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Research Report

Growth Hormone Signaling Shapes the Impact of Environmental Temperature on Transcriptomic Profile of Different Adipose Tissue Depots in Male Mice

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Abstract

Growth hormone receptor knockout (GHRKO) mice are smaller, long living, and have an increased metabolic rate compared with normal (N) littermates. However, it is known that thermoneutral conditions (30–32°C) elicit metabolic adaptations in mice, increasing the metabolic rate. Therefore, we hypothesized that environmental temperature would affect the expression profile of different adipose tissue depots in GHRKO mice. For this, N (n = 12) and GHRKO (n = 11) male mice were maintained at 23 or 30°C from weaning until 11 months of age. RNA sequencing from adipose tissue depots (epididymal—eWAT, perirenal—pWAT, subcutaneous—sWAT, and brown fat—BAT) was performed. Thermoneutrality increased body weight gain in GHRKO mice, but not in N mice. Only a few genes were commonly regulated by temperature in N and GHRKO mice. The BAT was the most responsive to changes in temperature in both N and GHRKO mice. BAT *Ucp1* and *Ucp3* expression were decreased to a similar extent in both N and GHRKO mice under thermoneutrality. In contrast, eWAT was mostly unresponsive to changes in temperature. The response to thermoneutrality in GHRKO mice was most divergent from N mice in sWAT. Relative weight of sWAT was almost 4 times greater in GHRKO mice. Very few genes were regulated in N mice sWAT when compared with GHRKO mice. This suggests that this WAT depot has a central role in the adaptation of GHRKO mice to changes in temperature.

Keywords: GHRKO, UCP1, Gene expression

Growth hormone receptor knockout (GHRKO) mice are GH resistant, have decreased production of insulin-like growth factor 1 (IGF-1), and reduced adult body size (1,2). A striking characteristic of these mice is the increased life span and reduction of age-related diseases (2,3). GHRKO mice are obese compared with normal mice (1), with a disproportionate increase in subcutaneous white adipose tissue (sWAT) (4). Surprisingly, although visceral adipose tissue removal increases insulin sensitivity in normal mice, it decreases it in GHRKO mice (1,5).

The GHRKO mice have increased brown adipose tissue (BAT) depot (1,6) along increased expression of uncoupling protein 1 (UCP1) and genes related to thermogenesis (6). UCP1 uncouples the

oxidative phosphorylation chain from ATP production and therefore generates heat, affecting energy expenditure (7). The greater mass of BAT in GHRKO mice is associated with higher metabolic rate (6), reduced respiratory quotient (RQ), and increased heat generation (8). In the absence of GH signaling, the WAT and BAT gene expression signatures are altered, consistent with the increased metabolic activity in BAT (9,10). These changes are suspected to contribute to the extended longevity of GHRKO mice (6).

Mice experience a mild to moderate degree of stress at the standard animal room temperature of 20–23°C and activate thermogenesis to maintain body temperature (11), increasing the metabolic rate (11) and food consumption (12). GH-deficient mice exposed to thermoneutrality had reduced expression of genes associated with thermogenesis and energy expenditure, and morphological changes in BAT (13). Although GHRKO mice have higher VO_2 and decreased RQ, their body temperature is slightly reduced (14). However, a recent study showed that when GHRKO mice were subjected to thermoneutrality, their extended longevity was sustained in males and females had a slightly longer life span (15). Based on these data, we hypothesized that environmental temperature will exert tissue-specific effects on the expression profile of different adipose tissue depots in GHRKO mice.

Method

Animals and Tissue Collection

Normal (N; $n = 12$) and GHRKO ($n = 11$) male mice were bred and maintained under light-controlled conditions (12-hour light/12-hour dark cycle). Half of the mice were maintained at 23°C (room temperature, RT), and half were maintained at 30°C (warm room, WR) since weaning until euthanasia. At 10–11 months of age, mice were anesthetized and euthanized after overnight fasting, and the adipose tissue depots (epididymal—eWAT, perirenal—pWAT, subcutaneous—sWAT, and brown fat—BAT) were dissected, weighed, immediately frozen on dry ice, and stored at −80°C. Just before euthanasia, body weight and fasting glucose levels were measured. All animal procedures employed in the presented work were approved by and performed in accordance with the guidelines from the Laboratory Animal Care and Use Committee (LACUC) at the Southern Illinois University School of Medicine (Springfield, IL).

RNA Extraction and mRNA Sequencing

Full details of RNA-Seq methods are provided in Supplementary Material. Total RNA was extracted using a commercial column purification system (miRNeasy Mini Kit, Qiagen) and on-column DNase treatment (RNase-free DNase Set, Qiagen) following manufacturer's instructions. Transcriptomic profile of individual samples was performed using commercial RNA-sequencing kits (NEBNext mRNA Library Prep Master Mix and NEBNext Multiplex Oligos for Illumina, New England Biolabs, Ipswich, MA) and adapted according to previous descriptions (16).

All RNA-Seq data are available at the Sequence Read Archive (SRA) at NCBI under accession number PRJNA734016. The mapping of sequencing reads to the mouse transcriptome and mRNA abundance was performed as our previous publications (16). mRNAs were further processed for pathway analysis using the Generally Applicable Gene-set Enrichment (GAGE), for enrichment of KEGG pathways and gene ontology (GO) terms (biological processes, molecular function, and cellular component).

Statistical analyses for differentially expressed mRNAs were performed using the software R (3.2.2). Genes with a false discovery rate (FDR) < 0.05 and fold change (FC) > 2.0 were considered upregulated and with FDR < 0.05 and FC < 0.5 were considered downregulated.

Results

Body and Adipose Tissue Weight and Glucose Levels

Environmental temperature did not affect body weight in N mice, but GHRKO mice housed at 30°C were 22% heavier than those housed at 23°C (Figure 1A). Fasting glucose concentrations were lower in GHRKO mice than in N mice and were also reduced in mice housed at 30°C (Figure 1B). The relative weight of the BAT depot (%, g of fat/g of body weight) was increased in both N and GHRKO mice housed

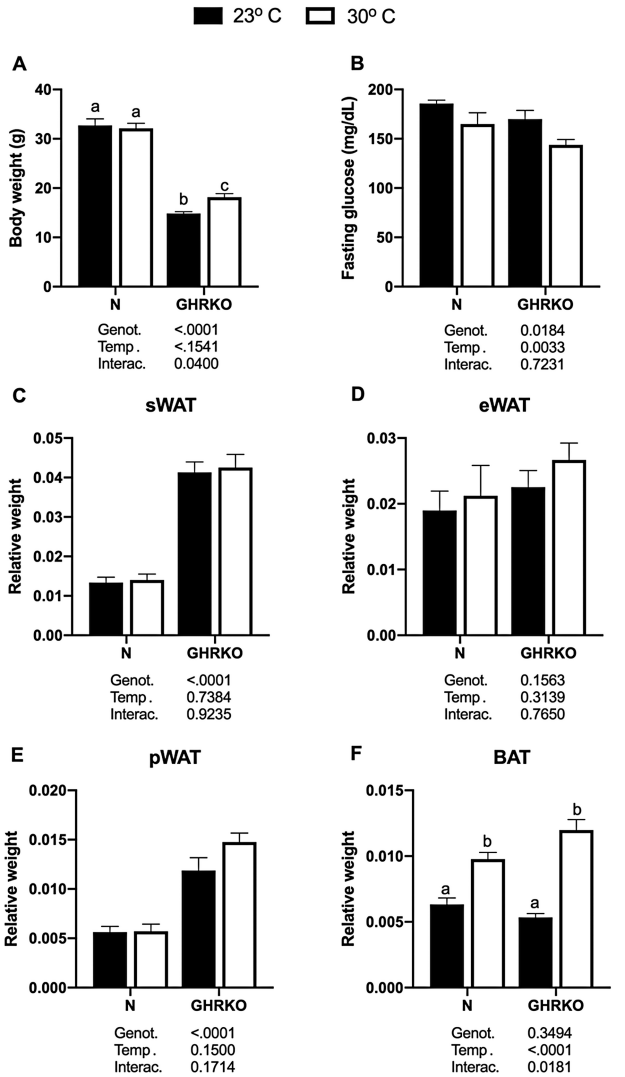

Figure 1. Body weight (A), fasting glucose (B), relative weight for subcutaneous (C), epididymal (D), perirenal (E), white adipose tissue (eWAT, pWAT, and sWAT, respectively), and brown adipose tissue (BAT; F) for normal (N) and growth hormone receptor knockout (GHRKO) mice maintained at 23 or 30°C since weaning until 11 mo of age. Different letters indicate statistical difference at $p < .05$.

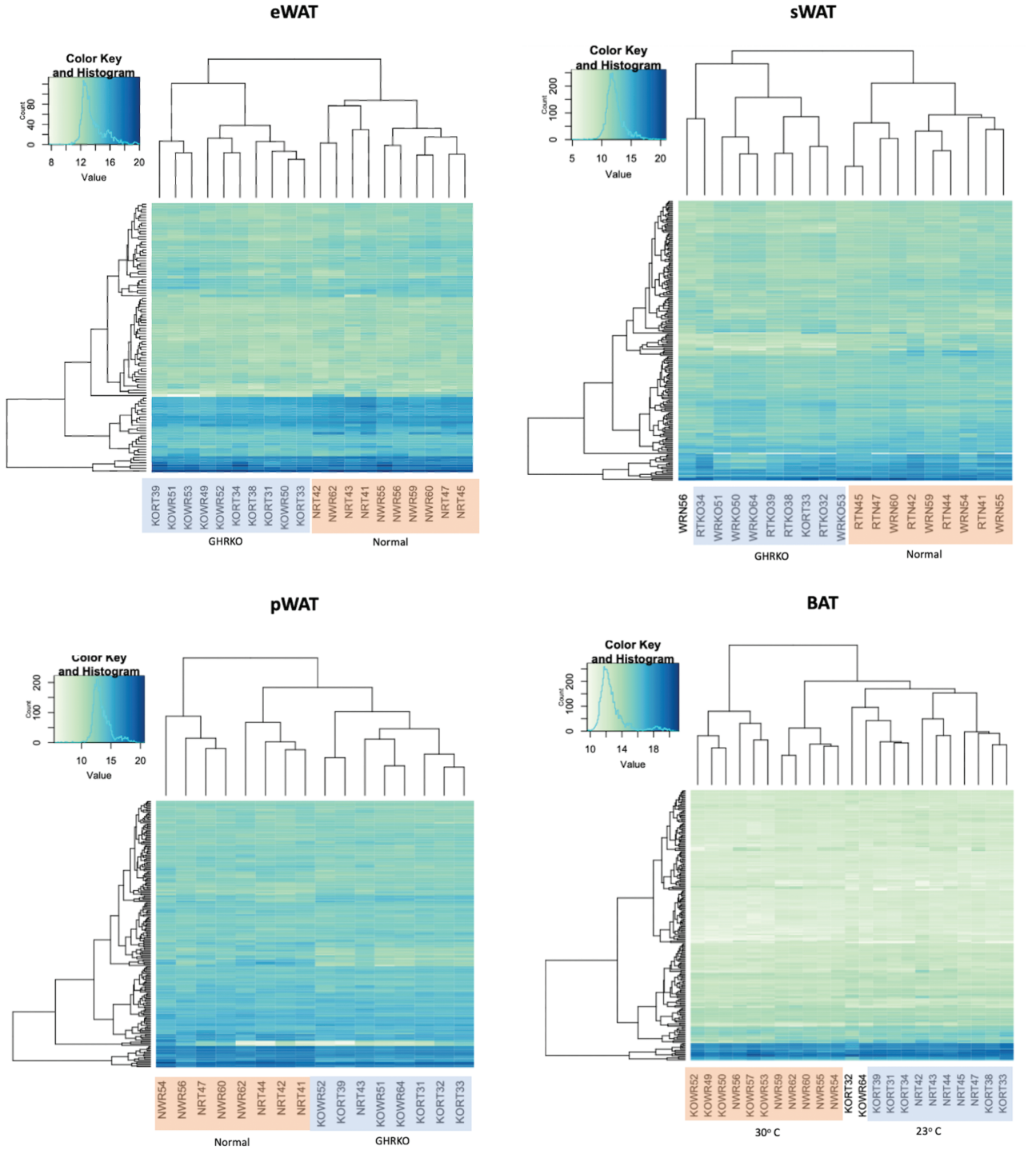

Figure 2. Unsupervised hierarchical clustering of expression levels for the top 200 most expressed genes in different adipose tissue depots of normal (N) and growth hormone receptor knockout (GHRKO) mice maintained at 23°C (room temperature, RT) or 30°C (warm room, WR) since weaning until 11 months of age.

at 30°C (Figure 1F). The sWAT and pWAT depots were heavier in GHRKO mice than in N mice, but their relative weight was not affected by temperature (Figure 1C and E). Relative weight of eWAT was not affected by either genotype or temperature (Figure 1D).

Transcription Pattern Among Adipose Tissue Depots

Results of principal component analysis for the most variable genes in different fat tissue depots, independent of temperature or genotype, identified a clear signature for each adipose tissue depot (Supplementary Figure 1). When each fat tissue depot was analyzed individually by unsupervised hierarchical clustering (Figure 2), there was a clear separation between GHRKO and N mice for eWAT and sWAT depots. In the pWAT and BAT depots, the separation between genotypes was less clear. Only for BAT, there was a clear general change of pattern for temperature, suggesting this was the tissue most affected by temperature changes.

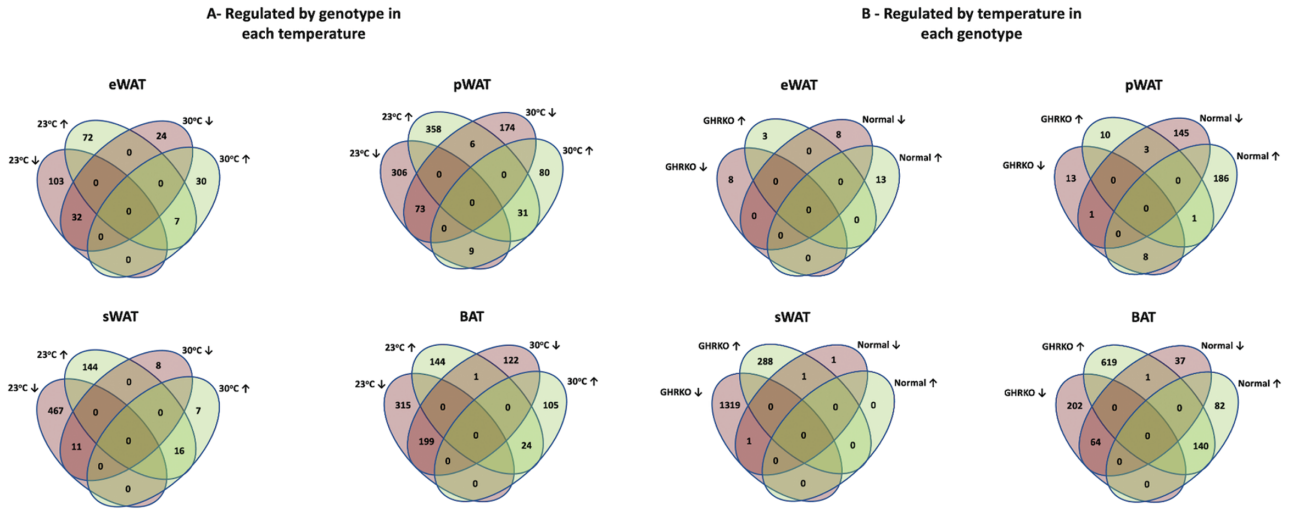

Figure 3. Venn diagrams for the number of differentially expressed genes (FDR < 0.05 and FC > 2 or FC < 0.5) by (A) genotype and (B) temperature in different adipose tissue depots of normal (N) and growth hormone receptor knockout (GHRKO) mice maintained at 23°C (room temperature, RT) or 30°C (WR) since weaning until 11 mo of age.

Differentially Expressed Genes According to Genotype and Temperature

In each of the examined adipose tissue depots, the number of differentially expressed genes regulated by GH resistance (GHRKO vs. N) was greater at 23°C than at 30°C (Figure 3A). Comparison of common genes regulated by GH resistance in different fat tissue depots identified only one gene, *Peg3*, which was commonly upregulated in all 4 tissues at 23°C. In contrast, 16 genes were commonly downregulated by GH resistance in all tissues at 23°C: *Scd2*, *Cish*, *Ghr*, *Ptges*, *Me1*, *Fabp5*, *Igf1*, *Acly*, *Slc1a3*, *Trf*, *Aqp11*, *Adcy5*, *Nabp1*, *Mogat2*, *Orm1*, and *Slc25a1*. At 30°C, we did not detect any genes that were commonly upregulated across the 4 depots and only 2 genes that were commonly downregulated by GH resistance, *Cyp2e1* and *Igf1*.

The number of genes regulated by exposing N or GHRKO mice to 30°C for each adipose tissue depot is shown in Figure 3B. It is clear that eWAT was mostly unaffected by temperature in both N and GHRKO mice. In contrast, BAT had the highest number of regulated genes in both N and GHRKO mice. Additionally, there was little overlap of genes regulated by temperature in both N and GHRKO mice, suggesting different mechanisms of adaptation. Surprisingly, in sWAT, there were only 2 genes regulated by temperature in N mice, whereas in GHRKO mice, there were 1 609 regulated genes.

KEGG Pathways Regulated According to Genotype and Temperature

The regulated pathways for each genotype are presented in Supplementary Tables 1–8. In mice exposed to 23°C, the proteasome pathway was commonly downregulated by GH resistance in all 4 adipose tissue depots, whereas there were no commonly upregulated pathways. At 30°C, the cardiac muscle contraction and oxidative phosphorylation pathways were commonly downregulated by GH resistance, and no pathways were commonly upregulated in all tissues.

No pathways were commonly regulated by temperature in all 4 adipose tissue depots and in both genotypes. The sWAT of GHRKO mice was surprisingly very responsive to thermoneutrality, with downregulation of several pathways related to immune response. This included chemokine signaling, cell adhesion molecules, antigen processing and presentation, as well as T- and B-cell receptor signaling (Supplementary Table 7). Of the examined depots, BAT was most affected by thermoneutrality independent of genotype. The commonly upregulated pathways included DNA replication, phagosome, cell adhesion molecules, complement, and coagulation cascades, whereas the commonly downregulated pathways included citrate cycle, fatty acid metabolism, oxidative phosphorylation, pyruvate metabolism, and PPAR signaling (Supplementary Table 6).

Discussion

The GHRKO mice are long lived, and many beneficial health effects of GH resistance have been linked to adipose tissue metabolism. In these mice, thermogenesis is stimulated, presumably in response to increased radiational heat loss. Therefore, it was of interest to determine how thermoneutral conditions would affect BAT and WAT transcriptomic signatures. Novel key findings in the present study suggest that GH signaling mediates the response to thermoneutrality, as few genes were commonly regulated by temperature in N and GHRKO mice.

In our earlier study, the extended longevity of male GHRKO mice was sustained when chronically housed under thermoneutral conditions (15). This, along with evidence from the current study, suggests that adaptation of fat tissue depots to thermoneutral conditions may facilitate maintenance of GHRKO mice metabolic advantages. We have confirmed that GHRKO mice have increased relative weight of sWAT and pWAT (17) and that BAT relative weight increases in thermoneutral conditions in GHRKO mice (15). In the present study, the BAT weight increase in GHRKO mice was similar in N mice. This suggests the increase in BAT weight, which presumably reflects suppression of thermogenesis, reduced fat oxidation, and accumulation of unused fat in BAT, does not depend on GH signaling.

Gene transcriptomic signature of adipose tissue depots were in general agreement with data from microarray analysis from a previous study on GHRKO mice (18). Importantly, we observed that *Igf1* expression was consistently downregulated in GHRKO mice

at 23 and 30°C, as expected. However, we observed increased BAT *Igf1* expression under thermoneutral conditions in GHRKO mice, but not in N mice. The same was not observed for other WAT depots. This suggested a divergent BAT response to thermoneutral conditions. The BAT was the most responsive to temperature changes regarding number of DEGs and clear grouping in the unsupervised hierarchical clustering analysis. This was expected, as BAT is responsible for thermogenesis and its relative mass increased in response to thermoneutral temperature in both N and GHRKO mice in the current study. Despite similar increase in weight, we observed more DEGs in BAT from GHRKO than N mice, with very few common DEGs. This suggests a divergent response of BAT to thermoneutrality in the absence of GH signaling. The key genes regulating BAT thermogenesis are UCP1 and UCP3, which uncouple the mitochondrial respiratory chain to increase heat production during exposure to cold (19). BAT UCP1 and UCP3 expression was decreased to a similar extent (~70% reduction) in both N and GHRKO mice under thermoneutral conditions in our study. Others have observed similar decrease in BAT UCP1 RNA and protein levels in N mice subjected to thermoneutrality (20). Surprisingly, BAT UCP2 expression increased 2-fold in GHRKO mice under thermoneutral conditions, but was unaffected in N mice. UCP2 shares amino acid homology with UCP1, but it is more widely expressed and its role is less clear both peripherally and centrally (21).

We observed that sWAT relative weight was almost 4 times greater in GHRKO mice, consistent with previous observations. The striking observation in the present study is that only 3 genes were regulated by temperature in sWAT from N mice, whereas 1 609 genes were regulated in GHRKO mice. Taken together, increased sWAT abundance and its enhanced response to thermoneutrality in GHRKO mice suggests a central role in adaptation of GHRKO mice to changes in temperature. Among the pathways upregulated exclusively in sWAT from GHRKO, we identified several pathways concerning glucose, amino acid, and fat metabolism, along with TCA cycle and oxidative phosphorylation. Interestingly, similar pathways were downregulated in sWAT of N mice exclusively. Previous work indicates that these same pathways are upregulated by cold exposure in N mice (22). Thus, the sWAT response to thermoneutral conditions in the absence of GH signaling is very divergent. Evidence suggests that sWAT is more prone to browning in mice (23). However, despite the robust response we observed, expression of *Ucp1* was not detected in sWAT from GHRKO mice. A previous study also detected the presence of UCP1 in BAT and pWAT, but not in other tissues of GHRKO mice (6).

Overall, our results suggest that BAT and WAT depots respond differently to thermoneutral conditions in GH-resistant mice. In our previous study, the extend longevity of GH-resistant mice was maintained when chronically housing these mice under thermoneutral conditions (15). Current results show that sWAT was the most responsive to temperature changes in GHRKO mice but was unaffected in N mice. Although BAT was responsive to thermoneutral conditions in both GHRKO and N mice, the genes and pathways regulated were divergent.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Author Contributions

A.S. and M.I.S.C. drafted the manuscript; A.S. and B.V. performed experiments and data analysis; Y.F., S.M. J.D., E.R.H., and K.N.H. performed experiments; A.B. and M.M.M designed experiments and performed data interpretation. All authors revised the manuscript and approved the final version.

Conflict of Interest

None declared.

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