Transcriptional Alterations of ET-1 Axis and DNA Damage in Lung Tissue of a Rat Obesity Model

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Obesity has been implicated in the development of many cancers. This can lead to genome damage, especially in the form of double-strand break, the presence of which is now easily detected through nuclear phosphorylation of histone H2AX (γ -H2AX) focus assay. Recently, the endothelin (ET) axis has also been shown to have a role in the growth and progression of several tumors, including lung cancer. The aim of this study was to evaluate the ET-1 system transcriptional alterations and γ -H2AX in lung tissue of Zucker rats subdivided into obese (O, $n=22$) and controls (CO, $n=18$) rats: under either fasting conditions (CO_{fc}-O_{fc}) or acute hyperglycemia (CO_{AH}-O_{AH}). Significantly higher *prepro-ET-1* ($p=0.05$) and ET-converting enzyme (*ECE*)-2 mRNA expression was observed in O with respect to CO. A significant positive association was observed between *prepro-ET-1* and *ET-A* in the whole rat population ($p=0.009$) or in the obese group alone ($p=0.007$). The levels of γ -H2AX in O and in O_{AH} rats were significantly higher ($p=0.019$) than in the corresponding CO and CO_{AH} rats ($p=0.038$). The study shows an inappropriate secretion of *ET-1* in O animals with a parallel DNA damage in their lungs, providing novel mechanisms by which ET receptor antagonist may exert organ protection.

Introduction

OBESITY IS A SIGNIFICANT public health problem since it affects many individuals and is linked with increased risk for numerous chronic diseases. It is a complex pathology with interacting and confounding causes, such as the environment, hormonal factors, and genetic predisposition (Wang and Nakayama, 2010). It is an independent risk factor for cardiovascular disease, which can dramatically increase the likelihood of negative outcomes (Kopelman, 2000). In the development of atherosclerosis and diabetes, obesity has been studied as a form of epidemic inflammation that predisposes the body to other forms of epidemic inflammation known to be involved in these disease states (Nathan, 2008). Recently, obesity has also been implicated in the development of many carcinomas, and its prevalence is reaching epidemic proportions in children and teenagers (Lavrador *et al.*, 2011) and there is also expanding evidence of the role of obesity in cancer development, treatment, and survival (Renehan *et al.*, 2008). Researchers are considering the biochemical and physiologic implications of obesity over that in the development of chronic diseases even in cancer where the rela-

tionships are evolving. Multiple mechanisms being studied include chronic hyperinsulinemia/insulin resistance, which is believed to create an environment favorable for tumor formation through changes in the availability of insulin growth factor. The endothelin (ET) axis has been shown to have a role in the growth and progression of several tumor types, including lung cancer (Bhalla *et al.*, 2009). There are three known isoforms, ET-1, ET-2, and ET-3, but ET-1 is the most clinically relevant, and it is derived from a 212 amino acid precursor known as *prepro-ET-1* (Rubany and Polokoff, 1994; Bhalla *et al.*, 2009). This undergoes proteolytic cleavage to produce Big-ET-1, which is then cleaved to active ET-1 by ET-converting enzyme (ECE). ET-1 exerts its effects by binding to two distinct G protein-coupled receptors: ET-A and ET-B (Rubany and Polokoff, 1994). The vasoconstrictor effects of ET-1 are mediated through ET-A, which are typically found in vascular smooth muscle, while the vasodilator effects are mediated by ET-B, mainly found in vascular endothelium (Battistini *et al.*, 1993). Both these receptors are present in many cell types other than vascular endothelium and smooth muscle (Pollock *et al.*, 1995). The lung is particularly rich in both ET-A and ET-B, suggesting

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that ET-1 plays an influential role in the physiology of the lung (Elshourbagy *et al.*, 1993). To date, the mechanistic relationship between obesity and the development of cancer remains largely unexplained. In this context, the DNA double-strand break (DSB) is considered the most deleterious DNA lesion affecting mammalian cells, as it can initiate and promote carcinogenesis. However, cells respond promptly by activating the DNA damage response (DDR), which precociously results in phosphorylation of Ser-139 of histone H2AX at the site of DNA damage (γ -H2AX foci) (Rogakou *et al.*, 1998). Thus, identification of γ -H2AX in the nuclei of a given cell population is considered an excellent marker of early DNA damage, and several authors used the γ -H2AX assay, this including either the focus version (immunofluorescence analysis of cell lines) or the positive nucleus version (immunohistochemistry analysis of paraffin-embedded tissue cells) to study the response of physical or chemical genotoxins and to correlate DSB formation with exposures or pathological conditions, including cancer (Bartkova *et al.*, 2005; Nuciforo *et al.*, 2007; Watters *et al.*, 2009; Redon *et al.*, 2011; Scarpato *et al.*, 2011, 2013). The Zucker rat model offers a reliable genetic model for research on obesity and metabolic syndrome due to the incorporation of transgenic and knockout technology (Cozzi *et al.*, 2009; Geurts *et al.*, 2009). In fact, the homozygous fa^{-}/fa^{-} rats, unlike the lean heterozygous fa^{+}/fa^{-} animals, present hyperlipidemia and hypertension and exhibit severe hepatic as well as peripheral insulin resistance. Thus, in an attempt to establish whether a connection exists between one of the most intriguing features of the metabolic syndrome and a well-validated marker of early DNA damage and carcinogenesis risk, we aimed to evaluate the ET-1 system transcriptional alterations in lung tissue of obese Zucker rats along with the measurement, in the same samples, of nuclear phosphorylation of histone H2AX (γ -H2AX).

Materials and Methods

Experimental animal model

The study included 40 male Zucker rats, 9–13 weeks of age, subdivided into two groups: obese rats (O, $n=22$) and age-matched lean rats (CO, $n=18$) as control. Rats were fasted for 12 h with unrestricted access to water. Part of each group was studied during fasting conditions (CO_{fc}, $n=8$; O_{fc}, $n=12$) and the remainder during the induction of acute hyperglycemia (CO_{AH}, $n=10$; O_{AH}, $n=10$). Hyperglycemia was induced by intraperitoneal glucose injection (2 g/kg). On the day of the study, anesthesia was induced by inhalation of 2% isoflurane and maintained by intraperitoneal administration of Zoletil (Virbac, srl) (tiletamine and zolazepam 40 mg/kg) and Xylazine (5 mg/kg) (Guiducci *et al.*, 2011). During the procedure, the animals were under general anesthesia and were euthanized by isoflurane overdose. Two small pieces of lung tissue were collected from each animal; one of them was immediately placed in ice-cold RNeasy lysis buffer (Qiagen S.p.A., Milano, Italy) and stored at -80°C for RNA extraction; the second one was washed from blood residues and fixed in formalin until processed for DNA damage analysis by a standard immunohistochemistry protocol. National guidelines for the care and use of research animals (D.L. 116/92, implementation of EEC directive 609/86) were followed.

Tissue handling, RNA extraction and quality, and cDNA synthesis

The lung tissue was homogenized with an automated tissue lyser through high-speed shaking in plastic tubes with stainless steel beads (Qiagen).

Total RNA was extracted by the acid guanidinium thiocyanate–phenol–chloroform method using an RNeasy Midi kit (Qiagen) following the manufacturer's instructions, as previously described (Cabiati *et al.*, 2012, 2013). The RNA concentration and purity were determined spectrophotometrically (BioPhotometer, Eppendorf, Milano, Italy) measuring spectral absorption at 260 nm. The reading ratio at 260 and 280 nm (A_{260}/A_{280}) provides an estimate of RNA purity with respect to contaminants absorbing in the UV spectrum, such as protein. The integrity and purity of total RNA was also detected by electrophoresis of samples on Gel Star Stain (Lonza, Basel, Switzerland) agarose gels. Samples showing clear and distinct 28S and 18S ribosomal RNA bands and having spectrophotometric optical density 260/280 ratios of 1.9–2.1 were used. A known amount of total RNA (1 $\mu\text{g}/\mu\text{L}$; Ambion, Inc., Austin, TX) was used as marker. The RNA samples were stored at -80°C for use in gene expression studies.

Following DNase treatment (RNase-Free DNase Set; Qiagen), first-strand cDNA was synthesized with iScript cDNA Synthesis kit (Bio-Rad Laboratories, Hercules, CA) using about 1 μg of total RNA as template. Reverse transcriptase reaction sequence consisted of incubation at 25°C for 5 min, followed by three different cycles at 42°C for 30 min, at 45°C and 48°C for 10 min, to better separate the strands. The reverse transcriptase enzyme was inactivated by heating to 85°C for 5 min. The cDNA samples obtained were placed on ice and stored at 4°C until further use.

Real-Time polymerase chain reaction

Real-Time polymerase chain reaction (PCR) reactions were performed in duplicate in the Bio-Rad C1000™ thermal cycler (CFX-96 Real-Time PCR detection systems; Bio-Rad Laboratories), as previously described (Cabiati *et al.*, 2012, 2013). For monitoring cDNA amplification, a third-generation fluorophore, EvaGreen, was used (SsoFAST EvaGreen Supermix; Bio-Rad Laboratories). PCR was performed in a volume of 20 μL per reaction; to minimize the influence of PCR inhibitors in Real-Time applications, all cDNA samples were diluted 1:5. Reaction mixture included 2 μL of template cDNA (10 ng/ μL), 0.2 μM of each primer (Sigma-Aldrich, St. Louis, MO), 1 $\times$ SsoFAST EvaGreen SuperMix (BioRad Laboratories), and sterile H_2O . Amplification protocol started with 98°C for 30 s followed by 40 cycles at 95°C for 5 s and 60°C for 30 s. To assess product specificity, amplicons were systematically checked by melting curve analysis. Melting curves were generated from 65°C to 95°C , with increments of 0.5°C per cycle. Multiple inter-run calibrators were always used to allow comparison of Ct values obtained in different runs. The primer pairs specific for each gene analyzed in this study (Table 1) were designed with Primer Express Version 2.0 (Applied Biosystems, Waltham, MA); whenever possible, intron-spanning primers were selected to avoid amplification of genomic DNA. Reaction conditions of all primer pairs used were optimized; that is, a gradient PCR was conducted to assess the optimal annealing temperature, while a standard

TABLE 1. SPECIFIC PRIMER SEQUENCES USED IN REAL-TIME POLYMERASE CHAIN REACTION EXPERIMENTS

<i>Genes</i>	<i>Primer sequence (5'-3')</i>	<i>GenBank accession no.</i>
<i>ACTB</i>	F: GTCGTACCACTGGCATTGTG R: CTCTCAGCTGTGGTGGTGAA	NM_031144
<i>SDHA</i>	F: CTCTTTTGGACCTTGTCTGCTTT R: TCTCCAGCATTTGCCTTAATCGG	NM_130428
<i>YWHAG</i>	F: TTCCTAAAGCCCTTCAAGGCA R: GGCTTTCTGCACTAGTTGCTCG	NM_019376
<i>ET-A</i>	F: CTCCACAGTAGTAGCACAT R: TAGCCAGTCCTCACAGTA	NM_012550
<i>ET-B</i>	F: GATACGACAACTTCCGCTCCA R: GTCCACGATGAGGACAATGAG	NM_017333
<i>ECE-1</i>	F: CTTCCGAGTCCTCTTGTTGTT R: CCTTCTGGCTGTATGTGGTT	NM_021776
<i>ECE-2</i>	F: TTCTAACAGCAACATCATC R: TCTCATTGGCAGTTCTAT	NM_001002815
<i>PreproET-1</i>	F: GTCTAAGCGATCCTTGAA R: AATTCCAGCACTTCTTGT	NM_012548

ACTB, β -actin; *SDHA*, succinate dehydrogenase complex, subunit A, flavoprotein; *YWHAG*, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide; *ET-A*, endothelin receptor A; *ET-B*, endothelin receptor B; *ECE-1*, endothelin-converting enzyme 1; *ECE-2*, endothelin-converting enzyme 2; *PreproET-1*, endothelin 1 gene.

curve obtained by scalar dilution of a cDNA pool (1:5, 1:25, 1:125, 1:625) was always generated to verify the PCR efficiency. The geometric mean of the three most stably expressed genes in the lung (*ACTB*, *SDHA*, *YWHAG*), established in a previous study (Cabiati *et al.*, 2012), was used for normalization of Real-Time PCR results. Relative quantification of each target gene studied was calculated by the $\Delta\Delta C_t$ method using Bio-Rad's CFX96 manager software. The results are expressed as mean $\pm$ standard error of the mean (SEM). In an effort to provide greater transparency of our results between research laboratories, this study was carried out to conform to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) (Bustin *et al.*, 2009).

Analysis of DNA damage (γ -H2AX) in paraffin-embedded sections of lung tissue

Tissue section preparation. Formalin-fixed lung tissues were washed in tap water to remove fixative, dehydrated in an ethanol series (70% for at least 1 day, then 95% and 100% for 1 h each), and immersed two times in isoparaffin for 1 h each (Panreac; Nova Chimica, Milan, Italy) to facilitate penetration of paraffin into tissue and included in paraffin. Paraffin-embedded lungs were then sectioned into 5- μ m slices using a Leica RM 2155 microtome (Leica Instruments, Wetzlar, Germany). Slices were then placed on Superfrost Ultra Plus[®] slides (Manzel-Glaser; Thermo-Fisher, Milan, Italy) to guarantee firm electrostatic adhesion of the sections. Two slides from each animal were set up, containing two paraffin-embedded lung sections each.

Immunohistochemistry protocol

Deparaffinization of slides was obtained by incubating them in three washes in xylene for 5 min each. Sections were rehydrated in two washes in an ethanol series (absolute, 95%, and 70%) for 10 min each followed by two 5-min washes in distilled water. For heat-mediated antigen unmasking, slides were placed in 10 mM sodium citrate buffer,

pH 6.0, heated to 95°C, and pulse-heated for 7, 4, and 4 min in the citrate buffer in the microwave, separating each pulse by a 2-min wash in sodium citrate buffer at 4°C to maintain tissue integrity. Slides were allowed to cool in three washes in distilled water for 5 min followed by a 5-min wash in 1 $\times$ phosphate-buffered saline (PBS). After a further wash in distilled water, slides were incubated in 3% hydrogen peroxide (Sigma-Aldrich) for 10 min, washed two times in distilled water and one time in 1 $\times$ PBS for 5 min each, and then incubated for 1 h in a blocking solution (10% fetal bovine serum [Life Technologies, Monza, Italy], 0.3% Triton-X, in 1 $\times$ PBS) at room temperature. After removing the blocking solution, slides were then incubated overnight at 4°C with the primary polyclonal rabbit anti- γ -H2AX (ser139) antibody (Abcam, Prodotti Gianni, Milan, Italy) diluted 1:200 in the blocking solution. The following day, slides were washed three times in 1 $\times$ PBS for 5 min each and incubated for 2 h at room temperature with an anti-rabbit horseradish peroxidase-conjugated secondary antibody (Sigma-Aldrich) diluted 1:100 in the blocking solution. Slides were washed three times in 1 $\times$ PBS for 5 min each, covered with the DAB substrate (Sigma-Aldrich) and incubated in the dark until staining developed. Next, slides were immediately immersed in distilled water, counterstained in hematoxylin, dehydrated, and mounted in glass coverslips for microscopy observation. The presence of γ -H2AX-positive nuclei within a hematoxylin-counterstained cell population was recognized by formation of a homogeneous brown precipitate covering partially or entirely the DSB-damaged nuclei, which were easily distinguished by their undamaged blue-colored nuclei (Fig. 1). A (semi)-quantitative analysis was performed with the aid of the ImageJ software (download at <http://imagej.nih.gov/ij/>), where the DNA DSB marker in each rat was expressed as the average ratio of γ -H2AX positive (brown-stained) to negative (blue-stained) nuclei corrected for the percentage of γ -H2AX positivity of the sections (i.e., how areas, in percent, showed a prevalence of brown nuclei). At group level, the results were then expressed as mean $\pm$ SEM.

FIG. 1. Photos of lung sections of Zucker rat after immunohistochemistry with γ -H2AX primary antibody detected with an anti-rabbit horseradish peroxidase-conjugated secondary antibody: (A, B), two areas showing several γ -H2AX-positive nuclei; (C, D), two areas showing only hematoxylin-stained nuclei (no γ -H2AX expression). (A–C), 400 $\times$ magnification; (D), 200 $\times$ magnification.

Statistical analysis

Data were then statistically elaborated by ANOVA test to detect differences among the rat groups using Statview 5.0.1 software released for Windows Statistical (SAS Institute, Inc., Cary, NC). Differences between more than two independent groups were analyzed by Fisher's test after ANOVA. The results were expressed as mean $\pm$ SEM; p -value was considered significant when <0.05 .

First-order regression analysis was also used to assess correlation among the biomarkers studied.

Results

Body weight, glycemia, and insulin level determination

Body weight, glycemia, and insulinemia were evaluated in both obese Zucker rats and control lean animals. As reported in Figure 2, the obese rats as a whole were 20% heavier (362 ± 9 g) than the CO group (294 ± 11 g) and showed higher levels of baseline blood glucose (28%, $p=0.05$) and insulin (59%, $p=0.003$). Glycemia values of CO_{fc} and O_{fc} rats were 101 ± 15 and 299 ± 44 mg/dL, whereas those of CO_{AH} and O_{AH} were 324 ± 22 and 398 ± 46 mg/dL (data not shown). To assess insulin resistance in lean and obese animals, the HOMA index was measured. Fasting blood glucose and insulin levels were used to determine the HOMA index using the standard formula: [fasting insulin (μ IU/mL)*fasting glucose (mg/dL)]/405. Obese Zucker rats displayed elevated

HOMA-IR levels compared with lean Zucker rats at fasting conditions (CO_{fc}: 5.32 ± 2.07 ; O_{fc}: 41.28 ± 9.5). To avoid confounding effect due to the bolus of glucose in the hyperglycemic animals, the HOMA-IR was measured only in the fasting group (CO_{fc} and O_{fc}). The CO and O rat groups were studied under fasting conditions or during acute hyperglycemia for 3 h investigating the ability to response to a load of glucose (Fig. 3).

Assessment of Real-Time PCR conditions and gene expression profiling

To optimize the thermocycle profile of each gene, optimal annealing temperature and RNA concentration were assessed for each designed PCR primer. Dilution series were run for all candidate genes to quantify Real-Time PCR efficiency that resulted in the range of 95–105% and a linear standard curve, R^2 , greater than [0.990]. The global changes in mRNA expression of the ET-1 system in the lungs of both obese and lean control Zucker rats are reported in Figure 4. Higher *prepro-ET-1* ($p=0.05$) and *ECE-2* mRNA ($p=ns$) expression was observed in O with respect to CO. *ECE-1* mRNA expression resulted undetectable in the lung tissue of Zucker rats, whereas *ET-A* and *ET-B* expressed at similar levels in both CO and O. In Figure 5, the log₂-fold change values related to *ET-1*, *ECE-2*, *ET-A*, and *ET-B* were reported. When the two groups were further subdivided into fasting and hyperglycemic rats, no significant

FIG. 2. Levels of body weight, blood glucose and insulin in CO and O rats. CO, control lean; O, obese.

FIG. 3. Area under curves (AUC) for glucose in control lean and obese Zucker rats during fasting (CO_{fc} and O_{fc}) or acute hyperglycemia (CO_{AH} and O_{AH}).

difference was observed in the transcriptomic profile of ET-1 system between CO and O, although O_{AH} showed a more marked expression pathway than in all other groups except for *ET-B* gene (Fig. 6). A significant difference between O_{fc} and O_{AH} was observed for *prepro-ET-1* ($p=0.006$),

FIG. 5. Fold change values related to *ET-1*, *ECE-2*, *ET-A*, and *ET-B*.

ECE-2 ($p=0.035$), and *ET-A* ($p=0.048$) (Fig. 6). In the whole rat population, a significant positive association was observed between *prepro-ET-1* and *ET-A* ($p=0.009$). After stratifying animals according to obesity, we observed that the correlation remained at a significant level only in the obese group ($p=0.007$) (data not shown).

γ -H2AX in the lung tissue

The results of the γ -H2AX assay performed in the lungs of the fatty and lean animals are graphically visualized in

FIG. 4. mRNA expression of *prepro-ET-1*, *ECE-2*, *ET-A*, and *ET-B* in pulmonary tissues of control lean and obese Zucker rats. *ECE*, endothelin-converting enzyme; *ET*, endothelin.

FIG. 6. mRNA expression of *prepro-ET-1*, *ECE-2*, *ET-A*, and *ET-B* in pulmonary tissues of control lean and obese Zucker rats during fasting (CO_{fc} and O_{fc}) and acute hyperglycemia (CO_{AH} and O_{AH}). fc, fasting; AH, acute hyperglycemia.

Figures 7 and 8. We found that the average value of the obese group, as a whole, was significantly ($p=0.019$) higher (0.090 ± 0.018) than that of the control group (0.020 ± 0.021). When the data were viewed in the animals categorized only according to their glycemia level (irrespective of obesity), we did not observe statistically significant differences between fasting rats (0.071 ± 0.020) and rats with acute hyperglycemia (0.047 ± 0.018). Interestingly, both O_{fc} (0.085 ± 0.023) and O_{AH} (0.099 ± 0.029) rats showed higher levels of nuclear phosphorylation than the corresponding lean rats (0.036 ± 0.036 and 0.013 ± 0.024 , respectively), although significance was reached only among hyperglycemic animals ($p=0.038$). However, γ -H2AX levels of fasting and hyperglycemic animals stratified by their obesity condition did not differ significantly from each other. No correlation was observed between γ -H2AX levels and HOMA (data not shown).

Discussion

The data obtained in the present study showed increased transcriptional levels of ET-1 system in obese animals. The subdivision in fasted and hyperglycemic rats stressed the higher levels of expression of the genes analyzed in the lung tissue of hyperglycemic rats compared to those in the fasting group, confirming that glucose can interfere with the production of *ET-1*, by stimulation, as previously reported (Wu *et al.*, 2000). We also observed increased levels of DNA damage, in the form of DSB, in the lungs of obese rats. Recently, the ET axis has been shown to have a role in the growth and progression of several tumor types, including lung cancer (Bhalla *et al.*, 2009). Solid tumors that use the ET axis can be grouped into two distinct groups. First, there are those that overexpress *ET*, upregulate *ET-A*, and downregulate *ET-B* receptors. The second group consists of those that overexpressed *ET-1* and upregulated *ET-A* and

FIG. 7. Levels of γ -H2AX in Zucker rats classified according to obesity condition (lean, CO; obese, O) or glycemia status (fasting, fc; acute hyperglycemia, AH). $p=0.019$, O rats versus CO rats (ANOVA test).

FIG. 8. Levels of γ -H2AX in lean (CO) and obese (O) Zucker rats stratified according to glycemia status (fasting, CO_{fc} and O_{fc} ; acute hyperglycemia, CO_{AH} and O_{AH}). $p=0.038$, O_{AH} rats versus CO_{AH} rats (ANOVA test).

ET-B receptors, such as lung tumors (Ahme *et al.*, 2000). The rat model used in this study is a valuable model for the study of obesity in humans since it offers many features similar to those of human obesity, such as moderate hypertension, hypertriglyceridemia, and insulin resistance, all features of metabolic syndrome (Stapleton *et al.*, 2008). In this study, the overexpression of *ET-1*, together with the *ET-A* and *ET-B* upregulation, was observed in *O_{AH}*, in line with previous studies on solid tumors (Nakamuta *et al.*, 1993; Kajima and Nihei, 1995; Nelson *et al.*, 1996; Shankar *et al.*, 1998; Bagnato *et al.*, 1999). Interestingly, the positive correlation observed in obese rats between expression of *ET-1* and *ET-A* receptor mRNA suggests that the condition of obesity may drive the activity of *ET-1* in the lung mainly toward vasoconstriction. With regard to DSB and glycometabolic conditions, it would seem that hyperglycemia predisposes the lungs of rats, especially obese animals, to undergo DNA lesions to a greater, although not to a significant extent, than during fasting conditions. Consistent with our findings, activation of the DDR response has been reported in a multistep inflammation-based lung cancer rat model, with higher levels of γ -H2AX being observed in precancerous tissues than in tumors (Blanco *et al.*, 2007). Even more relevant is a recent study in which, compared to normal weight controls, an eight-fold increase in γ -H2AX nuclear foci was observed in peripheral lymphocytes of overweight/obese children and adolescents; after repair of the initial amount of DNA lesions took place, the prevalence of genetically unstable cells harboring micronuclei was reduced to only two-fold (Scarpato *et al.*, 2011). This confirms that the obesity condition is sustained by a chronic systemic inflammation, as the generation of endogenous molecules that are able to damage the DNA of the cells is not limited to the adipose tissue alone but can also occur and/or diffuse to other organs (i.e., the lung). To support the data obtained here, recent studies indicate that the number of individuals suffering from asthma or other respiratory pathologies would correlate with the increasing number of obese subjects (Rosenkranz *et al.*, 2010; Sutherland *et al.*, 2012). Thus, an obesity-dependent inflammation of the respiratory tract, where the distribution of fat mass plays a central role (Scott *et al.*, 2012), can explain the significant difference in the degree of DNA damage between lung cells of obese and lean rats.

Our findings are in line with the observation that human umbilical vein endothelial cells grown at high-glucose concentrations exhibited a greater number of γ -H2AX-positive nuclei than cells grown at normal glucose levels, this being the consequence of overproduction of the p300 transcriptional coactivator together with its increased binding to *ET-1* (Chen *et al.*, 2010).

Conclusion

The study shows an inappropriate secretion of *ET-1* in *O* animals with a parallel DNA damage in their lungs, providing novel mechanisms by which *ET* receptor antagonist may exert organ protection.

Limitations

The major limitation of this study is the lack of a concomitant evaluation of protein levels: the relatively small amount of tissue available has necessarily led us to focus on the transcriptomic pattern and to the evaluation of DNA

damage. It is interesting to note that in a previous study of ours (Cabiati *et al.*, 2014), carried out in Zucker rat cardiac tissue, we observed that the pattern obtained at the mRNA level was confirmed by ET protein concentration in the heart of CO and O. Moreover, to avoid confounding effects related to hormonal cycle, the study was performed using only male animals.

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Disclosure Statement

No competing financial interests exist.

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