A Novel Rat Model with Obesity-Associated Retinal Degeneration

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PURPOSE. A strong association between retinal degeneration and obesity has been shown in humans. However, the molecular basis of increased risk for retinal degeneration in obesity is unknown. Thus, an animal model with obesity and retinal degeneration would greatly aid the understanding of obesity-associated retinal degeneration. The retinal abnormalities in a novel rat model (WNIN-Ob) with spontaneously developed obesity are described.

METHODS. Histologic and immunohistochemical examination were performed on retinal sections of 2- to 12-month-old WNIN-Ob rats, and findings were compared with those of lean littermate controls. RNA from retinas of 12-month-old WNIN-Ob and lean littermate rats was used for microarray and qRT-PCR analysis.

RESULTS. The WNIN-Ob rats developed severe obesity, with an onset at approximately 35 days. Evaluation of retinal morphology in 2- to 12-month-old WNIN-Ob and age-matched lean littermate controls revealed progressive retinal degeneration, with an onset between 4 to 6 months of age. Immunohistochemical analysis with anti-rhodopsin, anti–cone opsin, and PSD-95 antibodies further confirmed retinal degeneration, particularly rod cell loss and thinner outer plexiform layer, in the obese rat retina. Gene expression by microarray analysis and qRT-PCR established activation of stress response, tissue remodeling, impaired phototransduction, and photoreceptor degeneration in WNIN-Ob rat retina.

CONCLUSIONS. WNIN-Ob rats develop increased stress in retinal tissue and progressive retinal degeneration after the onset of severe obesity. The WNIN-Ob rat is the first rat model to develop retinal degeneration after the onset of obesity. This novel rat model may be a valuable tool for investigating retinal degeneration associated with obesity in humans. (Invest Ophthalmol Vis Sci. 2009;50:3456–3463) DOI:10.1167/iovs.08-2498

The problems of excess weight and obesity have been recognized globally both in developed and in developing countries.1–3 Excess weight is the second leading modifiable risk factor for death in the United States,3,6 where 64% of adults age 20 years and older are overweight and 30% are obese. In the past 20 years, the rates of obesity have tripled in developing countries.6,7 Eye problems are probably the latest addition to the list of complications associated with obesity.1,2 The ocular complications of obesity include diabetic retinopathy, high intraocular pressure, cataracts, macular degeneration, floppy lid syndrome, and exophthalmos.1,8 Although these complications can have serious consequences, the effects of overweight and obesity on the eye are not well known to patients or to health care providers.

There is evidence of the increased risk for retinal degeneration and diabetic retinopathy in obese persons. Obese men are more than twice as likely to have dry macular degeneration than men with a normal basal metabolic index.9 An association between higher basal metabolic index and early macular degeneration has been reported.10 Similarly, abdominal obesity in patients with early or intermediate stages of macular degeneration increases the risk for progression to advanced macular degeneration.11 Reduction in the macular pigments, lutein, and zeaxanthin in obese persons further supports the association between obesity and retinal degeneration.12 Patients with Bardet-Biedl syndrome (BBS) develop retinal dystrophy, obesity, polydactyly, renal malformation, and learning disabilities.13,14 In several populations, obesity is one of the basic components of metabolic syndrome, which is implicated in microvascular changes in the retina.15

Retinal degeneration, including age-related macular degeneration (AMD) and diabetic retinopathy (DR), is a major cause of irreversible blindness in the developed and the developing world.16,17 The alarming increase in the prevalence of obesity further exacerbates the concern about retinal degeneration. In the past 20 years, considerable progress has been made in our understanding of the molecular basis of retinal dystrophies. However, there is no experimental evidence to establish the mechanism by which obesity increases the risk for retinal...
dystrophies. Although retinal abnormalities are found in tubby mouse and mouse models of BBS, obesity develops along with or much later than retinal changes. Therefore, an animal model that develops retinal degeneration after the onset of obesity, similar to obesity-associated retinopathy in patients, would immensely aid our basic understanding of the relationship between retinal degeneration and obesity.

Wistar is the oldest strain of rats used in biomedical research. The National Institute of Nutrition (NIN) in India has an inbred stock of Wistar rats dating back to 1920 that is christened WNIN (Wistar maintained at NIN). A spontaneously developed obese rat was isolated from WNIN rats, and a colony of WNIN-Obese (WNIN-Ob) rats was generated by selective breeding. These rats (Fig. 1(i)) are maintained at the National Center for Laboratory Animal Sciences at NIN. The inheritance pattern and the biochemical and phenotypic characteristics of obesity in WNIN-Ob rats have been characterized in detail. Starting at 35 to 40 days of age, rats of the WNIN-Ob phenotype are different from their lean littermates in terms of body weight. Their body weight increased progressively until the age of 12 months (Fig. 1(ii)). By this age they weigh as much as 1.2 kg, in contrast to their lean littermates that weigh 500 to 600 g. WNIN-Ob rats demonstrate low fertility, and their average lifespan is 20 to 24 months. The colony is maintained by mating carriers (+/−), which, on crossing, gives three genotypes: lean (+/+/), carrier (+/−), and WNIN-Ob (−/−)—in a classical Mendelian ratio of 1:2:1, respectively. The present study describes retinal degeneration in this spontaneous obese rodent model.

MATERIALS AND METHODS

Reagents and Antibodies

The details of antibodies used for this study are as follows: rabbit-anti-opsin, red/green (1:250 dilution; Chemicon, Temecula CA), mouse monoclonal anti-rhodopsin (1:200 dilution; Chemicon), mouse postsynaptic density protein-95 (PSD-95; 1:250 dilution; Sigma-Aldrich, St. Louis, MO), anti-rabbit Alexa Fluor-555 (1:2500 dilution; Invitrogen-Molecular Probes, Carlsbad, CA), and anti-rabbit Alexa Fluor-488 (1:1000 dilution; Invitrogen-Molecular Probes).

Animals and Tissue Collection

All procedures involving rats were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Ethics Committee at the National Institute of Nutrition. Animals were kept in a 12-hour light/12-hour dark cycle with ambient light intensity and temperature at the National Center for Laboratory Animal Science, National Institute of Nutrition. Two- to 12-month-old WNIN-Ob rats and their lean littermates were fasted overnight before they were euthanatized at the end of the dark cycle. All studies described here were carried out by evaluating at least three animals.

Histology and Immunohistochemistry

Eyeballs were collected from 2- to 12-month-old animals and fixed in 4% paraformaldehyde in phosphate-buffer (pH 7.2), followed by embedding and sectioning for plastic and cryosections using standard protocols, as described previously. Three eyes were used for each age group. Plastic sections were stained with hematoxylin and eosin. Immunohistochemistry was performed on cryosections using respective primary antibody, as described previously. The slides were mounted in antifade reagent containing DAPI (ProlongGold; Invitrogen) and were observed under an epifluorescence microscope (E800; Nikon, Tokyo, Japan). Images were captured using appropriate filters. Higher magnification images were captured with a confocal microscope (Zeiss, Oberkochen, Germany).

RNA Preparation

Retinas dissected from the eyes of WNIN-Ob rats and age-matched littermate controls were collected in reagent (Trizol; Invitrogen) for RNA isolation. RNA was isolated according to the manufacturer’s instructions.

Global Gene Expression by Microarray Analysis

Five micrograms of total retinal RNA isolated from two pooled retinas was used to synthesize double-stranded cDNA with the use of first- and second-strand cDNA synthesis kits (Affymetrix, Santa Clara, CA) ac-
According to the manufacturer’s guidelines. Purification of ds-cDNA, labeling, hybridization, washing, and staining were carried out according to the Affymetrix protocol. The rat gene expression chip (230.20; Affymetrix) was used for hybridization. Three independent samples for each WNIN-Ob and control group were analyzed.

Gene chips were scanned (Microarray Suite 5.0 [Affymetrix] operated by GCOS software version 2.0), and expression data were normalized with the use of robust multiarray average. Data were based on three gene chips per each control and WNIN-Ob groups. Ratios of average signal intensity (log2-transformed data) were then calculated for the probe sets (WNIN-Ob relative to lean control) and were converted to fold change. The contrast between experimental and control was extracted selecting those genes with an adjusted P of 0.05 and a threefold difference.

**Quantitative Real-Time PCR**

To remove any trace of genomic DNA, RNA was treated with RNase free DNase (RQ1; Promega, Madison, WI) according to the manufacturer’s protocol. Equal amounts of RNA (7 μg) were reverse transcribed for each sample (control and WNIN-Ob) using oligo-dT (12–18) primer to first-stand cDNA with reverse transcriptase (SS II; Invitrogen) according to the manufacturer’s recommendations. Quantitative real-time PCR was then performed on cDNA templates using gene specific primers, as described previously. Specificity of primers was confirmed by melt curve analysis and by gel electrophoresis. Primer sequences are available from the authors on request. PCR data were analyzed to estimate the relative expression of different genes based on the difference in threshold cycle (Ct) between control and WNIN-Ob groups after normalization to various housekeeping genes—hypoxanthine phosphoribosyltransferase (Hgprt), β-actin, succinate dehydrogenase (SdhA), and ribosomal protein L19 (Rpl19)—as described.

**RESULTS**

**Morphologic Evaluation**

We have evaluated the retinal morphology of 2- to 12-month-old WNIN-Ob rats and compared our findings with those of lean littermate controls. No significant changes were observed in the morphology of 2-month-old WNIN-Ob animals compared with controls (Figs. 2(i), A, B), whereas the retinas of 4- to 12-month-old WNIN-Ob rats had significant alterations. At 4 months, the thickness of the outer nuclear layer (ONL) in the central retina of WNIN-Ob rats was reduced to approximately 7 to 8 nuclei compared with 10 to 12 nuclei in lean littermate controls (Figs. 2(i), C, D). The retinal changes found in WNIN-Ob rat at 6 months were not much different from the changes seen at 4 months age (data not shown). By 9 to 12 months, ONL thickness was reduced to 4 to 6 rows of nuclei in WNIN-Ob retinas compared with 10 to 12 nuclei inagematched lean littermate controls (Figs. 2(i), E, F). Evaluation of retinal cell loss in the central and peripheral regions of 2- to 12-month-old WNIN-Ob and control rat retina demonstrated progressive degeneration of the photoreceptor cells in the central retina. In peripheral retina, a concomitant decrease

![Figure 2](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933246/)
occurred in the number of photoreceptor nuclei (Figs. 2(ii), (iii)). Further histologic evaluation of retinas at age 9 to 12 months demonstrated abnormalities in the inner retinal layers. The gross structure of the inner retina did not demonstrate significant abnormalities at ages 4 to 6 months, but the thickness of the outer plexiform layer (OPL) and inner plexiform layer appeared to decrease in the 9- to 12-month-old WNIN-Ob rat retina compared with controls (Figs. 2(i), E, F). Concomitant with the decrease in the thickness of the OPL, a few photoreceptor nuclei appeared to migrate into this layer, suggesting disruption of its structure. By morphologic evaluation, the onset of retinal degeneration in the WNIN-Ob rat appeared to occur between 4 and 6 months age, and variation was observed in the severity of degeneration at a given age.

In addition to analyzing WNIN-Ob rats and lean littermate controls, we evaluated the retinal histology of 3- to 12-month-old WNIN parent strain rats. These rats did not show significant changes in retinal morphology (data not shown), suggesting that the retinal degeneration observed in the WNIN-Ob rat is neither sporadic nor a nonspecific observation in this strain of rats.

**Immunohistochemistry**

Immunohistochemical analysis of WNIN-Ob and lean littermate control rat retinas was carried out using rod and cone photoreceptor specific marker antibodies and anti-PSD-95 antibodies. Immunostaining with rhodopsin antibodies showed reduction in the brightness of the rhodopsin-specific signal in the WNIN-Ob rat retina compared with that of lean controls at 9 months (data not shown) and 12 months (Figs. 3A–D). Immunostaining with cone opsin (MW) antibodies indicated no significant alteration in the number of cones in the WNIN-Ob rat retina (Figs. 3E–H); staining the retinal sections with shorter wavelength cone opsin (SW) gave similar results (data not shown). The number of cones in the retinas of WNIN-Ob and lean control rats was found to be similar at ages 9 to 12 months (Fig. 3(ii)).

To further evaluate the decrease observed histologically in the thickness of OPL, we labeled the retinal sections from WNIN-Ob and control rats with postsynaptic density protein-95 (PSD-95) and compared the immunofluorescence (Fig. 4). In the 12-month-old WNIN-Ob rat retina, the immunofluorescence of PSD-95 was weaker (Figs. 4C, D) than the PSD-95 signal observed in age-matched lean littermates (Figs. 4A, B). This indicated a loss of synaptic terminals and further sup-

**FIGURE 3.** Immunohistochemical evaluation of rod and cone photoreceptor markers in WNIN-Ob rat compared with controls at 12 months. (i) Retinal sections of (A, C, E, G) control and (B, D, F, H) WNIN-Ob rats were labeled with (A–D) rhodopsin and (E–H) cone opsin. Nuclei are labeled with DAPI. Scale bar, 50 μm. (ii) Histogram demonstrates the number of cones in WNIN-Ob and control rat retinas. Values represent mean ± SD of three independent observations (P = 0.2).

**FIGURE 4.** Immunofluorescence images of 12-month-old control (A, B) and WNIN-Ob rat (C, D) retinal sections labeled with PSD-95. Labeling of PSD-95 is prominent and intense in OPL layer of controls, whereas in the WNIN-Ob rat the fluorescence signal of PSD-95 is less intense.
ported the observed reduction in the thickness of OPL in WNIN-Ob rat retina.

**Differential Expression of Genes in WNIN-Ob Retina**

Having observed retinal degeneration in the WNIN-Ob rat, microarray analysis was performed on the WNIN-Ob rat retina at age 12 months to assess the global gene expression profile of the WNIN-Ob rat retina under degenerative conditions. The rat gene expression chip (250.2.0; Affymetrix) used in this experiment contains 30,000 probes representing 26,000 genes. Based on absent and present calls, approximately 60% of the probe sets were reported as present. We have calculated the expression values (log2 transformed) for each gene using robust multarray average. Principal component analysis confirmed that biological replicate samples were grouped together. After gene filtering, 13,570 genes were identified by removing any that did not appear to be differentially expressed in any of the three independent samples in each group. We then extracted the contrast of experimental versus control, selecting those genes with an adjusted P of 0.05 and a threefold difference. This resulted in 423 genes, of which 369 showed lower levels of expression, whereas the expression of 54 genes was found to be significantly higher than in controls. Most downregulated genes were involved in phototransduction pathways or related to retinal structural proteins, carrier proteins, or transcription factors (Supplementary Table S1; both Supplementary Tables are online at http://www.iovs.org/cgi/content/full/50/7/3456/DC1). Most upregulated genes were related to apoptotic stress response and inflammatory mechanisms (Supplementary Table S2).

Quantitative real-time PCR analysis was carried out to validate the observations by microarray analysis and to verify the changes observed by histologic and immunohistochemical examination by quantifying the expression of selected retinal genes in 12-month-old lean and WNIN-Ob rats. Expression of rod-specific genes such as rhodopsin (*Rho*), rod transducin (*Gnat1*), rod arrestin (*Sag*), peripherin (*Rds*), retinal outer segment membrane protein (*Rom1*), and cGMP-dependent phosphodiesterase 6B (*Pde6B*) was found to be significantly lower in WNIN-Ob rat retina than in lean controls (Fig. 5). Levels of expression of the retinal transcription factor, cone-rod homeobox gene (*Crx*), and elongation of very long chain fatty acids 4 (*Elov4*) genes expressed in rods and cones also decreased significantly in 12-month-old WNIN-Ob rats (Fig. 6). Obesity-associated genes *tubby* and *Bbs7* also showed a lower level of expression in the retinas of WNIN-Ob rats than in those of lean controls (Fig. 6), but the decrease was not significant.

Interestingly, expression of cone cell marker genes, SW cone opsin (*Opn1SW*), MW cone opsin (*Opn1MW*), cone arrestin (*Arr3*), and cone transducin (*Gnat2*) was unaltered or significantly increased (Fig. 7). Expression of stress response genes, glial fibrillary acidic protein (*Gfap*), annexin-1, ceruloplasmin (*Cp*), hemeoxygenase-1 (Hypo-1), and vascular endothelial growth factor (Vegf) were significantly higher in 12-month-old WNIN-Ob rat retinas than in those of lean controls (Fig. 8). These results indicate the preservation of cone cells, degener-
mediated survival factors that are synthesized by rods.\textsuperscript{37-39} Therefore, this rat model with an altered cone cell number, despite significant rod cell loss, may aid in understanding possible means to preserve cones in the absence of rods.

Retinal abnormalities were observed in a few rodent models with obesity. The \textit{tubby} mice develop neurosensory abnormalities and obesity, but neurosensory abnormalities develop much earlier than the onset of obesity. The obesity of \textit{tubby} mice is relatively mild, has late onset, and progresses slowly.\textsuperscript{19,20} Mouse models for Bardet-Biedl syndrome develop obesity and retinal abnormalities in addition to a spectrum of other phenotypic features.\textsuperscript{20,21} Among other obese rodent models, ocular complications, particularly pronounced retinal changes, were found in diabetic obese (fa/la) rats when they were fed a sucrose diet for 68 weeks.\textsuperscript{22} Mice lacking functional tubby-like protein-1 (TULP-1) develop retinal degeneration but not obesity.\textsuperscript{43} The phenotype observed in WNIN-Ob rats is unique because of the early age of onset and the severity of obesity and retinal degeneration.

The pattern of gene expression in the WNIN-Ob rat retina supports photoreceptor degeneration. Downregulated genes appear to be involved predominantly in transcriptional regulation (\textit{Grx}, neural fold homeobox, high mobility group box, zinc finger proteins) and visual transduction (\textit{Gnb}, \textit{Gnat}, \textit{Cng}, \textit{Pde6b}, \textit{Rbo}, phosphatases, retinol binding protein). In addition, rod cell structural proteins (\textit{Rho}, \textit{Rom}, peripherin) were also observed to be significantly decreased. A number of these downregulated genes are also implicated in various forms of inherited retinal degeneration (RetNet, www.sph.uth.tmc.edu/Retnet/home.htm). Lower levels of expression of rod cell specific genes may reflect the loss of photoreceptors rather than specific downregulation of these genes.

The upregulated genes are mostly related to apoptotic, stress response, tissue remodeling pathways (annexin-1, cathepsin, glutathione S-transferase, \textit{Cp}, \textit{Gfat}) indicating activation of the stress response in the WNIN-Ob rat retina compared with the lean littermate control rat retina. The expression pattern of some genes observed in the WNIN-Ob rat retina is similar to the gene expression changes observed in \textit{rd1} mice and injured rat retina.\textsuperscript{44,45} Levels of expression of obesity-related genes, tubby-like, and Bbs7 were found to be lower on microarray and qRT-PCR analysis, indicating a possible direct role for these obesity genes in maintaining normal retinal physiology.\textsuperscript{41-43,46} Unlike many studies involving microarray analysis, we found that only a limited number of genes are differentially expressed in the Ob-rat retina compared with lean littermate controls. Most of the genes showing altered expression are likely to have a significant role in retinal physiology. The expression profile of genes observed in the WNIN-Ob rat retina is similar to the findings reported in \textit{Bbs4} knockout mice.\textsuperscript{43} These observations suggest a direct association between obesity and retinal degeneration in these rats.

Further studies are needed to determine the causal relationship between obesity and retinal degeneration in the WNIN-Ob rat and to determine whether obesity, either in itself or through its metabolic consequences, alters retinal structure and function in these rats. No reports relate metabolic changes (similar to those seen in this model, such as hyperphagia, hypertriglyceridemia, hypercholesterolemia) to retinal changes, particularly photoreceptor degeneration, in a temporal manner. Nevertheless, studies are under way in our laboratories wherein we are inducing these metabolic changes in the parent strain of rat, which are not obese, by manipulating the diet. The effect of these metabolic changes without overt obesity might help us to address their relationship with retinal degeneration. This animal model may aid in investigating the association between obesity and retinal degeneration.
and the possibility of modifying obesity-associated risk by altering diet. The WNN1-Ob rat may thus be explored in greater detail for its usefulness as a model to study obesity-associated retinal degeneration. Evaluation of retinal vasculature and lipofuscin content in WNN1-Ob rats may provide valuable information. Given the impact of retinal dystrophies on blindness and the increased risk for retinal dystrophy caused by obesity, it is highly desirable that we understand the molecular basis of obesity-associated retinal degeneration. In this context, the observations made in this study provided such an opportunity.

Although obesity may be a risk factor for many ocular conditions, including retinal degeneration, the present literature is inadequate in establishing any convincing association. Nevertheless, the reduced levels of macular pigments in obese persons support the association of increased risk for retinal degeneration in this population. Reductions in macular lutein and zeaxanthin may be caused by decreased dietary intake in obese persons or by competition between retina and adipose tissue for the uptake of lutein and zeaxanthin. However, whether weight loss, by physical activities and dietary manipulations, reduces the risk for eye diseases remains unresolved. Because of the potential public health impact of obesity, there is a greater need to understand its ocular effects. The WNN1-Ob rat may aid investigators to address these issues.

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References


