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Research Article

Biochemical and Behavioral Analysis of Aging in Wistar Rats: A Longitudinal Case Study

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Abstract
Aging processes involve complex physiological and behavioral changes that require systematic investigation. This study examined biochemical and behavioral alterations in male Wistar rats during the transition from youth to maturity. Five healthy male Wistar rats were monitored longitudinally from 12 weeks (youth) to 24 weeks (maturity) in controlled laboratory conditions. Primary outcomes included HDL cholesterol, non-HDL cholesterol, triglycerides, and plasma glucose levels. Secondary outcomes assessed rearing behavior and locomotion patterns. Mature rats showed significantly decreased HDL cholesterol (2.3±0.6 vs 25.9±4.5 mg/dL), increased non-HDL cholesterol (83.2±24.5 mg/dL), and elevated triglycerides (311.4±84.8 mg/dL) compared to young rats. Plasma glucose remained unchanged. Rearing behavior significantly decreased in mature rats (9.6±7.5 vs 29±9.8 events), while locomotion was unaffected. The study demonstrates significant age-related biochemical and behavioral changes in Wistar rats, providing valuable insights for biomedical research and animal welfare considerations.

Keywords: Aging biomarkers; Wistar rat model; Behavioral analysis; Lipid profile; Biochemical changes.

Introduction

Aging is a physiological process that is dynamic, irreversible, and integral to the individual development of organisms. This process manifests itself from conception until the cessation of biological life and is characterized by a progressive decline in physiological function [1,2,3,4,5]. As organisms age, there is an elevated susceptibility to chronic pathological conditions, including cancer, cardiovascular pathologies, along with and neurodegenerative conditions [3,6,7].

Animal models serve as indispensable tools for aging research, particularly at the biochemical and behavioral levels. Wistar rats provide an excellent model for studying age-related changes due to their well-characterized lifespan and physiological similarities to human aging processes. Previous studies have demonstrated that lipid metabolism undergoes significant alterations during aging, with changes in cholesterol fractions and triglyceride levels serving as important biomarkers of metabolic dysfunction. Similarly, behavioral modifications, particularly in exploratory activities such as rearing behavior, demonstrate correlations with age-dependent neurological changes and can indicate alterations in anxiety and cognitive function [8,9,10].

Despite existing knowledge about individual aging markers, there remains a gap in understanding

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the concurrent biochemical and behavioral changes during the transition from youth to maturity in controlled longitudinal studies. The objective of this investigation was to investigate the physiological and behavioral modifications in male Wistar rats throughout aging from 12 to 24 weeks, focusing on lipid profile alterations and behavioral patterns.

Material and methods

Experimental Design

This represents a prospective longitudinal investigation that emerged as a secondary analysis from a larger doctoral research project investigating metabolic alterations induced by sucrose consumption in rodent models. During the execution of the main protocol, we observed unexpected metabolic changes in the control group related to aging processes, which prompted the development of the current study.

The animal experimental protocol received approval from the Institutional Ethics Committee of FMABC University Centre (protocol code 04/2022 and approval date 2022).

Animals

We used adult male Wistar rats (n = 5) that were monitored from 12 weeks of age (young adults; average weight: 402±25g) to 24 weeks (mature adults; mean weight: 466±37g). These rats were obtained from the animal facility of the FMABC University Centre with CEUA/FMABC committee approval (approval no. 04/2022).

The animals were randomly selected considering weight and age criteria. The sample size (n=5) was determined based on ethical research principles to minimize animal use. Small samples can be justified for preliminary, exploratory, or pilot studies intended for testing procedures or hypothesis generation, though results should be interpreted with caution and not generalized. This study emerged as an unexpected finding from a larger doctoral research protocol investigating sucrose-induced metabolic alterations, and the sample size was predetermined by the availability of control animals from that study. In this case, our findings open doors to new research that may confirm and expand these preliminary results with larger samples and more robust experimental designs, while acknowledging the potential limitations of the current sample size [11,12].

The subjects were maintained in a standardized environment featuring a temperature of 22±2°C, a 12-hour photoperiod cycle, and individual polypropylene cages equipped with stainless steel covers and environmental enrichment. Their diet consisted of Nuvilab CR-1 irradiated, and they had unrestricted access to drinking water and feed.

Experimental Protocol

The animals underwent a 12-hour fasting period for blood sampling at both time points, which were predetermined by the timeline of the larger doctoral research protocol. At 12 weeks of age, a small blood sample (0.5 ml) was obtained via gingival plexus puncture under 2% isoflurane anesthesia in O2 at 100%. Since the animals needed to remain alive for the continuation of the main study, this minimally invasive sampling method was chosen to ensure animal welfare and recovery. At 24 weeks of age, corresponding to the endpoint of the larger protocol, a larger sample (5 ml) was collected during euthanasia by caudal vena cava puncture under the same anesthetic protocol (2% isoflurane in O2 at 100%), followed by diaphragmatic incision to ensure euthanasia as required by the main study design. This difference in sampling methodology does not compromise sample quality, as both procedures were performed under identical anesthetic conditions and following the same fasting protocol, with the sampling site being the only variable determined by the animal's survival requirements in the larger study.

Determination of Cholesterol, Triglycerides, and Plasma Glucose

Biochemical assessments were conducted in the clinical laboratory of the FMABC University Centre using the Cobas (Roche®) 8000 equipment. The evaluation included parameters related to cholesterol (HDL and non-HDL), triglycerides, and glucose.

Behavioral Analysis - Open Field

To evaluate the behavioral parameters of rats (n = 5), we submitted them to the open-field test at 12 weeks of age and again at 24 weeks. The apparatus consisted of a white acrylic box (80 x 80 x 30 cm) equally divided into 16 squares, located in a quiet testing room with standardized lighting conditions (40 lux). Each subject was individually transferred from its housing cage and positioned in the center of the apparatus, allowing free exploration for 5 minutes. The following parameters were systematically recorded: locomotion (ambulação - total number of squares crossed with all four paws), rearing behavior (levantamento - number of vertical activity episodes where the animal stood on hind legs), grooming behavior (limpeza - duration and frequency of self-cleaning episodes), and fecal emissions. The number of peripheral, medium, and central quadrants visited during locomotion was quantified to assess exploratory patterns. The arena was thoroughly cleaned with 70% ethanol between each animal to eliminate olfactory cues and prevent interference between sessions [13].

Statistical Analysis

Normality of data distribution was evaluated through the Shapiro-Wilk test. Comparisons between the two age groups were performed using Student's t-test for paired samples. Statistical analyses were conducted using GraphPad Prism 8.0 software. A significance level of $p < 0.01$ was adopted instead of the conventional $p < 0.05$ due to the small sample size (n=5), requiring greater statistical stringency to ensure that only robust and meaningful effects were considered significant and to reduce the risk of Type I errors in exploratory analyses with limited statistical power.

Results

The research results indicate significant differences between adult and young rats in terms of health and behavior markers. Adult rats showed a significant reduction in HDL cholesterol levels (2.3±0.6 mg/dL) compared to young rats (25.9±4.5 mg/dL, Figure 1). Furthermore, there was a significant increase in non-HDL cholesterol (83.2±24.5 mg/dL) and triglycerides (311.4±84.8 mg/dL) in adult rats compared to young rats (31.4±3.8 mg/dL and 38.5±14.6 mg/dL, respectively, Figure 2). These changes suggest a deterioration in the lipid profiles associated with health.

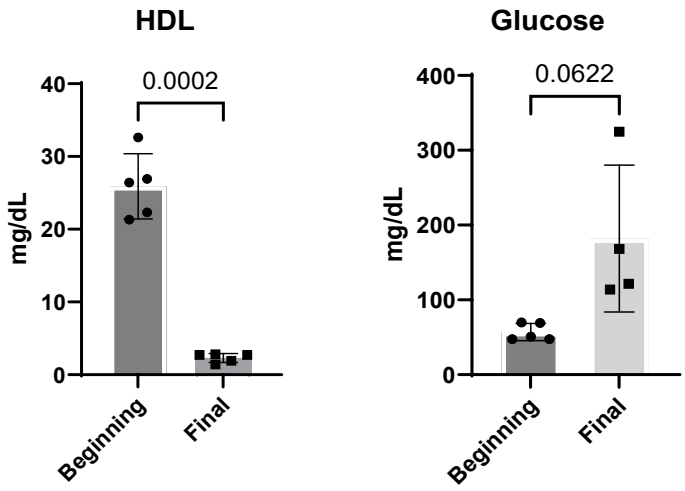

Figure 1. Comparison of HDL cholesterol and glucose levels between young (12 weeks) and adult (24 weeks) male Wistar rats. Data are presented as mean ± standard deviation (n=5). **p < 0.01 for HDL cholesterol comparison; p > 0.01 for glucose comparison (Student's t-test).

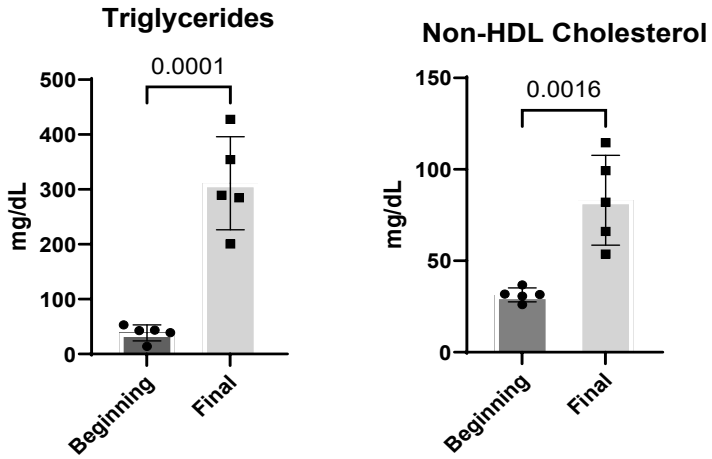

Figure 2. Comparison of non-HDL cholesterol and triglyceride levels between young (12 weeks) and adult (24 weeks) male Wistar rats. Data are presented as mean ± standard deviation (n=5). **p < 0.01 for both parameters (Student's t-test).

Plasma glucose showed high variability but no significant difference between groups (182.1±98.2 mg/dL in adults vs. 53.9±10.7 mg/dL in young rats, Figure 1). The large standard deviation observed in the adult group may be attributed to individual metabolic variations and the small sample size, which increases susceptibility to outlier effects. It is worth mentioning that the samples were obtained from another experiment. We followed ethical principles when using the groups from the previous experiment.

However, in the open field test, adult rats showed a significant reduction in "rearing" behavior compared to young rats (9.6±7.5 events vs. 29±9.8 events). This decrease suggests a higher level of fear, anxiety, and depression associated with aging in adult rats (Figure 3). However, no statistical difference was found in the locomotion of the animals (Figure 3).

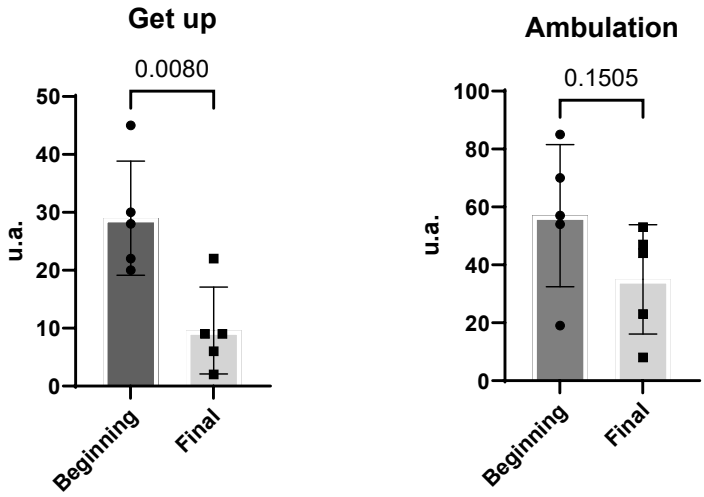

Figure 3. Behavioral analysis in open field test comparing young (12 weeks) and adult (24 weeks) male Wistar rats. Rearing behavior (vertical activity) and locomotion (horizontal activity) are shown. Data are presented as mean ± standard deviation (n=5). **p < 0.01 for rearing behavior; p > 0.01 for locomotion (Student's t-test).

Discussion

The present study demonstrates significant age-related changes in lipid metabolism and behavior in male Wistar rats between 12 and 24 weeks of age. It is widely recognized that aging causes several changes in the lipid profile, including an increase in LDL cholesterol. This increase is associated with a high cardiovascular risk, including diseases such as atherosclerosis, heart attack, and stroke. In addition, other lipid profile components, such as triglycerides and HDL cholesterol, also undergo changes with age, impacting vascular health and the risk of cardiovascular events [14,15].

Our findings of decreased HDL cholesterol (from 25.9±4.5 to 2.3±0.6 mg/dL) and increased non-HDL cholesterol (from 31.4±3.8 to 83.2±24.5 mg/dL) and triglycerides (from 38.5±14.6 to 311.4±84.8 mg/dL) align with previous aging studies in rodent models. In rat studies, biochemical and lipid changes related to aging are also observed. However, we still know little about these specific changes since most studies look at the lipid profile of animals after medicinal or dietary interventions at certain periods. This leaves aside the changes inherent in the aging process itself [16,17,18,19].

A classic study with Fischer-344 rats demonstrated potential changes in enzymatic parameters, which may be associated with alterations in the lipid profile correlated with the animal's aging, specifically in the activities of the tissue lipoprotein lipase [20]. Our results support these findings, showing similar metabolic deterioration during the transition from young adult to mature adult stages.

The observed reduction in rearing behavior (from 29±9.8 to 9.6±7.5 events) corroborates previous research linking lipid profile alterations with behavioral changes. Alteration of the lipid profile due to aging may be associated with behavioral changes. Recent studies indicate a correlation between elevated levels of residual cholesterol and depression. This association suggests that focusing attention on cholesterol can be useful in deepening our understanding of this disorder. In addition, it is noted that a cholesterol-rich diet can have age-dependent behavioral effects, like

anxiety, and influence central neurochemical changes [21,22].

However, several limitations must be acknowledged in our study. The small sample size (n=5) limits statistical power and generalizability of findings. The high variability observed, particularly in glucose measurements, may reflect individual metabolic differences that are magnified by the limited sample size. Additionally, the non-uniform study design, arising from a secondary analysis of control animals from a larger protocol, introduces potential confounding factors. Despite these limitations, exploratory studies with small sample sizes remain valid for publication as they serve to generate hypotheses for future research and contribute to the preliminary understanding of complex biological phenomena [11,12]. Subsequent investigations employing expanded cohorts and uniform experimental designs are essential to validate these initial observations.

Conclusions

This study demonstrated biochemical and behavioral changes in Wistar rats during the transition from young adult (12 weeks) to mature adult (24 weeks) stages. The observed alterations in metabolic parameters and behavioral patterns provide preliminary evidence for age-related physiological modifications in this animal model. Further research employing expanded sample populations and uniform methodological frameworks is essential to corroborate these observations and establish clearer correlations between rat developmental stages and human age equivalents.

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