Age-Related Changes in the Concentration of Plasma Cholesterol and Triglycerides in Two Groups of Rats with Inherited Widely Different Levels of Spontaneous Physical Activity

E.W. RASMUSSEN AND A.T. HOSTMARK

SUMMARY The plasma concentration of cholesterol and triglycerides was determined in two groups of rats with inherited widely different levels of spontaneous physical activity. Active as well as passive rats of each sex were divided into two subgroups, one with free access to wheel running activity (exercising group), and another for which admittance to the drum was closed (nonexercising group). Plasma concentrations of cholesterol and triglycerides were followed from the age of 3–8 months in females and 3 months to 1 year in males. A pronounced increase with age in the plasma concentration of these lipids was observed in the active male rats. In the passive male rats and in all females, there were no major changes in plasma levels of cholesterol and triglycerides. Corresponding groups of exercising and nonexercising rats had similar plasma levels of these components. The data for male rats show a positive association between the inherited tendency to perform spontaneously a high level of physical activity and an age-related increase in plasma lipids. However, running activity per se does not seem to have any influence on the level of plasma cholesterol and triglycerides in these rats.

REPORTS ON the chronic effects of physical activity on plasma lipid concentration are conflicting. In man, Lopez-S et al. observed a minimal decrease in plasma cholesterol level following long-term physical training. Also Wood et al. reported a somewhat lower plasma cholesterol concentration in physically active men, 35–39 years of age, compared to that found in a control group of relatively inactive men. However, physically active men above this age did not have a significantly lower plasma cholesterol concentration than inactive controls. In the rat, Papadopoulos et al. observed that plasma cholesterol decreased in response to exercise; however, others found no such effect. Even an increased concentration of cholesterol in response to long-term exercise was observed by Herbert et al. in old rats maintained on a high sucrose diet. A lowering effect of long-term physical activity on plasma triglyceride concentration appears to be a more consistent finding. In a study by Fraberg, however, no such effect of physical activity on the plasma triglyceride level was found.

It has been demonstrated in this laboratory, through selective breeding, that the level of spontaneous physical activity in rats is inheritable. In offspring of active parents, the level of spontaneous wheel-running activity is several-fold higher than that observed in offspring of passive parents. In an attempt to elucidate the effect of voluntary physical activity on the level of plasma lipids, we have determined the concentration of cholesterol and triglycerides in the plasma of two groups of selected female and male rats with widely differing levels of spontaneous wheel-running activity. The plasma lipid concentration was followed from the age of 3–8 months in females and from 3 months to 1 year in males, i.e., for about one-third to one-half of their normal life-span. The rats were kept individually in a small living cage closely connected to a revolvable drum. To evaluate whether differences in plasma lipids between active and passive rats were due to the different levels of physical activity or to other factors, the active and the passive groups were divided into two subgroups, one in which the rats had free access to the revolving drum (exercising rats) and one in which admittance to the drum was closed with a metal plate (nonexercising rats).

Methods

Animals and Treatment

Unselected Wistar rats of a local strain were used as the parent generation. Their level of spontaneous wheel-running activity was determined. The most active male rats were mated with the most active females. Similarly, the most passive males were mated with the most passive females. In the successive generations, rats were selected and mated as in the parent generation. Mean activity level of the total number of female and male offspring of active and passive rats in each of the generations F, to F, is shown in Table 1. In all descendant generations, pronounced differences were observed between offspring of active and passive rats. In this study, rats belonging to the ninth generation were used.

The complete drum apparatus consists of three main parts: the drum itself, a small living cage, and a dividing
was 55-70% and the room temperature was 20-22°C. The relative air humidity causes wear and tear on the apparatus. Therefore, the complete rotation, clockwise or counterclockwise, is registered. The high level of running activity in active rats continued to have free access to the revolving drum (exercising group), while those of the other group were kept in the small living cage outside the drum (nonexercising group).

### Female rats

<table>
<thead>
<tr>
<th>Generation</th>
<th>Active</th>
<th>Passive</th>
<th>Active</th>
<th>Passive</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_1$</td>
<td>9.89 ± 1.07 (16)</td>
<td>7.64 ± 1.26 (16)</td>
<td>6.01 ± 0.86 (16)</td>
<td>1.71 ± 0.58 (16)*</td>
</tr>
<tr>
<td>$F_2$</td>
<td>9.87 ± 0.20 (20)</td>
<td>3.11 ± 0.54 (11)*</td>
<td>6.74 ± 0.78 (18)</td>
<td>1.11 ± 0.38 (15)*</td>
</tr>
<tr>
<td>$F_3$</td>
<td>12.58 ± 0.69 (16)</td>
<td>3.38 ± 0.57 (16)*</td>
<td>5.13 ± 1.14 (16)</td>
<td>0.55 ± 0.06 (16)*</td>
</tr>
<tr>
<td>$F_4$</td>
<td>8.35 ± 1.08 (16)</td>
<td>0.94 ± 0.13 (15)*</td>
<td>4.71 ± 1.12 (16)</td>
<td>0.25 ± 0.04 (16)*</td>
</tr>
<tr>
<td>$F_5$</td>
<td>13.97 ± 0.97 (16)</td>
<td>1.44 ± 0.25 (12)*</td>
<td>7.90 ± 1.20 (19)</td>
<td>0.51 ± 0.11 (14)*</td>
</tr>
<tr>
<td>$F_6$</td>
<td>15.99 ± 1.02 (15)</td>
<td>3.39 ± 0.62 (14)*</td>
<td>7.89 ± 1.22 (16)</td>
<td>0.33 ± 0.06 (18)*</td>
</tr>
<tr>
<td>$F_7$</td>
<td>15.64 ± 1.42 (16)</td>
<td>3.45 ± 0.99 (16)*</td>
<td>8.85 ± 1.76 (16)</td>
<td>0.31 ± 0.05 (16)*</td>
</tr>
<tr>
<td>$F_8$</td>
<td>16.93 ± 0.76 (17)</td>
<td>1.89 ± 0.55 (16)*</td>
<td>11.65 ± 1.01 (16)</td>
<td>0.40 ± 0.08 (14)*</td>
</tr>
<tr>
<td>$F_9$</td>
<td>15.23 ± 1.17 (15)</td>
<td>5.41 ± 1.00 (17)*</td>
<td>11.83 ± 1.24 (16)</td>
<td>1.06 ± 0.41 (16)*</td>
</tr>
</tbody>
</table>

Female and male rats with high, alternatively low, levels of spontaneous wheel-running activity were selected. Mean activities of their offspring are presented. Level of wheel-running activity for each rat was determined during the 10th to the 19th day after the rat had been placed into a drum apparatus. Results are expressed as mean ± sem; numbers in parentheses = number of rats.

* $P < 0.001$ vs. active.

The rats were placed into the living cage connected to the drum apparatus when they were about 6 weeks old. After 7 days, the number of drum revolutions was recorded for the first time, and the counters were adjusted to zero. Three days later, the number of revolutions was recorded again. During these first 10 days in the apparatus, the running activities increased rapidly (Fig. 3). Recordings of revolution number followed by zero adjustment of the counter were then made every 3rd day throughout the study. The mean drum-running activities presented in Table 1 are calculated from the successive three registrations made after the first 10 days in the apparatus. Based on these activities, pairs of rats within each of the four groups (i.e., active and passive of each sex) were selected so as to provide equal mean levels of wheel running activity at the start of the experiment (Table 2). In one of each pair of subgroups, the rats continued to have free access to the revolving drum throughout the study. Such rats are referred to as exercising rats.

### Male rats

Rats of the remaining subgroup were housed in the small living cage connected to the drum, but the opening to this was closed with a metal plate. These rats are referred to as nonexercising. Since both female and male rats were separated into active and passive groups and each of these groups was divided into exercising and nonexercising subgroups, we finally obtained eight groups as seen from Table 2.

<table>
<thead>
<tr>
<th>Spontaneous wheel-running activity (km/24 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exercise group</th>
<th>Nonexercising group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female rats</td>
<td>16.41 ± 1.42 (6)*</td>
</tr>
<tr>
<td>Male rats</td>
<td>11.83 ± 1.80 (8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exercise group</th>
<th>Nonexercising group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female rats</td>
<td>3.36 ± 0.56 (6)</td>
</tr>
<tr>
<td>Male rats</td>
<td>0.56 ± 0.20 (7)</td>
</tr>
</tbody>
</table>

* Results are expressed as mean ± sem; numbers in parentheses = number of rats.

Active, alternatively passive rats were divided into subgroups with similar mean levels of spontaneous wheel-running activity. Rats in one of these groups continued to have free access to the revolving drum (exercising group), while those of the other group were kept in the small living cage outside the drum (nonexercising group).
are given as the mean ± SEM. Student's t-test was used to
and total glycerol were carried out in duplicate. All results
ance at 340 nm was determined manually on a Vitatron
trifugation, and the glycero! assay was carried out on 100
in plastic cuvettes (Sarstedt, Germany). The fall in absorb-
pyruvate kinase, and lactic dehydrogenase, using enzyme
esterase and oxidase, using commercial kits (Boehringer).
and free glycerol, which was determined in a separate
fjd saponification of 100 µl of plasma with KOH-ethanol,
and triglycerides at the age of 14, 22, and 34 weeks, and in
also at 1 year. Number of rats in each group is given in Table 2.
Two SEM are indicated by the length of the vertical bar, which
sometimes was too small to be shown. ♦ = exercising rats, ○ =
nonexercising rats.

**TABLE 3** Composition of Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole meal of wheat, coarse</td>
<td>65.5</td>
</tr>
<tr>
<td>Lactic acid casein</td>
<td>6.8</td>
</tr>
<tr>
<td>Milk powder of whole milk</td>
<td>4.9</td>
</tr>
<tr>
<td>Dried brewer's yeast</td>
<td>3.9</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>3.4</td>
</tr>
<tr>
<td>Grass meal</td>
<td>5.1</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1.5</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1.0</td>
</tr>
<tr>
<td>Oleum arachis</td>
<td>2.9</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>1.0</td>
</tr>
<tr>
<td>Whole meal of herring</td>
<td>2.9</td>
</tr>
<tr>
<td>Seaweed meal</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* In addition, whole corn was given every 2nd day to prevent hypertr-

All rats were given a standard laboratory diet as a dry
mixture (Table 3) and water ad libitum. Protein supplied
22%, fat 20%, and carbohydrate 58% of calories.

**Plasma Lipid Determination**

Plasma lipids were determined when the rats were 14,
22, and 34 weeks old and, in males, also when they were
1 year old (Fig. 1). Sampling was performed by puncture
of the tail vein after an overnight fast. To obtain vasodi-
latation, the rats were placed under an infrared lamp for
2-3 minutes before venipuncture. The syringes were
moistened with heparin (1:10 dilution of heparin, 5000
U/ml). Samples of about 1 ml of blood were centrifuged
twice for 10 minutes at 2500 rpm in a table centrifuge and
the plasma was frozen at −20°C. In young rats, blood
sampling by venipuncture is difficult. Necrosis of the tail
may occasionally occur. The first blood samples therefore
were taken 5 weeks after the rats had been separated into
exercising and nonexercising groups, at the age of 14
weeks, and do not give the baseline value. Total chole-
sterol was determined in 50 µl of plasma with cholesterol
esterase and oxidase, using commercial kits (Boehringer).
The plasma concentration of triglycerides was estimated
as the difference between total glycerol, determined after
saponification of 100 µl of plasma with KOH-ethanol,
and free glycerol, which was determined in a separate
plasma sample (Eggstein and Kreutz10). Plasma-free gly-
cerol was determined enzymatically with glycero! kinase,
pyruvate kinase, and lactic dehydrogenase, using enzyme
kits for neutral fat determination (Boehringer). To 100
µl of plasma we added 200 µl of 0.6 N perchloric acid in
1-ml centrifuge tubes. The mixture was centrifuged at
10,000 g for 10 minutes in a Sorwall RG5 refrigerated
(4°C) centrifuge. One hundred and fifty microliters of the
supernatant fluid was neutralized with 10 µl of 6 N KOH.
The solution was freed of potassium perchlorate by cen-
trifugation, and the glycerol assay was carried out on 100
µl of the supernatant fluid (total assay volume, 0.635 ml)
in plastic cuvettes (Sarstedt, Germany). The fall in absorb-
ance at 340 nm was determined manually on a Vitatron
photometer. Determinations of cholesterol, free glycerol,
and total glycerol were carried out in duplicate. All results
are given as the mean ± SEM. Student's t-test was used to
estimate the significance of differences between experi-
ments.

**Results**

**Plasma Cholesterol and Triglyceride Levels in Active and Passive Rats**

When the first blood sample was obtained from rats at
14 weeks of age, the plasma level of cholesterol was
significantly ($P < 0.001$) higher in exercising passive
(60.4 ± 3.0 mg/100 ml, $n = 7$) than in active (40.8 ± 2.9
mg/100 ml, $n = 8$) male rats. Also, the plasma concen-
tration of triglycerides was higher ($P < 0.02$) in exercising
passive (0.281 ± 0.025 mm, $n = 7$) than in active (0.148
± 0.027 mm, $n = 8$) males. At the age of 22 weeks,
however, active and passive rats had nearly equal values
(Fig. 1). Surprisingly, when the rats were 34 weeks old,
the plasma levels of both cholesterol and triglycerides had
increased appreciably in active males but had changed
little in their passive counterparts. Thus, at this age,
exercising active male rats had plasma cholesterol ($P <
0.02$) and triglyceride ($P < 0.005$) levels approximately
twice as high as those of the corresponding passive group.
Even more pronounced was the difference in plasma lipid
level observed in 1-year-old male rats. At this age, the
triglyceride level was 3- to 4-fold higher in active than in
passive males.

Nonexercising rats had plasma cholesterol and trigly-
ceride levels which were not significantly different from
those found in their exercising counterparts. This was
PLASMA LIPIDS IN SELECTED RATS/Rasmussen and Höstmark

E.15O

### FIGURE 2
Concentration of cholesterol and triglycerides in plasma of active and passive female rats. Symbols are as in Figure 1.

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observed both in active and in passive male and female rats.

Although there appeared to be a slight age-related increase in plasma lipids in active female rats (Fig. 2) and no such increase in passive ones, there were no significant differences between the two groups during the experiment.

### Age-Related Changes in Spontaneous Wheel-Running Activity

The level of spontaneous wheel-running activity of active rats increased rapidly during their first weeks in the drum apparatus (Fig. 3). In active female rats, the activity level then appeared to increase slightly during the rest of the experimental period. In contrast to this, the activity level of active male rats gradually decreased during the later part of the experimental period. Passive male rats showed a low level of activity for the whole period. In passive females, a higher activity level was observed, and no significant alteration with increasing age was noted.

### Body Weight Curves

A somewhat steeper increase in body weight was observed in passive than in active rats of both sexes (Fig. 4). Nonexercising male rats had significantly ($P < 0.01$) higher weights than those of the corresponding exercising group up to 34 weeks of age. In 1-year-old male rats, values for exercising and nonexercising males were similar. Nonexercising and exercising female rats had similar body weights throughout the observation period.

### Discussion

The question whether chronic physical activity influences the level of blood lipids is controversial. Discrepancies between results obtained in various studies might be explained partly by different experimental conditions used, such as work type, intensity and duration of exercise, dietary regimen, and animals used. In most studies, the effect of forced, strenuous exercise was investigated.

The results presented here demonstrate a dramatic difference between male rats with a low level of voluntary physical activity with regard to age-related changes in plasma cholesterol and triglyceride concentration. It should be pointed out that baseline values for the plasma lipids were not obtained, due to difficulties with blood sampling in young rats. It seems very likely, however, that exercising and nonexercising rats had similar plasma lipid values at the start of the experiment, since these subgroups were taken from homogeneous main groups of active and passive rats.

The fact that plasma cholesterol and triglycerides showed a pronounced increase with age in active male rats while the level remained low in passive male rats is

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**Figure 3** Age-related changes in spontaneous wheel running activity. Levels of spontaneous wheel running of active (○) and passive (●) female and male rats were determined in several periods during the observation period. Each point represents the mean for eight rats. The length of the vertical bar indicates 2 SEM. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$ vs. passive rats.
somewhat surprising. In man, physical activity is believed to have a beneficial influence on coronary heart diseases, the incidence of which is positively correlated to the level of blood lipids. Accordingly, it might be suggested that a high level of physical activity would prevent an age-related increase of the plasma lipids. Whether lifelong physical activity of moderate intensity, in man, may reduce the increase of plasma lipids occurring with age is not known. It appears, however, that a high level of spontaneous physical activity in rats does not prevent an age-related increase in plasma cholesterol and triglyceride concentration. Since the level of wheel-running activity of active male rats decreased with time, it might be suggested that the age-related increase in plasma lipids was due to a concomitant decline in the level of physical activity. However, nonexercising and exercising rats had very similar levels of plasma lipids in all groups tested, in both males and females, and in active as well as in passive rats. It seems very likely, therefore, that the pronounced age-related elevation in plasma triglyceride and cholesterol concentration observed in active male rats is not associated with the decline in spontaneous physical activity occurring with increasing age, but depends upon other, probably inherited, factors. It is pointed out that the difference in physical activity between active and passive rats was pronounced. Active male rats ran approximately 12 km/24 hours, while passive rats ran only about 0.5 km/24 hours. The difference in activity level between exercising and nonexercising genetically active male rats should be even greater. It must be concluded, therefore, that, in this study, running activity per se had no influence upon the concentration of plasma cholesterol and triglycerides.

As suggested by the works of Lopez-S et al. and Wood et al., physical exercise in man causes an increase in the concentration of plasma high-density lipoproteins. Whether physical activity in the selected rats used in this study leads to an increase in this lipoprotein fraction is presently under investigation.

It should be noted that, if lipid values observed in 14-week-old male rats were the only ones considered, one might have been led to the erroneous conclusion that physical inactivity in these rats was causally associated with an increase in plasma cholesterol and triglyceride concentration, since at this age passive rats had a significantly higher plasma lipid level than did the active ones. The explanation of the prolonged pronounced differences between active and passive male rats with respect to plasma lipid values, whether related to lipid production, transport, or elimination, calls for further studies.

Acknowledgments

The excellent technical assistance by Ida Goffeng Bay, Lisbeth Sorenson, and Einar Eilertsen is gratefully acknowledged.

References

IN 1879, Burdon-Sanderson and Page\textsuperscript{1} described the effects of injury to the surface of the frog heart and noted that, during activity, the injured site became positive with respect to the uninjured surface. Since then, many authors have studied the effects of injury, including those of ischemia, on local extracellular electrograms of the heart. Gradually, it was established that S-T elevation as recorded with condenser-coupled amplifiers could be due to effects of injury to the surface of the frog heart and noted that, during activity, the injured site became positive with respect to the normal extracellular electrogram, making the ischemic extracellular space positive with respect to the normal area. This was confirmed by the work of Prinzmetal et al.\textsuperscript{10} The mechanism of S-T elevation, however, seems not quite as well established. Samson and Scher\textsuperscript{8} have stated that it is caused by transient recovery of electrical activity in the ischemic zone. After 2 hours, the zone of unresponsiveness was larger than after 15 minutes of occlusion, and the overall amplitude of DC potentials had decreased further, possibly because of healing over.

SUMMARY We recorded transmembrane potentials from subepicardial ventricular cells and local extracellular DC electrograms in isolated perfused pig hearts before and after occlusion of the left anterior descending artery. The first change was a decrease in the resting membrane potential, reflected by T-Q depression in the electrogram. After 3 minutes, action potentials shortened and their amplitude decreased, resulting in S-T elevation until, finally, cells in the center of the ischemic zone became totally unresponsive at resting potentials of about $-65$ mV. This rendered the extracellular complex monophasic. Determination of extracellular potential distribution at 150-250 epicardial sites after 15-30 minutes of occlusion showed an increase of T-Q depression and S-T elevation toward a central area, with maximum values of $-15$ and $+35$ mV, respectively. Comparison of amplitude and configuration of intramural and epicardial potential profiles revealed that the potential distribution was homogeneous throughout ischemic parts of the wall. Extracellular epicardial current originated, therefore, from the epicardial intracellular compartment. Maximal current density during late systole was $1 \mu$A/mm$^2$, flowing in the border zone towards normal myocardium. After 1 hour of occlusion, there was a marked decrease of extracellular DC potentials which could be attributed to transient recovery of electrical activity in the ischemic zone. After 2 hours, the zone of unresponsiveness was larger than after 15 minutes of occlusion, and the overall amplitude of DC potentials had decreased further, possibly because of healing over.

Mechanism and Time Course of S-T and T-Q Segment Changes during Acute Regional Myocardial Ischemia in the Pig Heart Determined by Extracellular and Intracellular Recordings

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injury between healthy cells and ischemic cells which rendered the extracellular space in the ischemic area negative with respect to the normal area. This was confirmed by the work of Prinzmetal et al.\textsuperscript{10} The mechanism of S-T elevation, however, seems not quite as well established. Samson and Scher\textsuperscript{8} have stated that it is caused by a shortening of the ischemic action potential. In the absence of activation delay of the ischemic cells, a systolic current of injury would be expected to flow between the ischemic and normal areas, making the ischemic extracellular space positive with respect to the normal extracellular space. Samson and Scher found that the injured area produced near normal action potentials during depolarization and they therefore stated that a lack of responsiveness in the injured area could not account for true S-T elevation, as was originally suggested by the results of Eyster et al.\textsuperscript{4} Recent studies from this laboratory\textsuperscript{11} have shown, however, that transmembrane action potentials from ischemic cells can vary from "near normal" to responses of very small amplitude, depending on localization and duration of ischemia.
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