insulin-stimulated glucose uptake depending on their length of incubation. After a short incubation period (≤12 h), AGEs significantly promote glucose uptake and accelerate intracellular lipid droplet formation; however, resistance develops when the incubation time is extended to 24−48 h. Although AGEs stimulate insulin signaling in the early phase (≤12 h), our data indicate that insulin resistance develops in the later phase due to oxidative stress and the production of the proinflammatory cytokine, TNF-α.

Obesity is a critical risk factor for the development of insulin resistance.[26] Among T2D patients, there is a high percentage of cases with obesity-linked insulin resistance, and this percentage is increasing because of diet changes in recent years.[27] Obesity-linked insulin resistance may occur in liver, muscle, and adipose tissue; however, it is unclear which tissue is first affected. Usually, insulin resistance appears earliest in liver and muscle, followed by adipose tissue.[28] Interestingly, these clinical phenomena are similar to our present findings.

Insulin resistance in liver or muscle not only is caused by obesity but also can cause it. Glucose uptake in muscle is 10 times greater than in adipose tissue. Previous studies have shown that adipocytes may enhance glucose uptake and promote fat accumulation due to glucose malabsorption in muscle under conditions of insulin resistance.[29] Our study suggests that when adipocytes are exposed to AGEs, uptake of glucose is enhanced and intracellular triacylglycerol synthesis is accelerated through the activation of insulin signaling. It remains to be seen whether the findings from AGE-treated cultured adipocytes are applicable to humans. Data from clinical trials have shown that the human body can absorb 10% of AGEs from food; of the amount absorbed, one-third will be retained in the body and the other two-thirds will be excreted in urine.[30,31] Case studies of 18 obese children/adolescents have found that both insulin sensitivity and plasma AGE levels are lower compared to lean children/adolescents, and the difference might be related to the accumulation of AGEs in adipose tissues.[31] Schalkwijk et al.[32] also showed that plasma AGE levels in obese patients are lower than those in normal controls, but the accumulation of AGEs in visceral adipose tissue is significantly higher than that in normal controls. Injection of AGEs into *db/db* mice also

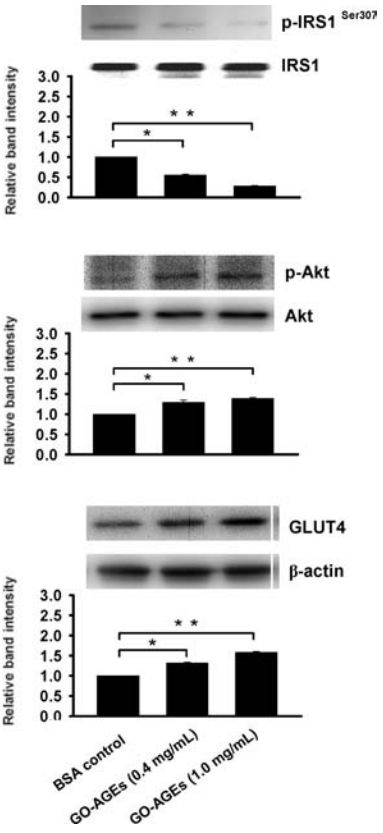

Figure 3. Effects of GO-AGEs on expression of insulin signaling and GLUT4 in 3T3-L1 adipocytes. Cells were treated with or without BSA or GO-AGEs for 24 h followed by insulin (100 nM) treatment for 30 min. Protein expression was quantified and expressed as multiples of BSA control. The results shown are representative of three independent experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$.

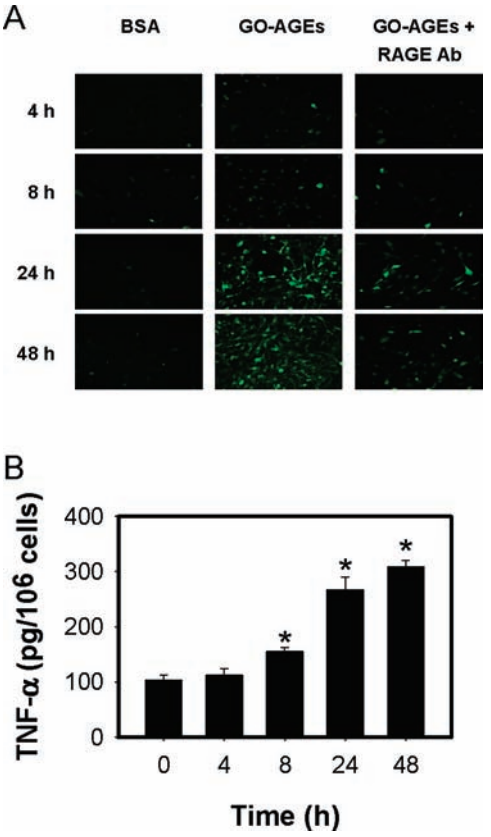

Figure 4. Induction of cellular oxidative stress and inflammation by GO-AGEs. (A) Effect of GO-AGEs on the intracellular ROS levels in 3T3-L1 adipocytes. (B) Cells treated under the same conditions were assayed for TNF-α production by ELISA as described in Materials and Methods. All appropriate controls and standards as specified by the manufacturer were used, and the data are expressed as picograms of TNF-α secretion per million cells. The results are expressed as mean ± SD, $n = 3$. $^{*}p < 0.05$.

results in accumulation of AGEs in adipose tissues; furthermore, with the same amount of food intake, both body weight and visceral fat weight of mice fed a low-AGE diet were found to be lower than those of mice in the high-AGE diet group.[33] This indicates that high-AGE diets facilitate the accumulation of fat in tissues.[10] In summary, previous studies have indicated that AGE accumulation in adipose tissues is frequently seen in obese patients, and our present study further demonstrates that AGE accumulation may be related to increased glucose uptake and triacylglycerol synthesis by adipocytes.

Adipose tissue in obese patients is usually characterized by chronic inflammation, which causes the secretion of proinflammatory factors (such as TNF-α and IL-6) and subsequently induces insulin resistance.[34] For example, TNF-α is secreted after the enlargement of adipocytes. Additionally, TNF-α can stimulate muscle cells and adipocytes to increase serine/threonine phosphorylation of IRS1;[35,36] decrease the expression of GLUT4, insulin receptor, IRSs, and peroxisome proliferator-activated receptor (PPAR) in adipocytes; increase the release of free fatty acids from adipocytes; and promote insulin resistance.[37] In addition, some studies have shown that the failure to control blood sugar levels in T2D patients may lead to ROS elevation, NF-κB activation, and expression of genes involved in inflammation, obesity, and insulin resistance.[37−39] According to reports from Miele et al.[13] and Unoki et al.,[15] AGEs can induce insulin resistance in muscle cells and adipocytes through the activation of protein kinase C, which interferes with IRS signaling and induces oxidative stress and inflammation. We noted similar phenomena by demonstrating that the generation of intracellular ROS and TNF-α by 3T3-L1 adipocytes increased significantly after 12 h of treatment with AGEs.

In conclusion, we showed that AGEs have different effects on glucose uptake in adipocytes at different time points. This suggests that the promotion of glucose uptake and triacylglycerol synthesis induced by AGEs at the initial phase may be related to the insulin resistance commonly seen in patients with type 2 diabetes.

AUTHOR INFORMATION

Corresponding Author

*Tel: 886-4-2287-9755. Fax: 886-4-2285-4378. E-mail: gcyen@nchu.edu.tw.

Funding Sources

This research work was supported by the National Science Council, NSC99-2628-B005-002-MY3, Taiwan, Republic of China.

ABBREVIATIONS USED

Ab, antibody; AU, arbitrary unit; AGEs, advanced glycation end products; BSA, bovine serum albumin; DCF-DA, 2′,7′-dichlorodihydrofluorescein diacetate; 2-DG, 2-^{14}C-deoxy-D-glucose; GLUT4, glucose

transporter 4; GO, glyoxal; GOLD, glyoxal-lysine dimer; MOLD, methylglyoxal-lysine dimer; IR, insulin resistance; NF-κB, nuclear factor-κB; OROSM, oil red O-stained material; RAGE, receptor for AGEs; RCS, reactive carbonyl species; ROS, reactive oxygen species; T2D, type 2 diabetes; TNF-α, tumor necrosis factor-alpha

REFERENCES

(1) Brownlee, M. Advanced protein glycosylation in diabetes and aging. *Annu. Rev. Med.* **1995**, *46*, 223–234.

(2) Singh, R.; Barden, A.; Mori, T.; Beillin, L. Advanced glycation end-productions: a review. *Diabetologia* **2001**, *44*, 129–146.

(3) Thornalley, P. J.; Langborg, A.; Minhas, H. S. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem. J.* **1999**, *344*, 109–116.

(4) Chellan, P.; Nagaraj, R. H. Protein crosslinking by the Maillard reaction: dicarbonyl-derived imidazolium crosslinks in aging and diabetes. *Arch. Biochem. Biophys.* **1999**, *368*, 98–104.

(5) Ahmed, M. U.; Thorpe, S. R.; Baynes, J. W. Identification of N-epsilon-carboxymethyllysine as a degradation product of fructoselysine in glycated protein. *J. Biol. Chem.* **1986**, *261*, 4889–4894.

(6) Krook, M.; Ghosh, D.; Strömberg, R.; Carlquist, M.; Jörnvall, H. Carboxyethyllysine in a protein: native carbonyl reductase/NADP(+)-dependent prostaglandin dehydrogenase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 502–506.

(7) Dunn, J. A.; McCance, D. R.; Thorpe, S. R.; Lyons, T. J.; Baynes, J. W. Age-dependent accumulation of N epsilon-(carboxymethyl)lysine and N epsilon-(carboxymethyl)hydroxylysine in human skin collagen. *Biochemistry* **1991**, *30*, 1205–1210.

(8) Brownlee, M. Biochemistry and molecular cell biology of diabetic complications. *Nature* **2001**, *414*, 813–820.

(9) Ogawa, S.; Nakayama, K.; Nakayama, M.; Mori, T.; Matsushima, M.; Okamura, M.; Senda, M.; Nako, K.; Miyata, T.; Ito, S. Methylglyoxal is a predictor in type 2 diabetic patients of intima-media thickening and elevation of blood pressure. *Hypertension* **2010**, *56*, 471–476.

(10) Unoki, H.; Yamagishi, S. Advanced glycation end products and insulin resistance. *Curr. Pharm. Des.* **2008**, *14*, 987–989.

(11) Watson, K. E.; Peters Harmel, A. L.; Matson, G. Atherosclerosis in type 2 diabetes mellitus: the role of insulin resistance. *Cardiovasc. Pharmacol. Ther.* **2003**, *8*, 253–260.

(12) Wei, Y.; Chen, K.; Whaley-Connell, A. T.; Stump, C. S.; Ibdah, J. A.; Sowers, J. R. Skeletal muscle insulin resistance: role of inflammatort cytokines and reactive oxygen species. *Am. J. Physiol.* **2008**, *294*, R673–R680.

(13) Miele, C.; Riboulet, A.; Maitan, M. A.; Oriente, F.; Romano, C.; Formisano, P.; Giudicelli, J.; Beguinot, F.; Van Obberghen, E. Human glycated albumin affects glucose metabolism in L6 skeletal muscle cells by impairing insulin-induced insulin receptor substrate (IRS) signaling through a protein kinase C alpha-mediated mechanism. *J. Biol. Chem.* **2003**, *278*, 47376–47387.

(14) Cassese, A.; Esposito, I.; Fiory, F.; Barbagallo, A. P.; Paturzo, F.; Mirra, P.; Ulianich, L.; Giacco, F.; Iadicicco, C.; Lombardi, A.; Oriente, F.; Van Obberghen, E.; Beguinot, F.; Formisano, P.; Miele, C. In skeletal muscle advanced glycation end products (AGEs) inhibit insulin action and induce the formation of multimolecular complexes including the receptor for AGEs. *J. Biol. Chem.* **2008**, *283*, 36088–36099.

(15) Unoki, H.; Bujo, H.; Yamagishi, S.; Takeuchi, M.; Imaizumi, T.; Saito, Y. Advanced glycation end products attenuate cellular insulin sensitivity by increasing the generation of intracellular reactive oxygen species in adipocytes. *Diabetes Res. Clin. Pract.* **2007**, *76*, 236–244.

(16) Riboulet-Chavey, A.; Pierron, A.; Durand, I.; Murdaca, J.; Giudicelli, J.; Van Obberghen, E. Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. *Diabetes* **2006**, *55*, 1289–1299.

(17) Jia, X.; Wu, L. Accumulation of endogenous methylglyoxal impaired insulin signaling in adipose tissue of fructose-fed rats. *Mol. Cell. Biochem.* **2007**, *306*, 133–139.

(18) Chao, L.; Marcus-Samuels, B.; Mason, M. M.; Moitra, J.; Vinson, C.; Arioglu, E.; Gavrilova, O.; Reitman, M. L. Adipose tissue is required for the antidiabetic, but not for the hypolipidemic, effect of thiazolidinediones. *J. Clin. Invest.* **2000**, *106*, 1221–1228.

(19) Takeuchi, M.; Makita, Z.; Bucala, R.; Suzuki, T.; Koike, T.; Kameda, Y. Immunological evidence that non-carboxymethyllysine advanced glycation end-products are produced from short chain sugars and dicarbonyl compounds in vivo. *Mol. Med.* **2000**, *6*, 114–125.

(20) Hsu, C. L.; Yen, G. C. Effects of flavonoids and phenolic acids on the inhibition of adipogenesis in 3T3-L1 adipocytes. *J. Agric. Food Chem.* **2007**, *55*, 8404–8410.

(21) Howes, K. A.; Liu, Y.; Dunaief, J. L.; Mailam, A.; Frederick, J. M.; Marks, A.; Baebr, W. Receptor for Advanced Glycation End Products and Age-Related Macular Degeneration. *Invest. Ophthalmol. Vis. Sci.* **2004**, *45*, 3713–3720.

(22) Goh, S. Y.; Cooper, M. E. Clinical review: The role of advanced glycation end products in progression and complications of diabetes. *J. Clin. Endocrinol. Metab.* **2008**, *93*, 1143–1152.

(23) Peppa, M.; Raptis, S. A. Advanced glycation end products and cardiovascular disease. *Curr. Diabetes Rev.* **2008**, *4*, 92–100.

(24) Takeuchi, M.; Yamagishi, S. Possible involvement of advanced glycation end-products (AGEs) in the pathogenesis of Alzheimer's disease. *Curr. Pharm. Des.* **2008**, *14*, 973–978.

(25) Bian, Z. M.; Elner, V. M.; Yoshida, A.; Kunkel, S. L.; Elner, S. G. Signaling pathways for glycated human serum albumin-induced IL-8 and MCP-1 secretion in human RPE cells. *Invest. Ophthalmol. Vis. Sci.* **2001**, *42*, 1660–1668.

(26) Zeyda, M.; Stulnig, T. M. Obesity, inflammation, and insulin resistance: a mini-review. *Gerontology* **2009**, *55*, 379–386.

(27) Kelly, G. S. Insulin resistance: lifestyle and nutritional interventions. *Altern. Med. Rev.* **2000**, *5*, 109–132.

(28) Kovacs, P.; Stumvoll, M. Fatty acids and insulin resistance in muscle and liver. *Best Pract. Res. Clin. Endocrinol. Metab.* **2005**, *19*, 625–635.

(29) Koschinsky, T.; He, C. J.; Mitsuhashi, T.; Bucala, R.; Liu, C.; Buenting, C.; Heitmann, K.; Vlassara, H. Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6474–6799.

(30) He, C.; Sabol, J.; Mitsuhashi, T.; Vlassara, H. Dietary glycotoxins: inhibition of reactive products by aminoguanidine facilitates renal clearance and reduces tissue sequestration. *Diabetes* **1999**, *48*, 1308–1315.

(31) Sebeková, K.; Somoza, V.; Jarcusková, M.; Heidland, A.; Podracká, L. Plasma advanced glycation end products are decreased in obese children compared with lean controls. *Int. J. Pediatr. Obes.* **2009**, *4*, 112–118.

(32) Schalkwijk, C.; Gaens, K.; Zandvoort, M. V.; Niessen, H.; Greve, J. W.; Rensen, S.; Buurman, W.; Stehouwer, C. N{epsilon}-(carboxymethyl)-lysine trapping in adipose tissue: implications for obesity-associated changes in adipokine Expression. *Circulation* **2009**, *120*, 518–519.

(33) Hofmann, S. M.; Dong, H. J.; Li, Z.; Cai, W.; Altomonte, J.; Thung, S. N.; Zeng, F.; Fisher, E. A.; Vlassara, H. Improved insulin sensitivity is associated with restricted intake of dietary glycoxidation products in the db/db mouse. *Diabetes* **2002**, *51*, 2082–2089.

(34) Bastard, J. P.; Maachi, M.; Lagathu, C.; Kim, M. J.; Caron, M.; Vidal, H.; Capeau, J.; Feve, B. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur. Cytokine Network* **2006**, *17*, 4–12.

(35) Hotamisligil, G. S.; Peraldi, P.; Budavari, A.; Ellis, R.; White, M. F.; Spiegelman, B. M. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* **1996**, *271*, 665–668.

(36) Weigert, C.; Hennige, A. M.; Brischmann, T.; Beck, A.; Moeschel, K.; Schaüble, M.; Brodbeck, K.; Häring, H. U.; Schleicher, E. D.; Lehmann, R. The phosphorylation of Ser318 of insulin receptor substrate 1 is not per se inhibitory in skeletal muscle cells but is necessary to trigger the attenuation of the insulin-stimulated signal. *J. Biol. Chem.* **2005**, *280*, 37393–37399.

(37) Muller, D. N.; Dechend, R.; Mervaala, E. M.; Park, J. K.; Schmidt, F.; Fiebeler, A.; Theuer, J.; Breu, V.; Ganten, D.; Haller, H.;

Luft, F. C. NF-kappaB inhibition ameliorates angiotensin II-induced inflammatory damage in rats. *Hypertension* **2000**, *35*, 193–201.

(38) Han, Y.; Runge, M. S.; Brasier, A. R. Angiotensin II induces interleukin-6 transcription in vascular smooth muscle cells through pleiotropic activation of nuclear factor-kappa B transcription factors. *Circ. Res.* **1999**, *84*, 695–703.

(39) Nathan, C. Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. *J. Clin. Invest.* **2003**, *111*, 769–778.