Free Rad. Res., Vol. 34, pp. 337-347
Reprints available directly from the publisher
Photocopying permitted by license only

Effect of Obesity and Troglitazone on Expression of Two Glutathione Peroxidases: Cellular and Extracellular Types in Serum, Kidney and Adipose Tissue

KOHTARO ASAYAMA[a,b,1], TAKAYA NAKANE[b], KAZUSHIGE DOBASHI[b], KOJI KODERA[b], HIDEMASA HAYASHIBE[b], NORIHIKO UCHIDA[b] and SHINPEI NAKAZAWA[b]

[a]*Department of Pediatrics, School of Medicine, University of Occupational and Environmental Health, Japan, 1–1 Iseigaoka Yahatanishi-ku, Kitakyushu 807–8555, Japan and* [b]*Department of Pediatrics, Yamanashi Medical University, 1110 Shimokato, Tamahocho, Nakakoma-gun, Yamanashi 409–3898, Japan*

Accepted for publication by Prof. N. Tanaguchi

(Received 23 March 2000; In revised form 18 August 2000)

To determine the effect of obesity on expression of cellular- (C-) and extracellular (EC-) glutathione peroxidase (GPX) in serum, kidney and adipose tissue, we measured GPX in serum, kidneys and adipose tissue of the obese Otsuka-Long-Evans-Tokushima Fatty (OLETF) rat and its lean counterpart (LETO). We also investigated the effect of troglitazone. Five each of OLETF and LETO rats were fed diet with or without 0.2% troglitazone for 10 days. Final body weight, kidney weight, blood glucose and serum tumor necrosis factor-α (TNF-α) level were higher in OLETF rats than in LETO rats. Serum and kidney GPX activities were higher, but adipose tissue GPX activity was lower, in OLETF rats than in LETO rats. Troglitazone treatment decreased adipose tissue GPX activity and abolished overproduction of TNF-α in OLETF rats. Immunoblot analysis, for the first time, revealed that both obesity and troglitazone suppressed the protein signals for C-GPX and EC-GPX in adipose tissue. Serum protein carbonyl groups were increased in OLETF rats and troglitazone completely blocked this increase. Increased serum GPX activity in obese rat was due to the increased secretion of EC-GPX from the kidney. Troglitazone protected against the enhanced oxidative stress induced by obesity independently of the serum GPX concentration.

Keywords: glutathione peroxidase, gene expression, adipose tissue, free radicals, obesity, peroxisome proliferator-activated receptor

INTRODUCTION

Glutathione peroxidase (GPX), an antioxidant enzyme, catalyzes the reduction of organic hydroperoxides and hydrogen peroxide (H_2O_2), using reduced glutathione as the substrate. Four types of selenium-dependent GPX have so far been reported; C-GPX (the classical type cellular GPX), EC-GPX (plasma or extracellular GPX) [1], phospholipid hydroperoxide GPX [2], and gastrointestinal GPX [3]. The C-GPX and EC-GPX are homotetramers with a similar catalytic action because of high homology in their active sites [4], and are the two isoforms that can be measured using *t*-butylhydroperoxide as a substrate [5].

1.Kohtaro Asayama, M.D., Department of Pediatrics, School of Medicine, University of Occupational and Environmental Health, Japan, 1–1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807–8555, Japan. Telephone: 81–93–691–7254, Fax: 81–93–691–9338 E-mail: kasayama@med.uoeh-u.ac.jp

C-GPX occurs in most cells [6], but not in plasma of man [7] and rats [5]. On the other hand, EC-GPX is a secretory-type glycoprotein [1] and represents whole GPX activity in plasma [5]. Kidney is the main source [8], but few other organs, such as lung [9], heart [10], gastrointestinal tract [11], placenta [12], testis [11] and epididymal body [11] express EC-GPX. We have previously demonstrated that GPX activity in kidney homogenate represents the C-GPX protein expression, and that serum GPX activity parallels the EC-GPX protein expression in kidney [5, 13]. Recently, EC-GPX is reported to be expressed abundantly in white adipose tissue of man [14], and rat [15].

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the steroid hormone nuclear receptor superfamily [16]. PPAR-γ is expressed primarily in adipose tissues [17–19], and is a master regulator of adipocyte differentiation [19]. Troglitazone, a thiazolidine derivative, is a synthetic ligand of PPAR-γ and is a new type antidiabetic agent which improves insulin sensitivity in hepatocytes, muscle fibers and adipocytes [20]. Adipose tissue is the largest organ in the body. Adipose mass is increased in obesity.

The present study was designed to determine whether EC-GPX secretion from adipose tissue increases in obesity resulting in a significant contribution to the increase in serum GPX activity. We measured GPX in serum, kidneys and adipose tissue of the Otsuka-Long-Evans-Tokushima Fatty (OLETF) rat, an animal model of slow-onset non-insulin dependent diabetes mellitus associated with mild obesity [21] and visceral fat accumulation [22], and its lean counterpart. We also investigated the effect of troglitazone on the GPX in serum and adipose tissue.

MATERIALS AND METHODS

Animal Treatments

Troglitazone was a kind gift from SANKYO Co., Tokyo, Japan. Both male OLETF rats and LETO rats were kindly provided by Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). The LETO rats are lean controls, which are obtained from the same colonies of Long-Evans rats, and therefore, have the same genetic background as OLETF rats. All animals were maintained in a temperature-controlled room (22 ± 1°C). They were fed with standard rat Purina chow pellets (Oriental Yeast, Osaka, Japan) and tap water ad libitum until they were subjected to the experiment. Then five each of OLETF and LETO rats were randomly assigned to the two different feeding groups. From 23 week and 3 day of age, OLETF and LETO rats were fed a powder chow (MF powder, Oriental Yeast Co., Tokyo, Japan) with or without 0.2% w/w of troglitazone for 10 days. Rats were sacrificed under pentobarbital anesthesia (50 mg/kg). For GPX activity assay, kidney was homogenized with 20 volumes (w/v) of 10 mM potassium phosphate/ 0.01% digitonin buffer (pH 7.4). Epididymal fat pad was homogenized with equal volume (w/v) of 50 mM Tris-HCl/ 2 mM EDTA/ 2 mM/ 5 mM 2-mercaptoethanol buffer (pH 7.4). After centrifugation (2000xg, 10 min), fat cake was discarded, and the supernatant was obtained.

Animals received humane care in compliance with the Yamanashi Medical University's guidelines and the National Research Council's criteria for humane care as outline in guide for the Care and Use of Laboratory Animals.

RNA Isolation and Reverse-Transcriptase Coupled Polymerase Chain Reaction (RT-PCR) Analysis for GPX

Total RNA was isolated from epididymal fat by the acidic guanidine isocyanate/ phenol/ chloroform extraction method using Ultraspec-II RNA isolation system (Biotecx Laboratories Inc., Houston, TX, USA). Reverse transcription was carried out on 1 μg of the treated RNA using Superscript Preamplification System (Gibco BRL), according to the manufacturer's instruction. PCR reaction was carried out by commercial kit (Takarashuzo Co., Osaka, Japan). Amplification was performed on a Thermal Cycler (Perkin Elmer Corp., Norwalk, CT) under the following conditions; 94°C (5 min) for 1 cycle, 94°C (30 sec), 54°C (1 min), 72°C (1min) for 35 cycles and 72°C (10 min) for 1 cycle. Oligonucleotide PCR primers for C-GPX (forward primer: 5'-GCT GCT CAT TGA GAA TGT CG-3'; reverse primer: 5'-TAG ACT GCT TGG ACA GCA GG-3'') and EC-GPX (forward primer: 5'-AAG AGA AGT CCA AGA CAG ACT GC-3'; reverse primer: 5'-ACA GGA TGT CCA TCT TCA CG-3') were designed using Gene Works version 2.4 software (Intelligenetics Inc., Campbell, CA, USA) based on data from Genbank (National Center for Biotechnology Information, NIH, Bethesda, MD, USA) [9, 23]. The PCR amplified products, which correspond to the nucleotide positions 183 to 667 for C-GPX and 207 to 770 for EC-GPX, were confirmed by restriction mapping. Oligonucleotide primers for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (forward primer: 5'-ACC ACC ATG GAG AAG GCT GG-3'; reverse primer: 5'-CTC AGT GTA GCC CAG GAT GC-3') were used as control. The PCR products were visualized by electrophoresis in a 2% agarose gel containing ethidium bromide (0.5 μ g/ml).

Immunoblot Analysis

The mono-specific polyclonal antibodies against rat C-GPX and rat EC-GPX have been described previously [5, 24]. Sodium dodecyl sulfate (SDS)-PAGE was performed by the method of Laemmli [25] using 12.5% slab gels. After electrophoresis, the proteins were transferred onto a polyvinylidene difluoride membrane, incubated overnight at 4°C with either anti-C-GPX or anti-EC-GPX antiserum at a dilution of 1:3000, followed by incubation with anti-rabbit IgG Fab fraction conjugated to horseradish peroxidase (MBL Co., Nagoya, Japan) for 60 min at room temperature. The peroxidase activity was visualized by an ECL western blotting analysis system kit from Amersham Life Science (Arlington Heights, IL).

Other Methods

The protein concentration was determined by means of the Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA), using bovine serum albumin as standard, according to the Bradford method [26]. The assay method for GPX activity was described previously [27]. One unit of enzyme was defined as μmol of NADPH oxidized per min per mg protein. Serum protein carbonyl groups (PCG) were estimated by the 2, 4-dinitrophenylhydrazine assay, [28] and were expressed as nmol per mg protein. The concentration of tumor necrosis factor-α (TNF-α) was assayed by commercial ELISA kit (ENDOGEN, Woburn, MA).

RESULTS

Final Body Weight, Kidney Weight, Blood Glucose Concentration and Serum TNF-α Concentration

Final body weight and kidney weight were significantly higher in OLETF rats than in LETO rats, whereas troglitazone treatment showed no effect on these parameters (Table I). Similarly, non-fasting blood glucose level was higher in OLETF rats than in LETO rats, and again troglitazone did not affect the blood glucose level. There was no interaction between the effects of strains (OLETF vs. LETO) and troglitazone.

TABLE I Final body weight, kidney weight and non-fasting blood glucose concentration

Experimental group	*Body weight (g)*	*Kidney weight (g)*	*Blood glucose (mg/dl)*
LETO			
Non-treated (n=5)	519.6 ± 12.0	1.23 ± 0.05	97.4 ± 6.0
Troglitazone (n=5)	514.6 ± 3.6	1.24 ± 0.03	111.2 ± 2.6
OLETF			
Non-treated (n=5)	600.0 ± 17.7	1.65 ± 0.06	134.0 ± 2.8
Troglitazone (n=5)	605 8 ± 14.2	1.60 ± 0.04	139.2 ± 16.0

Data are means ± s.e.m. The left kidney was weighed in each rat. Statistic significance was estimated by two-way factorial ANOVA. Body weight: $p<0.001$ for strain (OLETF vs. LETO), $p=0.59$ for troglitazone and $p=0.178$ for two factor interaction. Kidney weight: $p<0.001$ for strain, $p=0.927$ for troglitazone and $p=0.389$ for two factor interaction. Blood glucose, $p=0.001$ for strain, $p=0.215$ for troglitazone and $p=0.746$ for two factor interaction.

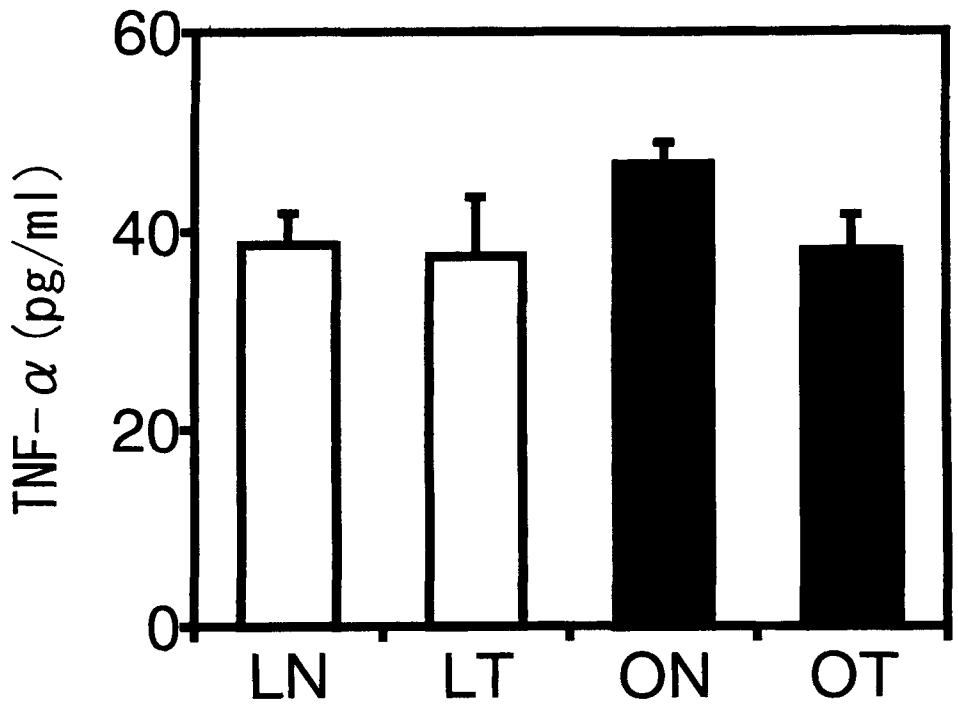

FIGURE 1 Serum level of tumor necrosis factor-α (TNF-α). LN; LETO rats without troglitazone, LT; LETO rats with troglitazone, ON; OLETF rats without troglitazone, OT; OLETF rats with troglitazone (n=5, each). Statistic difference was evaluated by the two-way factorial ANOVA: $p = 0.024$ for strain, $p = 0.013$ for troglitazone and $p=0.111$ for two factor interaction

Serum TNF-α concentration was significantly higher in OLETF rats than in LETO rats. Troglitazone treatment significantly suppressed serum TNF-α level in OLETF rats (Fig. 1). There was no interaction between the effects of strains and troglitazone.

TABLE II GPX activities in various tissues

Experimental group	*Serum (IU/ml)*	*Kidney (IU/mg-protein)*	*Adipose tissue (IU/mg-protein)*
LETO			
Non-treated (n=5)	5.51 ± 0.40	10.97 ± 0.33	5.88 ± 0.52
Troglitazone (n=5)	5.69 ± 0.28	11.20 ± 1.97	3.93 ± 0.10
OLETF			
Non-treated (n=5)	7.12 ± 0.97	13.81 ± 0.32	4.08 ± 0.33
Troglitazone (n=5)	6.76 ± 0.65	14.24 ± 0.58	3.23 ± 0.23

Data are means ± s.e.m. Serum: p=0.002 for strain, p=0.814 for troglitazone and p=0.464 for two factor interaction. Kidney: p<0.001 for strain, p=0.572 for troglitazone and p=0.859 for two factor interaction. Adipose tissue: p=0.002 for strain, p<0.001 for troglitazone and p=0.115 for two factor interaction.

Activities of GPX in Serum, Kidney and Adipose Tissue

Both serum and kidney GPX activities were significantly higher in OLETF rats than in LETO rats, but conversely, adipose tissue GPX activity was significantly lower in OLETF rats than in LETO rats (Table II). Troglitazone treatment showed no effect on the activities of serum and kidney GPX, but significantly decreased adipose tissue GPX activity in both LETO and OLETF rats. There was no interaction between the effects of strains and troglitazone in the tissue GPX activities.

Immunoblot and RT-PCR for C-GPX and EC-GPX

Figure 2 summarizes immunoblot analysis for EC-GPX in serum. The immunoreactive signals for C-GPX were not detected in the sera of all four groups (data not shown), as was reported previously [5].

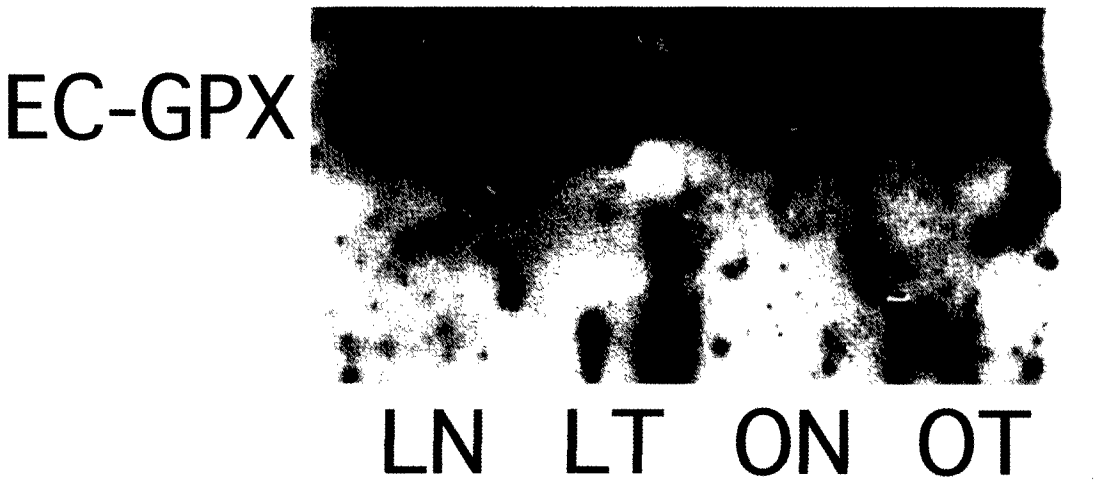

FIGURE 2 Immunoblot analysis of serum EC-GPX. Serum containing 50 μg of protein was loaded on each lane. The exposure time for detection of immunoreactive band in the enhanced chemiluminescence system was 1 min. A specific band was detected at 22.5 kDa level for EC-GPX. The data are representative blot out of 3 observations. The abbreviations are the same as in Fig. 1

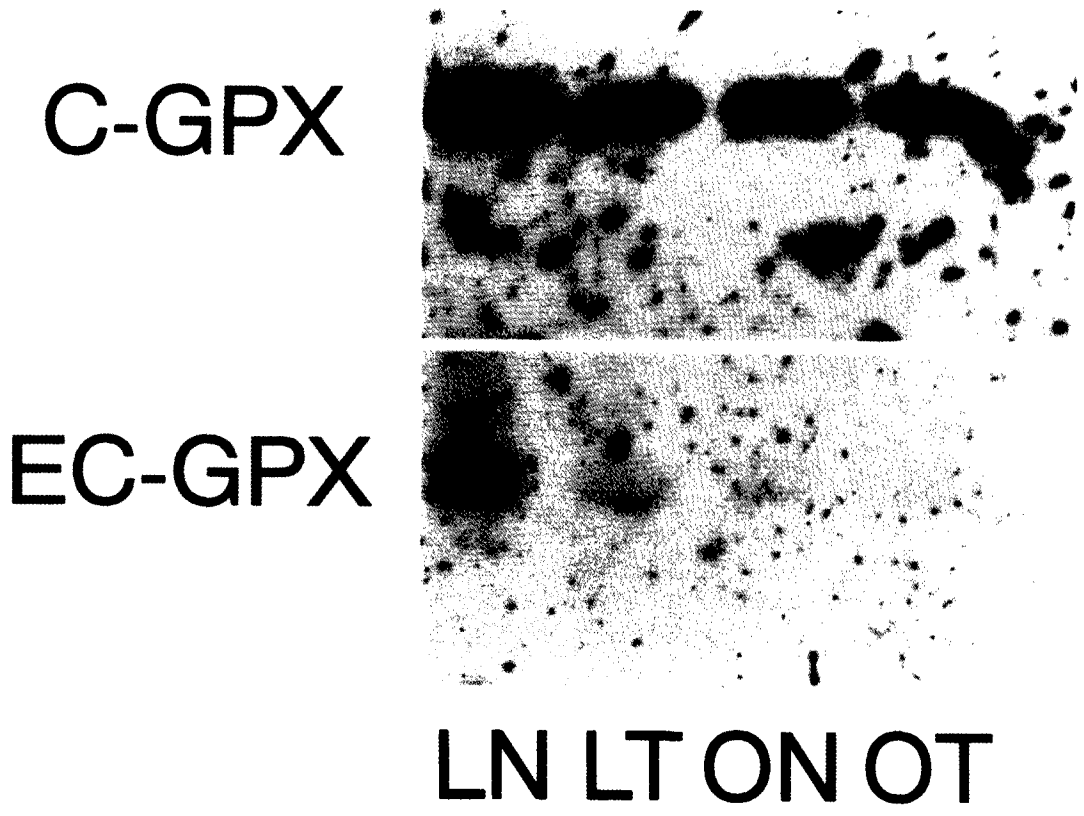

FIGURE 3 Immunoblot analysis of adipose tissue C-GPX and EC-GPX. For C-GPX, a sample containing 50 μg of protein was loaded on each lane. The exposure time for detection of immunoreactive C-GPX band in the enhanced chemiluminescence system was 1 min. For EC-GPX, a sample containing 150 μg of protein was loaded on each lane. The exposure time for detection of immunospecific EC-GPX band in the enhanced chemiluminescence system was 15 min. The abbreviations are the same as in Fig. 1

The adipose tissue homogenate contained much more immunoreactivity for C-GPX than that for EC-GPX. The amount of protein loaded on each lane of the electrophoresis gel was 5 times greater for detection of EC-GPX than for detection of C-GPX. The exposure time for visualization of the immunoreactive bands was 15 times longer for EC-GPX than for C-GPX. Figure 3 summarizes immunoblot analysis of adipose tissue C-GPX and EC-GPX. The immunoreactive signal for C-GPX was more intense in LETO rat without troglitazone than in OLETF rat without troglitazone. Troglitazone treatment appeared to suppress the signal for C-GPX in both LETO and OLETF rats. The immunoreactive signal for EC-GPX was close to the minimal detection limit of the present system. The signal for the LETO rat without troglitazone was more intense than those for the rest of groups. Both obesity and troglitazone appeared to suppress the signal for EC-GPX.

The results of RT-PCR are summarized in Figure 4. A single PCR product for C-GPX, and also for EC-GPX, is depicted in each lane, indicating that the two genes were expressed in the adipose tissue of OLETF and LETO rats both in the presence and absence of troglitazone.

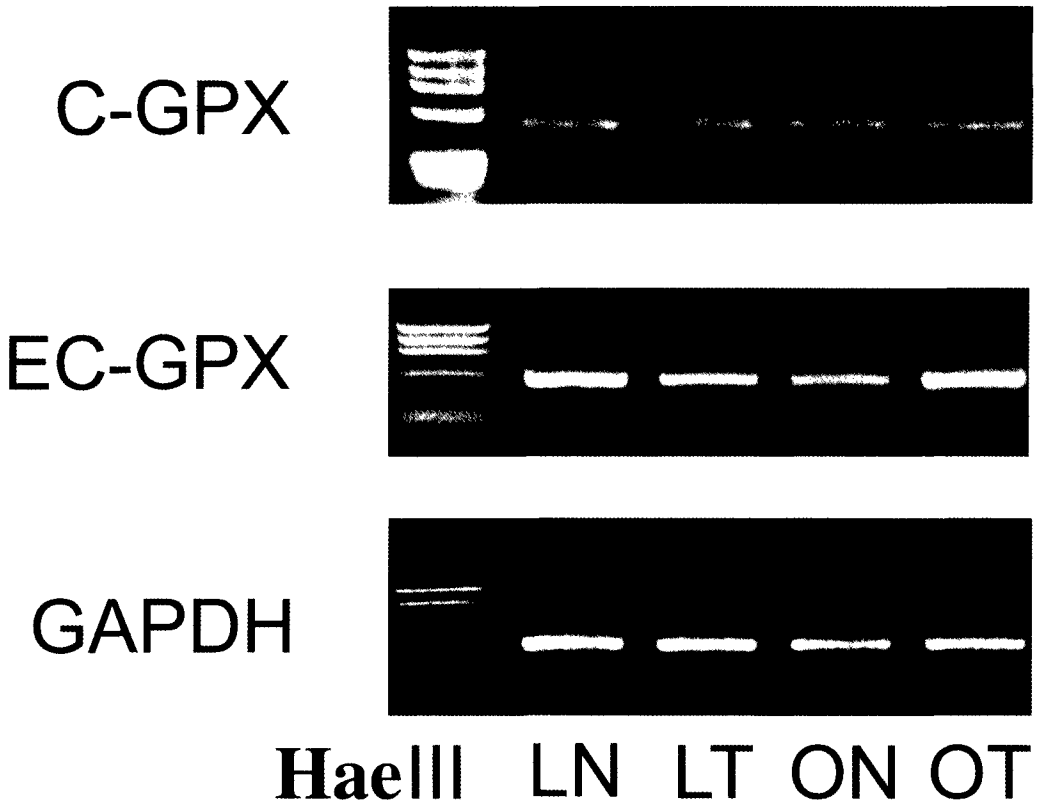

FIGURE 4 Reverse-transcriptase coupled polymerase chain reaction (RT-PCR) analysis of adipose tissue C-GPX and EC-GPX. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) are used as control. The abbreviations are the same as in Fig. 1

Protein Carbonyl Groups in Serum

Serum PCG were significantly higher in OLETF than in LETO rats (Fig. 5). The troglitazone treatment completely blocked the increase in PCG level in the OLETF rats.

DISCUSSION

The present RT-PCR analysis clearly depicted the presence of RNA message for both C-GPX and EC-GPX in adipose tissue. Previous studies demonstrated the occurrence of GPX activity [29] and the mRNA for C-GPX [30] and EC-GPX [14, 15] in adipose tissue. To our knowledge, the present study, for the first time, revealed that the protein signals for both C-GPX and EC-GPX existed in white adipose tissue of rat. The relative amount of immunoreactive product for EC-GPX remained in adipose tissue was much less than that of C-GPX, judging from the present results of immunoblot analysis. Thus, the GPX activity in adipose tissue homogenate solely represented the activity of C-GPX but not that of EC-GPX. We previously reported the results of immunoblot analysis for C-GPX and EC-GPX in rat kidney homogenate [5]. We found that immunoreactivity for EC-GPX remained in the kidney tissue was much less than that of C-GPX.

In the present study, we confirmed that EC-GPX was expressed in the adipose tissue of rat. Adipose mass is increased in OLETF rat as com-

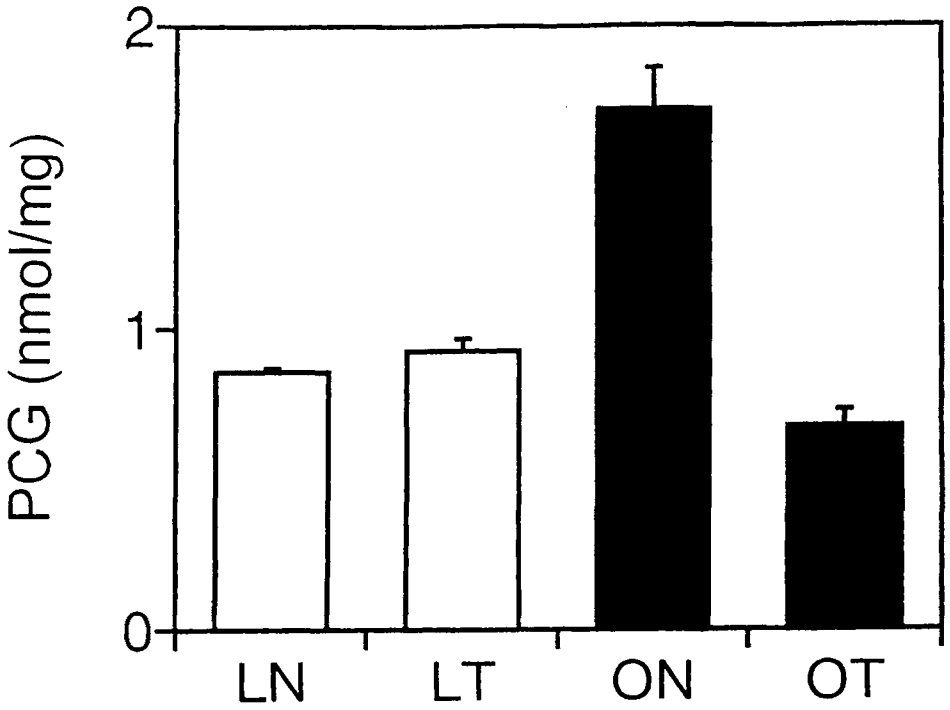

FIGURE 5 Serum concentration of protein carbonyl groups (PCG) (n=5, each). The abbreviations are the same as in Fig. 1. The two-way factorial ANOVA: $p = 0.006$ for strain, $p<0.001$ for troglitazone and $p<0.001$ for two factor interaction

pared to LETO rat [31]. However, decreased expression of EC-GPX in adipose tissue of obese rat, observed here, could offset the effect of increase in adipose mass due to obesity. Furthermore, troglitazone suppressed the expression of EC-GPX in adipose tissue but not in the kidney, and did not affect the serum GPX concentration. Thus, increased serum GPX activity in the obese rat appeared to be due to the increased secretion of EC-GPX from the kidney.

The GPX activity that represented the C-GPX [5] was increased in the kidney of the obese rats, along with the increase in EC-GPX synthesis. On the other hand, both obesity and troglitazone suppressed the expression of C-GPX and EC-GPX in adipose tissue. Thus, the expression of both C-GPX and EC-GPX appeared to be regulated in a parallel manner in the same tissues. We previously reported a similar effect of selenium deficiency on both C-GPX and EC-GPX in rat kidney [5], suggesting the common mechanism of expression regulation for both genes.

However, effect of obesity and troglitazone on the expression of both GPX in the adipose tissue was quite different from that in the kidney. The GPX activity was significantly increased in the kidney of the present OLETF rats. Assuming that kidney C-GPX and EC-GPX were subjected to a similar regulation for expression [5], not only the mass effect but also the enhanced expression of kidney EC-GPX *per se* contributed to the increase in the serum EC-GPX. Kidney GPX activity was also reported to be increased in ob/ob mouse [32, 33] and streptozotocin-induced diabetic rat [34, 35]. No effect of troglitazone on gene expression in kidney was because PPAR-γ was not expressed in kidney [19].

Troglitazone activates PPAR-γ in adipose tissue, thereby accelerating adipocyte differentiation. It increases the number of small adipocytes and decreases large adipocytes without changing the total mass of white adipose tissue [36]. High fat diet induces, while fasting and insulin-dependent diabetes mellitus repress the expression of PPAR-γ [37]. However, adipose tissue level of PPAR-γ is not found to be increased in gold-thioglucose and ob/ob mice [37], and in simple obesity in man [38]. Human obesity due to an activated mutant of PPAR-γ is characterized by lack of insulin resistance [39]. It is unlikely that the repression of GPX expression in the insulin-resistant diabetic OLETF rat is due to the activation of PPAR-γ. Thus, obesity and troglitazone appear to exert independent repressive effect on the adipose tissue GPX expression.

Oxidative stress is increased in sera of obese subjects [40], diabetic man [41, 42] and obese animals [43, 44]. Troglitazone treatment significantly suppresses the enhanced oxidative stress in obese [40] and diabetic man [42]. It also decreases plasma lipid peroxide level in streptozotocin-induced diabetic rats [45] and prevents oxidation of low density lipoprotein in healthy volunteers [46]. PCG, which are marker of protein degradation mediated by oxidative stress, were increased due to obesity and troglitazone completely blocked this increase without affecting serum GPX activity in the present study. Thus, troglitazone protects against the enhanced oxidative stress induced by obesity independently of serum EC-GPX level. Troglitazone possesses structural similarity to α-tocopherol [45] and it exerts antioxidant action [46]. Troglitazone administration reduced adipocyte size in white adipose tissue of Zucker rat, and suppressed TNF-α expression to normal level [36]. Infact, troglitazone abolished TNF-α overproduction in the present OLETF rat. Such beneficial effect of troglitazone on improving obesity-associated imbalance of intra-cellular metabolism in adipocytes may eventually result in the reduction of oxidative stress in the serum of obese animals.

Acknowledgements

This work was supported in part by Grant-in-Aid #08670866 and #10670714 from the Ministry of Education, Science and Culture of Japan. The authors would like to thank Keiko Kagami for her technical assistance.

References

[1] K. Takahashi, N. Avissar, J. Whitin, and H. Cohen (1987) Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. *Archives of Biochemistry and Biophysics*, **256**, 677–686.

[2] F. Ursini, M. Mariorino, and C. Gregolin (1985) The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochimica et Biophysica Acta*, **839**, 62–70.

[3] F.F. Chu, J.H. Doroshow, and R.S. Esworthy (1993) Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSH-Px-GI. *Journal of Biological Chemistry*, **268**, 2571–2578.

[4] B. Ren, W. Huang, B. Akesson, and R. Ladenstein (1997) The crystal structure of seleno-glutahione peroxidase from human plasma at 2.9 A resolution. *Journal of Molecular Biology*, **268**, 869–885.

[5] T. Nakane, K. Asayama, K. Kodera, H. Hayashibe, N. Uchida, and S. Nakazawa (1998) Effect of selenium dificiency on cellular and extracellular glutathione peroxidase: immunochemical detection and mRNA analysis in rat kidney and serum. *Free Radical Biology & Medicine*, **25**, 504–511.

[6] O.A. Levander (1987) A global view of human selenium nutrition. *Annual Review of Nutrition*, **7**, 227–250.

[7] N. Avissar, J.C. Whitin, P.Z. Allen; I.S. Palmer; and H.J. Cohen (1989) Antihuman plasma glutathione peroxidase antibodies: immunologic investigations to determine plasma glutathione peroxidase protein and selenium content in plasma. *Blood*, **73**, 318–323.

[8] N. Avissar; D.B. Ornt, Y. Yagil; S. Horowitz, R.H. Watkins; E.A. Kerl, K. Takahashi, I.S. Palmer and H.J. Cohen (1994) Human kidney proximal tubules are the main source of plasma glutathione peroxidase. *American Journal of Physiology*, **266**, C367–375.

[9] S. Yoshimura, K. Watanabe, H. Suemizu,. T. Onozawa, J. Mizoguchi, K. Tsuda, H. Hatta and T. Moriuchi (1991) Tissue specific expression of the plasma glutathione peroxidase gene in rat kidney. *Journal of Biochemistry*, **109**, 918–923.

[10] Chu, F.F.; Esworthy, R.S.; Doroshow, J.H.; Doan, K.; Liu, X.F. Expression of plasma glutathione peroxidase in human liver in addition to kidney, heart, lung, and breast in humans and rodents. *Blood*, **79**, 3233–3238; 1992.

[11] R.L. Maser, B.S. Magenheimer and J.P. Calvet (1994) Mouse plasma glutathione peroxidase: cDNA sequence analysis and renal proximal tubular expression and secretion. *Journal of Biological Chemistry*, **269**, 27066–27073.

[12] N. Avissar, C. Eisenmann, J.G. Breen, S. Horowitz, R.K. Miller and H.J. Cohen (1994) Human placenta makes

extracellular glutathione peroxidase and secretes it into maternal circulation. *American Journal of Physiology*, **267**, E68-E76.

[13] K. Dobashi, K. Asayama, T. Nakane, H. Hayashibe, K. Kodera, N. Uchida and S. Nakazawa (1999) Effect of peroxisome proliferator on extracellular glutathione peroxidase in rat. *Free Radical Research*, **31**, 181–190.

[14] K. Maeda, K. Okubo, I. Shimomura, K. Mizuno, Y. Matsuzawa and K. Matsubara (1997) Analysis of an expression profile of genes in the human adipose tissue. *Gene*, **190**, 227–235.

[15] P.D. Kingsley, J.C. Whitin, H.J. Cohen and J. Palis (1998) Developmental expression of extracellular glutathione peroxidase suggests antioxidant roles in decidum, visceral yolk sac, and skin. *Molecular Reproduction and Development*, **49**, 343–355.

[16] I. Issemann and S. Green (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature*, **347**, 645–650.

[17] O. Braissant, F. Foufelle, C. Scotto, M. Dauca and W. Wahli (1996) Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat. *Endocrinology*, **137**, 354–366.

[18] O. Braissant and W. Wahli (1998) Differential expression of peroxisome proliferator-activated receptor-α, -β, -γ during rat embryonic development. *Endocrinology*, **139**, 2748–2754.

[19] P. Tontonoz, E. Hu and B.M. Spiegelman (1994) Stimulation of adipogenesis in fibroblasts by PPAR2, a lipid-activated transcription factor. *Cell*, **79**, 1147–1156.

[20] B.M. Spiegelman (1998) PPAR-γ: adipogenic regulator and thiazolidinedione receptor. *Diabetes*, **47**, 507–514.

[21] K. Kawano, T. Hirashima, S. Mori, Y. Saitoh, M. Kurosumi and T. Natori (1992) Spontaneous long-term hyperglycemic rat with diabetic complications. Otsuka Long-Evans Tokushima Fatty (OLETF) strain. *Diabetes*, **41**, 1422–1428.

[22] M. Ishikawa and K. Koga (1998) Measurement of abdominal fat by magnetic resonance imaging of OLETF rats, an animal model of NIDDM. *Magnetic Resonance Imaging*, **16**, 45–53.

[23] S. Yoshimura, S. Takekoshi, K. Watanabe and Y. Fujii-Kuriyama (1988) Determination of nucleotide sequence of cDNA coding rat glutathione peroxidase and diminished expression of mRNA in selenium deficient rats. *Biochemical and Biophysical Research Communications*, **154**, 1024–1028.

[24] K. Asayama, S. Yokota, K. Dobashi, H. Hayashibe, A. Kawaoi and S. Nakazawa (1994) Purification and immunoelectron microscopic localization of cellular glutathione peroxidase in rat hepatocytes: quantitative analysis by postembedding method. *Histochemistry*, **102**, 213–219.

[25] U.K. Laemmli (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.

[26] M. Bradford (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Chemistry*, **72**, 248–254.

[27] K. Asayama, W.D. Dettbarn and I.M. Burr (1986) Differential effect of denervation on free radical scavenging enzymes in slow and fast muscle of rat. *Journal of Neurochemistry*, **46**, 604–609.

[28] R.L. Levine, D. Garland, C.N. Oliver, A. Amici, I. Climent, A.G. Lenz, B.W. Ahn, S. Shaltiel and E.R. Stadman (1990) Determination of carbonyl content in oxidatively modified proteins. In: L. Packer, A.N. Glazer, eds., *Methods in Enzymology*, Vol. **186**, New York, Academic Press, 464–478.

[29] S.A. Meyer, R.C. Ewan and D.C. Beitz (1983) Effect of selenium on the subcellular distribution of glutathione peroxidase in rat liver, epididymal fat pad and seminal vesicle. *Journal of Nutrition*, **113**, 394–400.

[30] J.F. Mitchell, F. Nicol, G.J. Beckett and J.R. Arthur (1997) Selenium and iodine deficiencies: effects on brain and brown adipose tissue selenoenzme activity and expression. *Journal of Endocrinology*, **155**, 255–263.

[31] K. Shima, K. Shi, T. Sano, T. Iwami, A. Mizuno and Y. Noma (1993) Is exercise training effective in preventing diabetes mellitus in the Otsuka-Long-Evans-Tokushima fatty rat, a model of spontaneous non-insulin-dependent diabetes mellitus? *Metabolism*, **42**, 971–977.

[32] J.R. Prohaska, L.E. Jr. Wittmers and E.W. Haller (1988) Influence of genetic obesity, food intake and adrenalectomy in mice on selected trace element-dependent protective enzymes. *Journal of Nutrition*, **118**, 739–746.

[33] I.D. Capel and H.M. Dorrell (1984) Abnormal antioxidant defense in some tissues of congenitally obese mice. *Biochemical Journal*, 219, 41–49.

[34] T. Dohi, K. Kawamura, K. Morita, H. Okamoto and A. Tsujimoto (1988) Alterations of the plasma selenium concentrations and the activities of tissue peroxide metabolism enzymes in streptozotocin-induced diabetic rats. *Hormone and Metabolic Research*, **20**, 671–675.

[35] K. Asayama, H. Hayashibe, K. Dobashi, T. Niitsu, A. Miyao and K. Kato (1989) Antioxidant enzyme status and lipid peroxidation in various tissues of diabetic and starved rats. *Diabetes Res*, **12**, 85–91.

[36] A. Okuno, H. Tamemoto, K. Tobe, K. Ueki, Y. Mori, K. Iwamoto, K. Umesono, Y. Akanuma, T. Fujiwara, H. Horikoshi, Y. Yazaki and T. Kadowaki (1998) Troglitazone increases the number of small adipocytes without the change of adipose tissue mass in obese Zucker rats. *Journal of Clinical Investigation*, **101**, 1354–1361.

[37] A. Vidal-Puig, M. Jimenez-Linan, B.B. Lowell, A. Hamann, E. Hu, B.M. Spiegelman, J.S. Flier and D.E. Moller (1996) Regulation of PPAR- gene expression by nutrition and obesity in rodents. *Journal of Clinical Investigation*, **97**, 2553–2561.

[38] D. Auboeuf, J. Rieusset, L. Fajas, P. Vallier, V. Frering, J.P. Riou, B. Staels, J. Auwerx, M. Laville and H. Vidal (1997) Tissue distribution and quantification of the expression of mRNA of peroxisome proliferator-activated receptors and liver × receptor-γ a in humans. *Diabetes*, **46**, 1319–1327.

[39] M. Ristow, D. Muller-Wieland, A. Pfeiffer, W. Krone and C.R. Kahn (1998) Obesity associated with a mutation in a genetic regulator of adipocyte differentiation. *New England Journal of Medicine*, **339**, 953- 959.

[40] C.J. Tack, P. Smits, P.N. Demacker and A.F. Stalenhoef (1998) Troglitazone decreases the proportion of small, dense LDL and increases the resistance of LDL to oxidation in obese subjects. *Diabetes Care*, **21**, 796–799.

[41] H. Kaji, M. Kurasaki, K. Ito, T. Saito, K. Saito, T. Niioka, Y. Kojima, Y. Ohsaki, H. Ide, M. Tsuji, T. Kondo and Y. Kawakami (1985) Increased lipoperoxide value and glutathione peroxidase activity in blood plasma of type 2 (non-insulin-dependent) diabetic women. *Klinische Wochenschrift*, **63**, 765–781.

[42] L. Cominacini, U. Garbin, P.A. Fratta, M. Campagnola, A. Davoli, E. Foot, G. Sighieri, A.M. Sironi, C.V. Lo and E. Ferrannini (1998) Trogilitazone reduces LDL oxidation and lowers plasma E-selectin concentration in NIDDM patients. *Diabetes*, **47**, 130–133.

[43] C. Douillet, M. Bost, M. Accominotti, F. Borson-Chazot and M.Ciavatti (1998) Effect of selenium and vitamin E supplementation on lipid abnormalities in plasma, aorta, and adipose tissue of Zucker rats. *Biological Trace Elemental Research*, **65**, 221–236.

[44] K. Asayama, H. Hayashibe, K. Dobashi, N. Uchida and K. Kato (1990) Lipid peroxidation and antioxidant enzyme status in various tissues of gold-thioglucose-induced obese mice. *Journal of Clinical Biochemistry and Nutrition*, **9**, 119–127.

[45] X. Qiang, J. Satoh, M. Sagara, M. Fukuzawa, T. Masuda, Y. Sakata, Y. Muto, K. Takahashi and T. Toyota (1998) Inhibitory effect of troglitazone on diabetic neuropathy in streptozotocin-induced diabetic rats. *Diabetologia*, **41**, 1321–1326.

[46] L. Cominacini, M.M. Young, A. Capriati, U. Garbin, A.F. Pasini, M. Campagnola, A. Davoli, A. Rigoni, G.B. Contessi and V. Lo Cascio (1997) Troglitazone increases the resistance of low density lipoprotein to oxidation in healthy volunteers. *Diabetologia*, **40**, 1211–1218.