ABSTRACT

AGE-RELATED CHANGES IN PHYSICAL ACTIVITY AND BIOMARKERS OF CARDIOVASCULAR HEALTH IN FEMALE SPRAGUE-DAWLEY RATS

By Abigail L. Marker

Age plays a mediating role and can intensify cardiovascular health changes resulting from sedentary and physically active lifestyles. The purpose of this study was to monitor age-related changes in physical activity and biomarkers of cardiovascular health in animals provided access to: 1) a standard cage (SED), 2) 1-hr twice weekly in a large box (PA), and 3) alternate day running wheel (EX). Physical activity declined in all three groups with age. Biomarkers of cardiovascular health were maintained with age in all groups, with a few exceptions: total cholesterol and systolic blood pressure increased in SED and PA at 16 months. Animals in the EX group decreased their physical activity significantly with age, but daily activity was significantly greater compared with both PA and SED groups. Animals in PA and SED groups had extremely low activity levels throughout their life spans and showed signs of cardiovascular disease after age 16 months.
AGE-RELATED CHANGES IN PHYSICAL ACTIVITY AND BIOMARKERS OF CARDIOVASCULAR HEALTH IN FEMALE SPRAGUE-DAWLEY RATS

A Thesis
Submitted to the Faculty of Miami University
In partial fulfillment of
The requirements for the degree of
Master of Science
Department of Physical Education, Health and Sport Studies

By
Abigail Lea Marker
Miami University
Oxford, OH
2005

Advisor________________________
Dr. Helaine Alessio

Reader_________________________
Dr. Jeffrey Potteiger
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Methods</td>
<td>2</td>
</tr>
<tr>
<td>III. Results</td>
<td>5</td>
</tr>
<tr>
<td>IV. Discussion</td>
<td>8</td>
</tr>
<tr>
<td>V. References</td>
<td>12</td>
</tr>
<tr>
<td>VI. Figures</td>
<td>15</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure

1. Distance covered per 24-hr in the running wheel + standard cage (EX), open field + standard cage (PA) and standard cage (SED)  
   a. Distance covered in the open field + standard cage (PA)  
   b. Distances covered in the standard cage (SED)  
   15

2. Body mass as a function of animal age  
   18

3. Resting systolic blood pressure as a function of animal age  
   19

4. Resting diastolic blood pressure as a function of animal age  
   20

5. Heart rate as a function of animal age  
   21

6. Resting blood serum total cholesterol at 6, 12, and 16 months of age  
   22

7. Resting blood serum low density lipoprotein-cholesterol  
   at 6, 12, and 16 months of age  
   23

8. Resting blood serum high density lipoprotein-cholesterol  
   at 6, 12, and 16 months of age  
   24

9. Resting blood serum triglycerides at 6, 12, and 16 months of age  
   25
I would like to thank my advisor, Dr. Helaine Alessio for her continued guidance and support throughout this process. I would also like to thank Natalie Schweitzer², Angela Snedden¹, Kelly Vonder Haar³, Kelly Michalak³, and Ann Hagerman³ for their assistance in data collection. Additionally, thanks to Drs. Jeffrey Potteiger and Melissa Chase for serving as committee members.

¹Department of Physical Education, Health and Sport Studies, ²Department of Zoology, ³Department of Chemistry and Biochemistry, Miami University, Oxford, OH
**Introduction**

Physical activity is vital for survival. In a natural setting, animals expend energy in physical activity by foraging for food, building shelters, and running from predators [1, 2, 3]. The fundamental physical activity level that exists in natural animal settings is likely to contribute to vigor, prevent some diseases, and enhance mean life span. Studies show that compared with laboratory animals that reside solely in a standard cage, animals with access to regular exercise have improved cardiovascular health [4, 5], and live approximately 3 months longer than the mean life span of animals confined to a cage [6, 7].

A great deal of scientific research relies on animal models [8]. Nevertheless, the use of laboratory animals poses some potential confounding problems due to the sedentary cage conditions in which virtually all animals are forced to reside. The health of animals in research studies may be compromised in part by the lack of access to physical activity outside of a standard cage. While injury or death from predators may be ruled out, laboratory animals that live solely in a cage may be at increased risk to a number of diseases such as obesity and cardiovascular disease, which are associated with extremely low physical activity levels [9, 10, 11].

Providing access to physical activity outside of a cage may have a significant impact on the health of laboratory animals and result in a research model that is pragmatic and generalizable to different species in natural living settings. Many long term studies have measured physical activity levels in laboratory rodents provided with access to running wheels [4, 6, 12-19]. Few studies have quantified the distance a laboratory rodent will travel in an open field/exploratory setting and home cage setting for a short period of time (6-15 min on average) [10, 19-22]. Typically, studies documenting open field/exploratory activity measure the frequency an animal explores or moves across specific areas in an arena [13, 15, 17, 18, 23-28]. Activity in these studies is defined and quantified as the number of nose pokes into holes in the floor or wall of an arena, crossing over light beams or crossing into a light or dark area of an arena. These types of activity measurements are limited and do not provide adequate information about daily activity level and its impact on the health of the animals.
If access to physical activity is important to health then it would be beneficial to compare different levels and types of daily physical activity over time in order to know the minimal amount of physical activity associated with disease prevention. The amount of physical activity that occurs in a standard cage has not been systematically measured over the life span of a laboratory rat. The amount of activity performed in an open field environment has not been studied as extensively as running wheel activity. The purpose of this study is to compare physical activity levels and select biomarkers of health over time in different settings: 1) standard cage, 2) twice weekly physical activity in a large box and 3) voluntary wheel activity, to better understand how physical activity levels are influenced by the way in which animals are housed and how they change with age. Furthermore, by monitoring body weight, blood pressure and blood lipids, we can learn how access to physical activity affects these biomarkers of health and disease over the life span.

Methods

One hundred and eight female Sprague-Dawley rats (Charles River) were used for this study and were cared for according to the ethical procedures and policies approved by the Miami University Animal Care and Use Committee. Animals were pair-weighted and assigned to one of three groups of 36: 1) a sedentary control group residing full time in their cages (SED), 2) a physical activity group with twice weekly physical activity outside of their cage (PA) and 3) a physically active group with access to regular voluntary wheel running (EX) every other day. Animals were housed in pairs within their designated group in climate-controlled rooms with a 12-h light/dark cycle.

All animals resided in pairs in standard polypropylene cages (0.454 m x 0.238 m), with no items added for enrichment. Handling procedures were similar for all animals, with weekly weighing, tumor analyses, and cage changes and biweekly blood pressure measurements. Animals in the PA group were removed from their cages twice per week for access to 1-h of voluntary physical activity in a large plastic box (1.21 m x 0.59 m). The box was equipped with four small
platforms of differing heights (0.15 m and 0.19 m) in each corner and two plastic tubes (0.66 m) along the lengths of the walls, to encourage exploration and activity. Animals were placed in the box in groups of six and were monitored during this time to ensure that animals were not aggressive. Animals in the PA group were video recorded once per month while in the physical activity box using a Sony® high speed 16mm digital camera with a 60 frames · second$^{-1}$ shutter speed. Video captured an overhead view displaying the complete outline of the large box. Two animals were randomly selected and marked with a colored marker before being placed in the box with the other animals for tracking purposes. Distance covered was tracked manually using a digital video recording of the activity of the animals. Distance covered in the large box by the marked animal was traced by placing tracing paper over a computer screen and following the animal for intervals of approximately 5-10 minutes when the lines were easily distinguishable and measurable. Tracings continued until the full 1-h session was analyzed and a total distance in meters per hour was calculated. The total distance covered by the two marked animals was averaged. All measurements were performed twice and checked for reliability.

Animals in the EX group were rotated so they were in cages that allowed for free access to wheels on alternate days. They were paired on non-wheel access days. The wheels (Nalgene, Rochester, New York) were connected to magnetic switches that recorded number of revolutions per day, translated into meters · 24-hr$^{-1}$.

In the SED group, a 24-h surveillance Sony® high speed 16mm digital video camera using infrared light for recording in the dark recorded the total distance covered in a standard cage. Two animals were randomly selected for this analysis. Recorded videos were evaluated in a similar way as the physical activity box recordings. Twenty-four hour surveillance was also performed and analyzed on the PA and EX groups monthly. The total distance covered in 24-h for the PA and EX groups was added to the total distance covered in the activity box in the PA group and the total distance traveled in the voluntary running wheel in the EX group.

Monthly systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were collected using a non-invasive tail cuff method. Animals were placed in a restrainer with the base of the tail placed through an ITTC NIBP Sensor (IITC Life Sciences, Woodland Hills, CA).
The animal was placed in the restrainer which was placed on a heated pad (38 °C). The sphygmomanometer and pressure cuff were inflated to approximately 200 mmHg and the IITC Model 31 NIBP amplifier and software (IITC Life Sciences, Woodland Hills, CA) measured pulse detection with a photoelectric sensor in a tail cuff. Once the initial threshold or 200 mmHg was reached, SBP was determined when blood flow resumed following occlusion. Heart rate was measured by the sensing cuff and amplifier, which measured the amplitude and time between pulses in the animal’s tail.

Blood was available for analyses from animals in each of the three groups for analysis only at 6, 12, and 16 months. At 6 and 12 months, 12 randomly selected rats from each treatment group were anaesthetized with isofluorane and a small cut was made on the anterior base of the tail. Within one minute, blood was collected, centrifuged, and serum plasma was deep frozen at –80°C until assayed for total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG) and glucose (GLU). At 16 months of age, 12 animals from each treatment group were sacrificed by decapitation and exsanguinations.

Analysis of variance with repeated measures (ANOVA-RM) was used to compare mean biomarkers of health collected monthly over time and group by time. A 3 (group) X 3 (times) ANOVA was used to compare mean total cholesterol, HDL-C, LDL-C, triglycerides, and glucose among the three treatment groups at 6, 12 and 16 months. Post hoc comparisons were made by Bonferroni. Biomarkers and distances covered were reported as mean ± SEM. A probability level of 0.05 was set for significance. Blood analysis was completed at 6, 12 and 16 months, while body mass, blood pressures, heart rate and distances were analyzed up to 19 months of age.
Results

*Voluntary wheel running, physical activity and sedentary activity distances · 24-hr⁻¹*

Mean distance covered in the running wheel by animals in the EX group peaked at age 3 months (15,192 ± 415 m · 24-hr⁻¹) and gradually declined to 1,244 ± 185 m · 24-hr⁻¹ at 19 months. Mean distance covered by the animals in the PA group also declined with age, peaking at 2 month (579 m · 24-hr⁻¹) and declining to 121 m · 24-hr⁻¹ at 19 months. Mean distance covered by the animals in the SED group declined with age, peaking at 2 months of age (290 m · 24-hr⁻¹) to 32 m · 24-hr⁻¹ at 19 months (Fig.1).

From 2-19 months of age, there were no differences in distance covered in the PA and SED groups (p>0.05), while the EX groups covered significantly greater distances compared with the PA and SED groups (p<0.05). There were no differences in mean distances covered between all three groups from 16-19 months (p>.05) (Figs. 1a and 1b).

*24 hr surveillance*

Separate 24-h surveillance was conducted in the EX and PA groups from 10-19 months of age to monitor activity levels when animals were not in the cages equipped with wheels (every other day) or when animals were not in the large box for additional physical activity outside of the standard cage. These distances were compared to the 24-h surveillance data from the SED groups. Surveillance analysis showed that there were no differences in distance traveled in the standard cage among the three groups during the 24-h surveillance.

*Day versus Nighttime Activity Box Distances*

Analysis of distance covered in a large box by the PA group showed that there was a decline in activity from 1-19 months. At 19 months, animals in the PA group were traveling 88.5 m · 24-hr⁻¹ in the large box compared to 289 m · 24-hr⁻¹ at 1 month. Nighttime activity box physical activity levels were monitored from 15-19 months. Analysis of distance traveled in the activity
box performed at nighttime showed no decline over the four months. When comparing the daytime versus the nighttime distance traveled in the activity box for the same months 15-19, there was no difference between the distances traveled at nighttime versus daytime. Animals covered the same distances respectively, $88.5 \text{ m} \cdot 24$-hr$^{-1}$, in the activity box during the daytime and during the nighttime at 19 months.

**Body Mass**

Body mass increased with age in a curvilinear manner in each of the three groups (Fig. 2). There were no significant differences in mean body mass among the EX, PA, and SED groups throughout the study (1-19 months of age). Body mass averages at 19 months of age were $468\pm14$, $470\pm16$ and $458\pm18$ in EX, PA, and SED groups, respectively.

**Blood Pressure and Heart Rate**

There were no significant differences in mean SBP, DBP and HR among the groups throughout the study ($p>0.05$) (Figs. 3, 4, 5). Mean SBP values at 19 months of age were $132\pm6$, $124\pm14$, $146\pm4$ mmHg in EX, PA, and SED groups. Mean DBP values at 19 months of age were $99\pm4.5$, $96\pm7$, $92\pm4$ mmHg in EX, PA, and SED groups. Mean HR values at 19 months of age were $406\pm13$, $416\pm24$, $417\pm22$ b · min$^{-1}$ in EX, PA, and SED groups.

Resting monthly mean SBP, DBP and HR changed over time in EX, PA and SED groups

$[F(16, 408)=2.82; F(16, 452)=2.48; F(16, 443)=2.88, p<0.05] \text{ (SBP)}$; $[F(16, 407)=2.65; F(16, 433)=6.31; F(16, 445)=5.78, p<0.05] \text{ (DBP)}$; $[F(16, 407)=6.75; F(15, 423)=3.22; F(16, 440)=3.35, p<0.05] \text{ (HR)}$. Post hoc analyses showed a similar trend for each of the groups for SBP. Systolic blood pressure values increased linearly up to 4 months of age and leveled off from 5-16 months of age. During this baseline period, mean SBP averaged 140 mmHg and decreased 6% to 132 mmHg at 19 months of age in EX; mean SBP averaged 137 for the PA group and decreased 9% to 124 mmHg at 19 months of age; and mean SBP for the SED group averaged 143 mmHg and increased 4% to 146 mmHg at 19 months of age. Mean DBP also increased linearly up to 4 months in all three groups. From months 5-19, mean DBP values
leveled out and each of the groups followed a similar trend in mean DBP values throughout the months. Mean values for months 5-19 were approximately 97 mmHg in each of the three groups. Heart rate values ranged from 440 bts · min⁻¹ to 460 bts · min⁻¹ from 2-6 months of age. After this period, HR values dropped and leveled out in each group until 17-19 months of age where HR values began to fluctuate and increased up to 19 months of age. In the older animals, however, HR values did not reach the levels noted in the beginning months of age.

**Blood Lipids**

There were no significant difference observed in mean serum TC among the groups at 6 and 12 months except EX > PA at 16 months of age (Fig. 6). Mean serum TC levels at 16 months were 69±8, 92±7, and 87±9 mg · dl⁻¹ in EX, PA, and SED groups, respectively. There were no significant differences in LDL-C among the three groups at 6, 12, and 16 months of age (Fig. 7). Mean LDL-C at 16 months of age were 22±2, 22±3, and 22±1 mg · dl⁻¹ for EX, PA, and SED groups. For HDL-C, there was a significant difference at month 12 among the three groups [F(2, 32)=21.89; p<0.05]. Post hoc analysis showed mean HDL-C with EX = PA > SED (p<0.05) at month 12. There were no differences in mean HDL-C at 6 and 16 months in the three groups [F(2, 28)=0.37; F(2, 26)=2.79; p>0.05]. Mean HDL-C at 16 months were 47±7, 70±7, and 59±8 mg · dl⁻¹ in EX, PA, and SED groups (Fig. 8). For 6, 12, and 16 months of age, there were no significant differences in mean TG [F(2, 31)=0.004; F(2, 31)=1.75; F(2, 23)=1.03, p>.05] (Fig. 9). At month 16 mean TG levels were 132±21, 173±26, and 176±27 mg · dl⁻¹ for EX, PA, and SED groups.

There was a significant time effect for mean TC in the PA and SED groups. Post hoc analyses showed mean TC with 16 > 6 months in the PA group and 16 > 6 months in the SED group (p<0.05). For mean LDL-C, there was a significant time effect in the EX, PA, and SED groups where post hoc analyses showed each of the three groups with 12 > 6 months (p<0.05). There was also a significant time effect for mean HDL-C in the EX, PA and SED groups. Post hoc analyses showed mean HDL-C with 12 > 6 and 16 months in the EX group, month 16 > 6 months in the PA group and month 16 > 12 months (p<0.05). There was no time effect between months 6, 12, and 16 in any of the three groups for mean TG.
Discussion

Animal research has contributed knowledge about health and disease processes across the animal kingdom. Most animal studies house animals in standard cages with little to no access to physical activity outside the cage. However, the ability to maintain health function and prevent disease over time is influenced significantly by physical activity [1, 9]. A typical standard animal cage results in an extreme level of physical inactivity that is atypical in comparison with the natural setting. Laboratory animals may be less healthy than animals allowed access to physical activity and exercise over their life span, simulating a more natural environment. Providing access to physical activity by providing running wheels or access to a large box may address potential health problems associated with sedentary behavior inherent in residing solely in a standard cage.

Physical activity and exercise levels are assumed to change over time, but only running wheel activity has been investigated in most long term studies. However, distance covered in the EX group decreased by approximately 92% (15,192±415 to 1,244±185 m · 24-hr⁻¹) from the time of peak activity to the end of the study. Many similar longitudinal studies also reported peak activity counts at 2-3 months in rats and 5-6 months in mice and an overall 83-89% decline in activity from the time of peak activity to the end of the study (22-23 months) [4, 6, 12-19].

Distance covered in the PA group decreased by 79% (579 to 121 m · 24-hr⁻¹) from the time of peak activity to the end of the study. Alessio et al. (2005) [4] reported male Sprague-Dawley rats decreased activity in an open field by approximately 50% (322 to 161 m · day⁻¹) from the time of peak activity to the end of the study. Additional studies that have tracked the distance covered in an open arena (10 minutes, monthly) found peak activity occurred at 3 months of age and declined approximately 50% thereafter until 22-23 months of age [13, 15, 17, 18, 23-28].

Compared with animals that had access to running wheels, the amount of activity performed in the SED group was minimal, decreasing by approximately 89% from the time of peak activity to the end of the study. Although no studies, to our knowledge, have quantified the distance covered by a sedentary animal in a standard cage, Yu et al. (1985) [29] counted the number of horizontal and vertical movements of a laboratory rat in a plastic cage over a 24-hour period.
from the beginning of the study (6 months) to the end of the study (32 months) and found a significant decrease in activity counts.

The substantial difference between the distance covered by the EX group compared to the PA and SED groups indicate a dramatically different total amount of energy expenditure. The open field access to physical activity provided to the PA group (1-h, twice weekly in a large box), in addition to physical activity accumulated in the standard cage over the remaining 23 hours of the day, did not elicit a significantly greater distance covered compared to the SED group. During the respective ages of peak activity, the EX group covered 97-98% more distance than the PA and SED groups. Peak distances covered in the EX group, only slightly exceeded the minimal distances covered in the PA group during the final months and peak distances covered in the SED group did not approach the minimal distances covered in the EX group.

Physical activity levels did not have an impact on mean body weight in the female Sprague-Dawley rats. It has been suggested that female species (both humans and animals) may be more resistant to periods of energy imbalance than males [30]. Widdowson (1976) [31] suggested that because females are responsible for the survival of their young, they have been more affected by natural selection pressures to resist the loss of body energy stores. Studies by Applegate et al. (1982) [32] and Oscai et al. (1973) [33] found that when female rats were subjected to endurance training (swimming, treadmill) they were able to gain weight at approximately the same rate as the sedentary control animals. In contrast, male rats subjected to regular exercise typically lose weight [4, 33-35]. Studies have also reported that female rats ran 50-80% more than males in any given week [32, 34]. Nevertheless, daily wheel running affected body mass in males and they had significantly less body mass than the sedentary males.

Cardiovascular risk factors are sensitive to physical activity and exercise. Blood lipids measured in this study followed a pattern where EX=PA=SED for 6 and 12 months of age. However, at 16 months of age, TG in the EX group was 25% less than that of the PA and SED groups and TC in the EX group was 21% less than that of the SED group and 25% less than the PA group. This was not as large as the 53-55% decrease in TC and TG reported by Suzuki and Machida (1995) [36] among an exercise and sedentary control group. At 6 versus 16 months, TC and TG
remained stable in the EX group, and yet increased in the PA and SED groups indicating a potential age-related benefit in maintaining healthy blood lipid levels via regular exercise. Our findings for HDL-C and LDL-C are inconsistent with established findings that exercise training increases HDL-C, and decreases LDL-C and triglyceride levels [37]. The atypical findings may have been influenced by a lack of standard control of diet and physical activity before blood sampling.

Generally, SBP, DBP, and HR followed a similar pattern where EX=PA=SED for months 2-16. The significant drop in SBP and DBP from baseline levels in the EX and PA groups at 17 months of age may, in fact, be evidence of the effects of increased physical activity throughout the lifespan that are evident in old age. The increased standard errors of the mean for SBP and DBP after 16 months suggest that variability increases with age.

Access to a regular running wheel appears to maintain some health biomarkers from young age into old age. Although distance covered per 24-hour in a running wheel decreased 92% in female rats from age 3 to 19 months, the activity level was significantly greater than both PA and SED animals and is likely to have influenced healthier SBP, HR, TC and TG at age 16 months and older in the EX group. Results suggest that PA and SED activity levels were not enough to elicit or maintain the health characteristics of the EX activity levels, specifically SBP, TC and TG. Although the PA group was provided access to physical activity outside of a standard cage, one hour of twice-weekly activity, there were minimal benefits relative to the SED group when either was compared with the EX group. This is most likely because the PA group accumulated very small amounts of physical activity over and above the animals in the SED group.

Results from the variables measured suggest that a lifetime of physical inactivity as seen in the PA and SED groups begins to manifest health risks that break away from the levels noted in the EX group at 16 months of age. Young animals (15 months of age and younger) appear to have a reserve capacity that works toward maintaining health regardless of access to physical activity, as indicated by biomarkers of blood pressure and lipids. Lifelong access to exercise like that of the EX group preserves vigor into old age. Female Sprague-Dawley rats seem to be resistant to changes in the specific phenotypic cardiovascular markers we assessed, regardless of activity.
levels. Effects of decreased physical activity levels may be manifested in other phenotypic or
genotypic variables that were not within the scope of this study. It may be more difficult to
assess diseased animals using the biomarkers examined in this study. Additionally, compared
with a rat’s cardiovascular system, the human cardiovascular system appears to be more sensitive
to sedentary-physical activity based upon measures such blood pressure, blood lipids and body
weight. In conclusion, compared with rats that had access to physical activity and exercise, SED
rats had higher TC and tended to have higher SBP and TG. These results suggest that the typical
way in which laboratory rats are housed predisposes them to diseases that are not typical in
animals with access to physical activity, which is more similar to a natural animal setting.
References


[27] Lhotellier, L., and C. Cohen-Salmon. Age related changes in activity and exploration


Fig. 1. Distance covered per 24-hr in the running wheel + standard cage (EX), open field + standard cage (PA) and standard cage (SED). Points represent mean distance covered (n=36, EX; n=2, PA and SED. From 17-19 months n=10-12, EX; n=2 PA and SED), with S.E.M smaller than the symbols used.
Fig. 1a. Distance covered in the open field + standard cage (PA). Points represent mean distance covered (n=2).
Fig. 1b. Distances covered in the standard cage (SED). Points represent mean distance covered (n=2).
Fig. 2. Body mass as a function of animal age. Animals were weighed weekly throughout the experiment. The points represent average body mass (n=36. From 17-19 months n=10-12) on the third week of every month, with S.E.M. smaller than the symbols used.
Fig. 3. Resting systolic blood pressure as a function of animal age. Resting systolic blood pressure was monitored biweekly. The points represent mean systolic blood pressure at the end of each month (n=36. From 17-19 months n=10-12), and the errors indicated are S.E.M.
Fig. 4. Resting diastolic blood pressure as a function of animal age. Resting diastolic blood pressure was monitored biweekly. The points represent mean diastolic blood pressure at the end of each month (n=36. From 17-19 months n=10-12), and the errors indicated are S.E.M.
Fig. 5. Heart rate as a function of animal age. Heart rate was monitored biweekly. The points represent mean resting heart rate at the end of each month (n=36. From 17-19 months n=10-12), and the errors indicated are S.E.M.
Fig. 6. Resting blood serum total cholesterol at 6, 12, and 16 months of age. The points represent mean serum total cholesterol (n=12 for months 6, 12; n=10 for month 16) and the errors indicated represent S.E.M.

*EX < PA  a 16 > 6  b 16 > 6.*
Fig. 7. Resting blood serum low density lipoprotein-cholesterol at 6, 12, and 16 months of age. The points represent mean serum LDL-C (n=12 for months 6, 12; n=10 for month 16) and the errors indicated represent S.E.M.

\[ a \] 12>6, 16  
\[ b \] 12>6, 16  
\[ c \] 12>6, 16
Fig. 8. Resting blood serum high density lipoprotein-cholesterol at 6, 12, and 16 months of age. The points represent mean serum HDL-C (n=12 for months 6, 12; n=10 for month 16) and the errors indicated represent S.E.M.

1 PA > SED  
2 EX > SED  
3 12 > 6  
4 12 > 6, 16  
5 16 > 6  
6 16 > 12
Fig. 9. Resting blood serum triglycerides at 6, 12, and 16 months of age. The points represent mean serum TG (n=12 for months 6, 12; n=10 for month 16) and the errors indicated represent S.E.M.