# Effects of three different housing systems on activity and physiological parameters in male Spontaneously Hypertensive Rats and Sprague Dawley Rats

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## SAMMANFATTNING

Laboratorieråttor spenderar majoriteten av sina liv i sin hembur. Standardburen som används till råttor, Makrolon typ IV, ger dem inte möjlighet till fysisk aktivitet i någon större utsträckning. Råttornas välfärd skulle förbättras genom inhysning i större burar som ger möjlighet till en ökad fysisk aktivitet. Syftet med denna studie var att undersöka effekten av större, alternativa burtyper, på parametrar som förändras i samband med en ökad fysisk aktivitet. Vi jämförde 24 råtthanar av stammen Sprague Dawley (SD) med 24 råtthanar av stammen Spontaneously Hypertensive Rats (SH). SH är en råttmodell som utvecklar ett förhöjt blodtryck samt insulinresistens. Råttorna hölls stamvis uppdelade i två kommersiellt tillgängliga burtyper med kontrollgrupper i standardburar. De kommersiella burtyperna var; Scantainer$^{NOVO}$ (NOV) (golvyta ca 2240 $cm^2$, fyra burar sammankopplades, total golvyta ca 8970 $cm^2$, höjd 32.5 cm) och Enriched Rat Cage System (ERC) (golvyta ca 6020 $cm^2$, höjd 46 cm), och standardburen var Makrolon typ IV (ST) (golvyta ca 2240 $cm^2$, höjd 18 cm). I NOV-burarna och ERC-systemet hölls åtta råttor av vardera stam och i ST-burarna hölls råttorna parvis i fyra burar med SD råttor och fyra burar med SH råttor. Varje vecka mättes kroppsvikt, foderkonsumtion och blodtryck. Under vecka tre och tio i studien utfördes ett arbetsprov på en lutande rullmatta, s.k. laddermill där råttorna fick klättra på tre ökande arbetsintensiteter (7, 9 och 11 m/min) med ett efterföljande uthållighetsprov. Blodprov togs för analys av laktatnivåer. Råttornas muskelstyrka testades på ett lutande plan. Efter tio veckor avlivades djuren, hjärta och binjurar vägdes och blodprov togs för analys av kortikosteron- och insulinhalt.

Vid ett tillfälle, v.2, sågs en lägre viktsökning hos SD råttor i NOV-burarna jämfört med dem i ST-burar. Majoriteten av råttorna kunde fullfölja båda arbetsproven på laddermill. Vid arbetsprov 2 sågs en lägre laktathalt hos SD råttor från NOV-burar jämfört med dem i ST-burar. Båda råttstammarna i ST-burar, samt SHR i ERC-burar hade ökad laktathalt i arbetsprov 2 jämfört med prov 1. Alla råttor i NOV-burar hade en ökad uthållighet i arbetsprov 2 jämfört med prov 1. Under v.10 sågs ett signifikant ökat systoliskt blodtryck hos SD råttor i NOV-buren jämfört med SD råttor i ST-burar. SH råttor uppvisade ökad relativ hjärtvikt och ökad insulinkoncentration jämfört med SD råttor, men ingen skillnad mellan burtyper. En signifikant positiv korrelation kunde ses mellan kroppsvikt och insulinkoncentration hos SH råttor. Vid lutande plan-testet presterade råttor från ST-burar sämst, endast 56% av råttorna klarade av att hålla sig kvar på en lutning överstigande 55°, jämfört med 88 % i NOV-burar och 88%  i ERC-burar.

Sammanfattningsvis visade studien att råttor från NOV- burar uppvisade de största tecknen på ökad fysisk aktivitet baserat på resultaten från arbetsproven. Inga effekter på övriga fysiologiska parametrar sågs, vilket tyder på att råttornas aktivitet i hemburarna ej varit tillräcklig för att påverka dessa parametrar. SH råttorna bibehöll sitt höga blodtryck och insulinhalt oavsett burtyp. Det fanns heller inga skillnader mellan burtyper i kroppsvikt, hjärtvikt eller binjurevikt. Detta visar att det går att hålla SH råttor i större burar utan att påverka djurmodellen. Studien visar att råttor ökar sin spontana aktivitet i hemburen, men

inte tillräckligt mycket för att ge en tydlig träningseffekt. Men ökad hembursaktivitet är ändå relevant för råttornas välfärd och behöver inte påverka forskningsresultaten.

## SUMMARY

Laboratory rats spend a majority of their lives in their home-cages. The cage often used for rats, Makrolon type IV, only gives them limited opportunities for physical activity. The welfare of the rats would increase by housing them in larger cages that allow an increased amount of physical activity. The aim of this study was to evaluate the effect of larger, alternative cages, on parameters that are affected by physical activity. We compared 24 male Sprague Dawley (SD) rats and 24 male Spontaneously Hypertensive rats (SH) which were housed strain-wise in two commercially available housing systems with controls in standard cages. The SH rat is an animal model that develops an increased blood pressure and insulin resistance. The commercially available housing systems were; Scantainer$^{NOVO}$ (NOV) (floor space approximately 2240 $cm^2$, four cages connected to each other with floor space of approximately 8970 $cm^2$, height 32.5 cm) and Enriched Rat Cage System (ERC) (floor space approximately 6020 $cm^2$, height 46 cm), and the standard cage was Makrolon type IV cage (ST) (floor space 2240 $cm^2$, height 18 cm). Eight rats per strain were kept in the NOV-cages and the ERC-systems, while rats were kept in pairs in the ST-cages. Every week the rats´ body weight, blood pressure and food consumption were recorded. During week 3 and 10 the rats performed an exercise test on an inclined treadmill, a so-called laddermill, where the rats climbed at three different intensities (7 m/min, 9 m/min and 11 m/min) followed by an endurance test. Blood samples were collected for analysis of blood lactate concentration. The rats' muscle strength was assessed in a so-called inclined plane test. After ten weeks the rats were euthanized, heart and adrenals were collected and weighed, and blood samples were taken for analysis of corticosterone- and insulin concentration.

A temporary reduction in body weight gain in week 2 was seen in SD rats kept in NOV-cages, compared to those in ST-cages. The majority of rats succeeded in both exercise tests on the laddermill. In exercise test 2, SD rats kept in NOV-cages had a lower blood lactate level compared those kept in ST-cages. Both rat strains kept in ST-cages and SH rats kept in ERC-system had an increased lactate level in exercise test 2 compared to test 1. All rats kept in NOV-cages had a significantly increased endurance capacity in the exercise test 2. During week 10, SD rats kept in NOV-cages had an increased systolic blood pressure compared to those in ST-cages. The SH rats had a greater insulin concentration and relative heart weight compared to SD rats but no difference was seen between cage types. There was a positive correlation between body weight and insulin concentration in SH rats. Rats kept in ST-cages had the poorest performance in the inclined plane test, only 56% of the rats succeeded in holding on at an inclination exceeding 55°, compared to 88% of the rats in NOV-cages and 88 % of the rats in the ERC-system.

In a summary the rats kept in NOV-cages showed the clearest signs of an increased home cage activity based on the results obtained from the exercise tests. No effects on other physiological parameters were detected, which indicates that the activity level of the rats in their home-cages was not intense enough to result in a change in these parameters. The SH rats maintained their increased systolic blood pressure and insulin resistance regardless of cage type. In addition, they had

no differences in body-, heart-, or adrenal weight between the different housing systems. This shows that it is possible to keep SH rats in larger cages without affecting the animal model. The study shows that rats increase their spontaneous activity in their home cages but not enough to create a distinct training effect. However, increased home cage activity is still relevant for animal welfare and does not have to interfere with scientific results.

## INTRODUCTION

The laboratory rat, *Rattus norvegicus*, is the second most used laboratory animal in research. The major part of the life of a laboratory rat is spent in its home-cage, and according to the Swedish Animal Welfare Act (4§ Djurskyddslagen, 1988: 534) animals in captivity should be kept in a way that promotes their health and wellbeing and gives them possibilities to perform their natural behaviors. Therefore it is important that the home cage gives the rat optimal conditions to ensure their health and wellbeing. Wild rats, being agile and active animals, can have a home range of up to 20-30 m radius area from their nest and they can move several km/day (Barnett, 1975, s. 30). Laboratory rats released into the wild quickly adapt to the surrounding, and show several natural behaviors like burrowing, patrolling their home range etc (Boice, 1977). In addition, if laboratory rats are given access to running wheels they can run up to 12 km/day (Rodnick *et al*, 1989). The cage type commonly used for housing rats in research facilities today, the Makrolon type IV cage, limits the rats´ opportunity to perform various types of activities, like rearing, climbing and running. In combination with an *ad lib* feeding regimen it often results in over weight and inactive animals, in contrast to the curious and active rats living in the wild. It has been shown that if rats are given a larger cage, they will increase their active behaviors, like running and climbing (Spangenberg *et al,* 2005). Taken together, this emphasizes the need to offer the rats housing types with increased opportunities for physical activity.

Physical activity can have positive effects on both physical and mental properties in rats. These includes a reduced body weight gain (James *et al,* 1984; Zendrian-Piotrowska *et al,* 1993; Wisløff *et al,* 2001; Hoffman *et al,* 1987; Lambert *et al,* 1996; Morimoto *et al,* 2000; Narath *et al,* 2001; Rodnick *et al,* 1990; Evenwel *et al,* 1979) body fat content, enhanced cardiovascular functional capacity and better glucose tolerance (Holloszy, 1988), and enhanced insulin sensitivity (Ivy*,* 1981; James *et al*, 1984). Physical exercise has also been shown to reduce anxiety-like behavior in rats (Fulk *et al*, 2004), diminish depressive behavior (Zheng *et al,* 2006) and improve their memory (Radak *et al,* 2006). A larger and more enriched cage will significantly improve the recovery after focal brain ischemia in rats (Johansson *et al*, 1996). Furthermore, stress is a sign of reduced animal welfare and increased physical activity can reduce the negative physiological effects seen in stressed rats (Morimoto *et al,* 2000). In summary, physical activity is important for the animal welfare and a housing system that allows for an increased physical activity should therefore improve the welfare of the rats. It has previously been shown that rats kept in large pens had a lower body weight gain, greater muscle strength and higher oxidative capacity and glycogen content in their muscles (Spangenberg *et al*, 2005). Larger housing systems give the rats more possibilities to perform a wider range of natural behaviors. When designing new cages it is important to consider the species-specific behavior, because different species have different needs, so that the environmental enrichment is relevant, and will be used by the species (Newberry, 1995). Rats housed in larger cages with bigger groups will also have an increased amount of social interactions, which in itself can be enrichment.

The spontaneously hypertensive (SH) rat is the result of a spontaneous mutation in the Wistar Kyoto rat strain (WKY). The SH rats develops an abnormal increase in its blood pressure starting between four and nine weeks of age, with an established hypertension at 13 weeks of age (Evenwel *et al,* 1979; Hoffman *et al,* 1987; Lajoie *et al*, 2004). The adult SH rat have a systolic blood pressure above 200 mm Hg, compared to normotensive WKY and Sprague Dawley rats that have a systolic blood pressure level of 120-140 mmHg ((National Research Council, 1996); Zhang *et al*, 2004). The SH rat is commonly used as a model of hypertension and other cardiovascular disorders associated with hypertension. The SH rats also have an increased plasma insulin concentration, which indicates insulin resistance (Mondon *et al,* 1988). Increased physical activity benefits the SH rat; Hoffman *et al.* (1987) found that nine week old SH rats had a delayed onset of hypertension following six weeks of voluntary running in running wheels, with an average running distance of 6-7 km/rat and day. They had a significantly lower systolic blood pressure that remained at a lower level almost throughout the study. There are other studies that also show lowering effects on systolic blood pressure by physical exercise (Ghaemmaghami *et al,* 1991; Véras-Silva *et al.* 1997). Increased activity in the home cage could affect parameters like the blood pressure, insulin sensitivity and body weight in the SH rat. If these parameters are affected by various cage types/ sizes, it shows the importance of the housing conditions for this animal model. The control strain, Sprague Dawley was chosen for this study because it is one of the most commonly used rat strains in research.

Exercise tests are often used to measure physiological effects of training. Standardized exercise protocols have been designed in several animal species, e.g. rats (Voltarelli *et al*, 2002), and horses (Nostell *et al*, 2006) besides humans (Carter *et al,* 1999). A trained individual produces less lactate at specific work intensity during an exercise test (Eydoux *et al*, 2000). Spangenberg *et al* have earlier performed a treadmill exercise test to evaluate the training effect in rats housed in larger cages and groups (unpublished data). Occasionally rats refuse to run on the treadmill (Dishman *et al*, 2000; Norton *et al,* 1990) and therefore have to be excluded from the study. This can jeopardize the statistical power of that study. In addition, mild electric shocks are often used as aversive stimuli to force the rats to run (Tipton *et al*, 1983; Wisløff *et al*, 2001), resulting in stress-induced responses in the rats affecting the physiological parameters that are being recorded. A study made using a laddermill showed that rats that refused to run on a treadmill, willingly climbed on the laddermill (Norton *et al*, 1990). Similar results using a laddermill were obtained in a pilot study (Cvek *et al,* 2007) where all rats climbed willingly encouraged only by a mild prodding.

The aim of this study was to evaluate the effect on home cage activity of two different commercially available housing systems for laboratory rats, in comparison to the standard Makrolon type IV cage. The larger space and bigger groups in the alternative cages should give the rats opportunity to enhance their spontaneous locomotor activity and thereby improve their welfare. Effects will be measured in body weight, heart and adrenal weight, blood pressure, muscle strength and performance in exercise tests.

## MATERIAL AND METHODS

### Animals and housing

The Uppsala committee for ethical review of animal studies approved the experimental protocol. Twenty-four male Sprague Dawley (SD) rats (Charles River, Germany) and 24 male Spontaneously Hypertensive (SH) rats (Charles River, Germany) were randomly assigned to three different housing systems. At arrival the rats were seven weeks old, the SD rats had a mean body weight of 232.8 ± 1.7 g and the SH rats 209.5 ± 1.3 g. Sixteen rats, eight of each strain, were housed in pairs in standard Makrolon type IV cages (length, width, height: 59x38x20 cm, floor space approximately 2240 cm$^2$) (ST) (figure 1). Each ST-cage was equipped with two black plastic tubes (Ø 6.5 cm, 14-16 cm long) as shelters. Another sixteen rats, eight per strain and cage, were housed in Enriched Rat Cage System (length, width, height: 87.9x68.5x46 cm, floor space approximately 6020 cm$^2$) (ERC). The ERC-housing system was equipped with a shelf (24x60 cm) and two stairs (8x18 cm and 8x22 cm) attached parallel to two of the walls of the cage (figure 2). The last sixteen rats, eight of each strain, were housed strain-wise in Scantainer$^{NOVO}$ (length, width, height: 59x38x32.5 cm, floor space approximately 2240 cm$^2$) (NOV), equipped with a shelf (16x23 cm), with the possibility to connect four subsequent NOV-cages to each other, giving a total floor space of 8960 cm$^2$ (figure 3). On each cage side there was a passage, with an opening size of 7x7 cm, placed 22 cm above the cage floor. The Enriched Rat Cage System (ERC) and Scantainer$^{NOVO}$ (NOV) were both manufactured by Scanbur A/S, Denmark.

*Figure 1. The Makrolon type IV -cage used in the study with a floor space of approximately 2240 cm$^2$.*

*Figure 2. The Enriched Rat Cage System used in the study with a floor space of approximately 6020 cm$^2$.*

The rats were given free access to standard pelleted diet (SDS RM 1, Scanbur BK AB, Sollentuna, Sweden) and tap water. Food intake was recorded several times/week and the rats were weighed once weekly. The bedding in all housing systems was aspen GLP bedding (Beekay Bedding, Sollentuna, Sweden). The room temperature was kept between 22-23 °C and the relative humidity was kept between 23-43 %. There was a 12 h light and dark cycle with a dark period between 12:00 and 24:00. The rats were housed in the different housing systems for ten weeks.

*Figure 3. Scantainer$^{NOVO}$-cages used in the study. Four separate cages connected by passages to each other giving a floor space of approximately 8960 cm$^2$.*

## Laddermill and exercise tests

To study effects of the different housing types on the exercise physiology of the rats, two exercise tests were performed. All rats were habituated to climbing on a treadmill (Exer 4, Columbus Instruments, Columbus, Ohio, USA) modified to a laddermill. The laddermill is a treadmill tilted to an inclination of 50°, with wooden rods attached to the belt to facilitate climbing (figure 4). The rats climbed at a low intensity (7 m/min) for two minutes once weekly to maintain the ability to climb. In addition, the rats were habituated to the laddermill at four occasions prior to the first exercise test. The upper end wall of the treadmill was removed, because the rats were more willing to climb towards a free opening, rather than a wall. By touching the base of the tail, the rats were encouraged to run. If the rats refused to run despite plenty of prodding they would fall into a bowl of ice-chilled water. The rats were rewarded with honey puff following each exercise-session cereals (ICA Honungspuffar, ICA Sverige AB, Solna, Sweden).

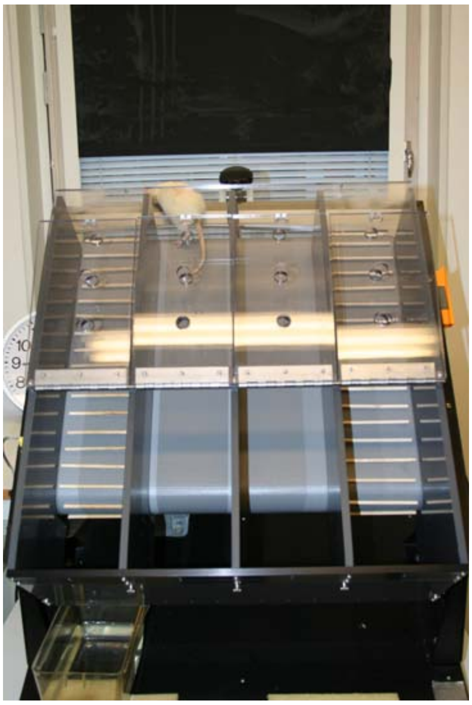

*Figure 4. Treadmill modified to a laddermill with a 50$^o$ slope.*

The exercise test protocol was based on a pilot study previously done on the laddermill (Cvek *et al,* 2007). The first exercise test was performed after the rats' acclimatization period of being housed for two weeks in their respective housing systems. A second exercise test was performed seven weeks later, i.e. after nine weeks of housing in the different systems. The rats were transported to the laddermill-room (separate from the housing room) individually in Makrolon type

III cages in groups of six. Preceding the start of the exercise-test a blood sample was taken from the saphenous vein in the hind leg for blood lactate levels in the rested state. Each rat started with a one minute “warm-up” at 7 m/min, followed by one minute of climbing on the laddermill at the same intensity. The rat was then immediately removed from the laddermill and another blood sample was taken from the hind leg for lactate analysis. This procedure was repeated at intensities 9 and 11 m/min. Each blood sample was taken within 60 sec after the rat was taken off the laddermill.  Following the last intensity an endurance test was performed, where the rat had to climb until exhaustion, which was defined as when the rat refused to climb for three times despite plenty of prodding. The inclination of the laddermill was 50° during the entire exercise test.

## Blood-pressure recordings

The systolic blood pressure was recorded non-invasively once per week by using a tail-cuff (ML125 NIBP (Non Invasive Blood Pressure), AD Instruments Pty, Australia) connected to a PowerLab and a personal computer. The data was analyzed with the software Chart5 for Windows. The tail-cuff was placed on the proximal portion of the rats’ tail (figure 5). The membrane of the pulse receiver of the recording equipment was situated on the ventral side of the rat´s tail as close to the tail artery as possible. The measurements took place in the room where the rats were housed and the starting order was randomized for every session. It was always the same person that restrained the rats, in a towel or in her arms, in order to create a calm, safe and non-stressful recording situation. Habituation to the procedure took place during three separate occasions prior to the data recording. The animals were not pre-heated before the recordings. After pulses in the tail were detected, the cuff was inflated to 200 mmHg for the SD rats and 280 mmHg for the SH rats, so that the pulses stopped. The cuff was then slowly deflated and the point when the pulses returned was recorded as the systolic blood pressure. Recordings without disturbances, e.g. movements or sniffing, that were made as close to the start of the recording session for each rat were chosen as the individual rat´s systolic blood pressure (mmHg).

*Figure 5. Blood pressure recording using a non-invasive tail-cuff.*

## Inclined Plane

The muscle strength of the rats was tested using an inclined plane test (first described by Rivlin and Tator, 1977). It consisted of a wooden box (60x30x15 cm), with a rubber mat on the floor of the box. The rat was placed on the rubber mat with its body axis perpendicular to the axis of the inclined plane and was not allowed to turn around (figure 6). The inclination of the box was measured by a digital water level attached to the box. The test consisted of two parts; the first one measured the maximal inclination at which the rat could keep holding on to the rubber mat. When the rat started to slide down, the inclination was recorded. The test started at 45° with an increase of the inclination by 1-2°/sec. The procedure was repeated three times and a mean value was calculated. In the second part, the endurance strength was measured. The rat was put in the box with a starting inclination of 55° for 60 sec, and the inclination increased by 5° for each following minute, until the rat could not hold on and started to slide down. The inclination and time was recorded. For both tests, each rat was acclimatized to the box for 15 sec followed by 5 sec at the starting inclination to give the rat a possibility to position itself. Each rat had a five-minute resting period between the maximal inclination test and the endurance test.

*Figure 6. Rat performing in the inclined plane test.*

## Euthanization

At the end of the study, the rats were euthanized by i.p. injection of pentobarbital (100 mg/ml). The circumference of the front leg, proximally of the elbow joint, and the circumference of the hind leg, proximally of the stifle joint, was measured. Front- and hind legs of both sides were measured and a mean value was calculated. Blood was collected by heart puncture to be analyzed for plasma levels of insulin and corticosterone. The heart and adrenal glands were dissected from connective tissues and weighed.

## Blood analyses

Blood samples taken during the exercise tests were collected in Analox tubes containing a lysing agent (fluoride, heparin and nitrite), and stored at 0° C until analysis. Lactate levels were analyzed on whole blood using the Analox Analyser (Analox Instruments LTD, London W6 0BA, UK, www.analox.com), and expressed as mmol/l. The blood samples taken at euthanization were stored at 0° C until all rats were euthanized, and thereafter immediately centrifuged for 20 min and the plasma and serum was stored at -20°C for further analyses. The insulin concentration in plasma was analyzed using a sandwich ELISA (Mercodia, Uppsala, Sweden, www.mercodia.se), ands expressed as µg/l plasma. The corticosterone concentration was analyzed using a RIA-kit (COAT-A-COUNT, PDC, Diagnostic Products Corporation, Los Angeles, USA), and expressed as ng/l plasma.

## Statistics

Comparisons within strains between housing systems were made using One-Way ANOVA, or Kruskal-Wallis ANOVA on Ranks for non-parametric data. Comparisons between strains were performed with t-test or Mann- Whitney Rank Sum test (non-parametric data). Data over time was analyzed using Repeated Measures ANOVA or paired t-test (Wilcoxon Signed Rank test for non-parametric data). Results were considered significant at $p<0.05$, and are presented as mean ± SEM.

# RESULTS

## Body and organ weights

During the second week of the study, the SD rats in the NOV-cages had a lower body weight than the SD rats housed in ST-cages ($p<0.05$) (figure 7A). There was a tendency for the same difference in the SD rats during week 3 ($p=0.057$). No other differences in body weight were found in SD rats and there were no significant differences in body weight in the SH rats between the different housing systems during the ten weeks of the study (figure 7B).

A B

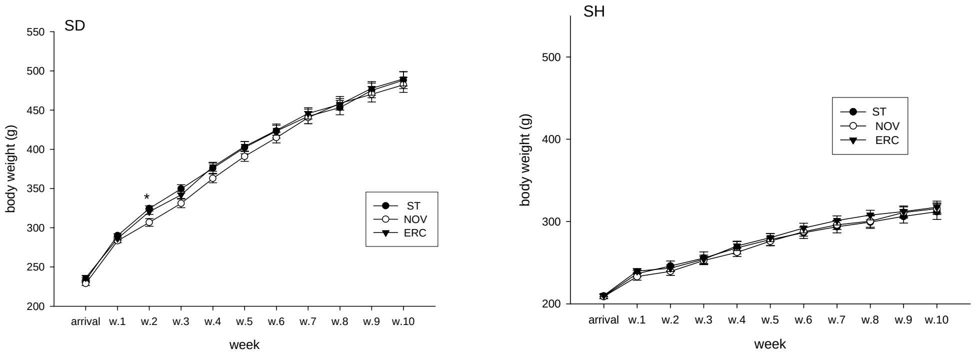


*Figure 7A-B. Weekly body weight gain of male Sprague Dawley (SD) rats (A) and Spontaneously Hypertensive (SH) rats (B) housed in the standard Makrolon type IV cages (ST), Scantainer $^{NOVO}$ (NOV) and Enriched Rat Cage (ERC). Data are presented as mean ± SEM.* denotes difference between ST and NOV $p<0.05$.*

The body weight ranges of the SD rats housed in ST-cages were; at arrival: 218.3-241.3 g, week 5: 374.8-438.9 g, and at week 10: 448.0-542.3 g. The body weight ranges of the SD rats kept in NOV-cages were; at arrival: 218.5-239.5 g, week 5: 368.6-423.1 g and at week 10: 459.0-530.1 g. The body weight ranges of SD rats kept in the ERC-system were; at arrival: 222.3-249.3 g, week 5: 382.6-429.1 g, and at week 10: 456.6-523.6 g. The corresponding body weight ranges in the SH rats kept in the ST-cages were: at arrival: 200.0-225.2 g, week 5: 256.8-315.1 g, and at week 10: 286.5-357.0 g. The body weight ranges of SH rats kept in NOV-cages were; at arrival: 195.0-221.5 g, week 5: 254.7-296.9 g and at week 10: 287.3-343.0 g. The body weight ranges of SH rats kept in ERC-system were; at arrival: 205.6-219.0 g, week 5: 261.8-305.5 g, and at week 10: 287.1-348.1 g.

*Table 1. Absolute (Abs) and relative (Rel) heart- and adrenal weight of male Sprague Dawley (SD) rats and Spontaneously Hypertensive (SH) rats, housed in the standard Makrolon type IV cages (ST), Scantainer $^{NOVO}$ (NOV) and Enriched Rat Cage (ERC). Data are presented as mean ± SEM*

| | N | Abs.heart weight (g) | Rel.heart weight (%) | Abs.adrenal weight (g) | Rel.adrenal weigth (%) |
|---|---|---|---|---|---|
| ST-SD | 8 | 1.41 ± 0.06 | 0.29 ± 0.02 | 0.047 ± 0.0021 | 0.010 ± 0.0004 |
| NOV-SD | 8 | 1.42 ± 0.04 | 0.30 ± 0.01 | 0.053 ± 0.0028 | 0.011 ± 0.0006 |
| ERC-SD | 8 | 1.36 ± 0.04 | 0.28 ± 0.01 | 0.052 ± 0.0016 | 0.011 ± 0.0004 |
| ST-SH | 8 | 1.25 ± 0.05 | 0.40 ± 0.02 | 0.034 ± 0.0022 | 0.011 ± 0.0007 |
| NOV-SH | 8 | 1.24 ± 0.05 | 0.39 ± 0.01 | 0.037 ± 0.0021 | 0.012 ± 0.0005 |
| ERC-SH | 8 | 1.22 ± 0.04 | 0.38 ± 0.01 | 0.039 ± 0.0007 | 0.012 ± 0.0002 |

The SD rats had a significant larger absolute heart weight than SH rats ($p<0.001$) but the opposite was true for relative heart weight ($p<0.001$). There were no significant differences in absolute or relative heart weight or adrenal weight between housing systems within the different strains (table 1). The SD rats had significantly larger absolute adrenal weight than the SH rats ($p<0.001$), but the opposite was found for the relative adrenal weight ($p<0.05$).

## Food consumption

There were no significant differences in food consumption between rat strains and different housing systems. In the NOV-cages there were four different food-trays, one for each single NOV-cage. In SD rats, there was a significant increase ($p<0.001$) in total food intake from the second to the tenth week of the study, in the first and fourth NOV-cage compared to the third NOV-cage, counting from the left. The mean food consumption of the SD rats was 25.7 ± 0.2 g/rat and day. Corresponding data on the SH rats was 19.0 ± 0.2 g/rat/day.

### Laddermill exercise test

Complete data registration on all rats in the two exercise tests was not possible, either because the rats refused to run or it was not possible to take blood samples. Complete data from the SD rats was obtained from five rats housed in ST-cages, six rats kept in the NOV-cages and eight rats housed in the ERC-system. In the SH complete data was obtained from five rats kept in ST-cages, seven rats kept in NOV-cage and six rats from the ERC-system. In total 37 out of 48 rats (77%) performed successfully in both exercise tests. The body weights of these rats at each exercise test are presented in table 2.

The first exercise test: There were no significant differences in blood lactate concentrations or in endurance during the first exercise test within strains between the different housing systems (figure 8A, 9A and table 3).

The second exercise test*:* At the intensity of 7 m/min the SD rats kept in NOV-cages had a significantly lower blood lactate concentration (4.5 ± 0.4 mmol/l) ($p<0.05$) compared to those in ST-cages (6.6 ± 0.7 mmol/l) (figure 8B). At the intensity of 11 m/ min there was an indication ($p=0.089$) that SH rats in the ERC-system had a lower blood lactate concentration (8.4 ± 0.4 mmol/l) compared to SH rats in ST-cages (11.5 ± 1.3 mmol/l) (figure 9B). No other differences were found in blood lactate concentration or in endurance. When merging both strains rats kept in NOV-cages had an increased endurance capacity (129.2 ± 14.0 s) compared to rats kept in ST-cages (80.3 ± 13.0 s) ($p<0.05$) (table 3).

Comparing first and second exercise test:

Rats housed in ST-cages: SD rats had an increased blood lactate concentration ($p<0.05$) in the second exercise test at the intensity 7 m/min, 6.6 ± 0.7 mmol/l versus 5.1 ± 0.8 mmol/l in the first test (figure 8A-B). At this intensity the SH rats also had an increased ($p<0.05$) concentration of blood lactate in the second exercise test (8.3 ± 0.3 mmol/l) compared to the first exercise test (6.0 ± 0.4 mmol). There was an indication ($p=0.052$) at the intensity 11 m/min for the SH rats to have an increased lactate concentration during the second exercise test (11.5 ± 1.3 mmol/l compared to 8.5 ± 0.5 mmol/l) (figure 9A-B). The SH rats significantly increased their endurance capacity in the second exercise-test ($p<0.05$) (table 3).

Rats housed in NOV-cages: There were no significant differences in lactate levels between the first and second exercise test for either SD rats or SH rats. Both strains did however significantly improve their endurance capacity ($p<0.05$) in the second exercise test (table 3).

Rats housed in ERC-system: There were no significant differences in lactate or endurance between the two exercise-tests in the SD rats (figure 8A-B). The SH rats had an increased blood lactate concentration in the second exercise test at the intensity 9 m/min ($p<0.05$) (8.4 ± 0.4 mmol/l compared to 6.6 ± 0.3 mmol/l in the first exercise test) (figure 9B). The SH rats significantly improved their endurance capacity ($p<0.05$) in the second exercise test (table 3).

*Table 2. The body weight (g) of male Sprague Dawley (SD) rats and Spontaneously Hypertensive (SH) rats housed in standard Makrolon type IV cages (ST), Scantainer$^{NOVO}$ (NOV) and Enriched Rat Cage (ERC) that successfully performed in the two exercise tests. Data measured at the time of the exercise test and presented as mean* ± SEM

| | Body weight (g) | |
|---|---|---|
| Cage | Exercise test 1 | Exercise test 2 |
| ST-SD | 339.2 ± 4.6 | 497.6 ± 9.3 |
| NOV-SD | 316.5 ± 5.9 | 474.0 ± 9.1 |
| ERC-SD | 334.1 ± 3.5 | 483.7 ± 8.7 |
| ST-SH | 253.2 ± 6.2 | 310.4 ± 8.2 |
| NOV-SH | 246.9 ± 5.2 | 312.5 ± 6.6 |
| ERC-SH | 248.2 ± 4.7 | 313.8 ± 6.4 |

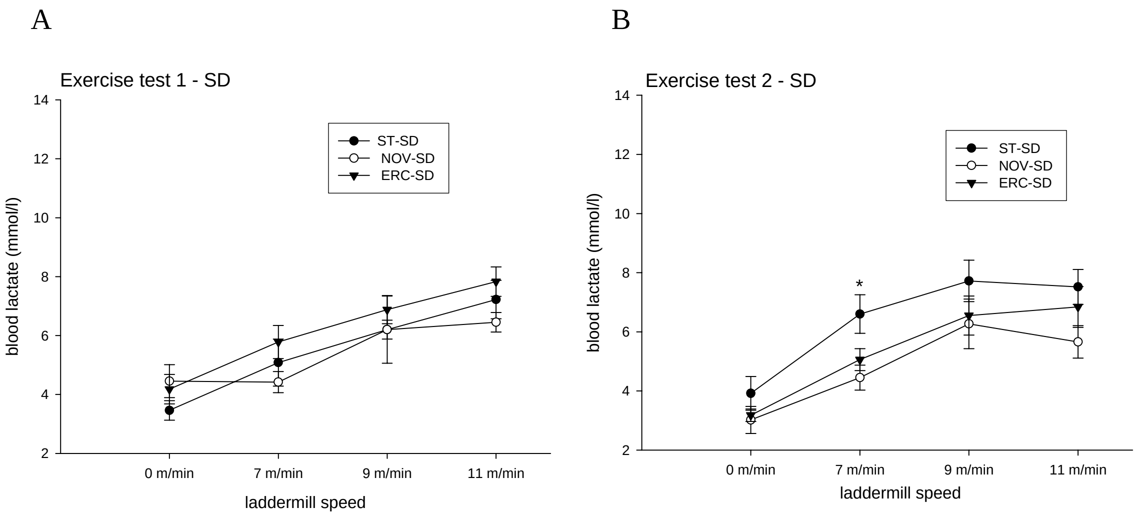


*Figure 8A-B. Blood lactate concentration (mmol/l) during the first (A) and second (B) exercise test in male Sprague Dawley (SD) rats. Rats housed in Standard Makrolon type IV cages (ST), Scantainer$^{NOVO}$ (NOV) and Enriched Rat Cage (ERC). Data are presented as mean ± SEM.*denotes significant difference in lactate concentration ($p<0.05$) between SD rats kept in ST-cages compared to SD rats kept in the NOV-cages.*

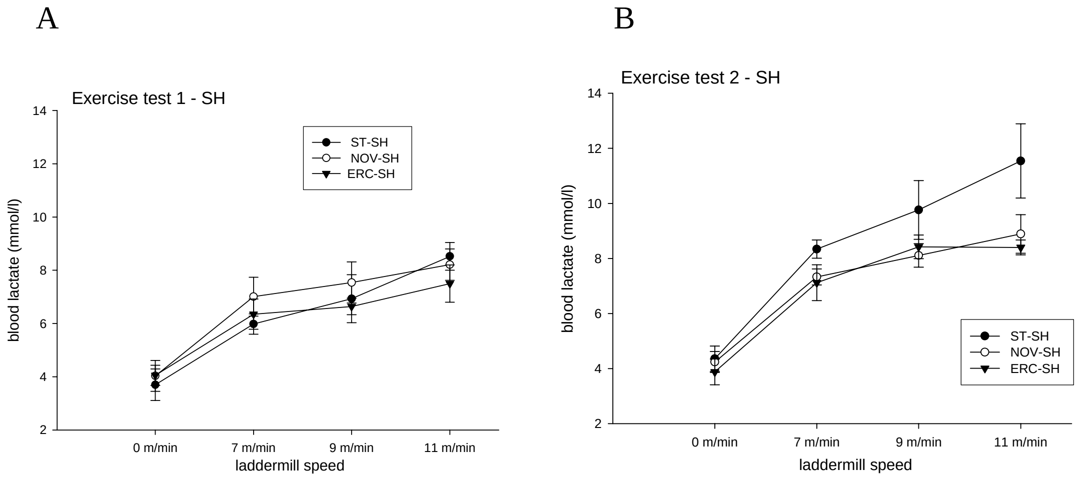


*Figure 9A-B. Blood lactate concentration (mmol/l) during the first (A) and second (B) exercise test in male Spontaneously Hypertensive (SH) rats. Rats housed in Standard Makrolon type IV cages (ST), Scantainer$^{NOVO}$ (NOV) and Enriched Rat Cage (ERC). Data are presented as mean ±* SEM.

*Table 3. Endurance test on laddermill in the two exercise tests in male Sprague Dawley (SD) and Spontaneously Hypertensive (SH) rats housed in Standard Makrolon type IV Cages (ST), Scantainerr$^{NOVO}$ (NOV), and Enriched Rat Cage (ERC). Data are presented as mean ± SEM*

| | Endurance (sec) | |
|---|---|---|
| Group | Exercise test 1 | Exercise test 2 |
| ST-SD | 46 ± 13 | 69 ± 22 |
| NOV-SD | 61 ± 11 | 127 ± 20* |
| ERC-SD | 69 ± 14 | 94 ± 15 |
| ST-SH | 40 ± 11 | 92 ± 16* |
| NOV-SH | 69 ± 14 | 132 ± 20* |
| ERC-SH | 54 ± 16 | 95 ± 9* |

* Significantly higher than exercise test 1 ($p<0.05$)

## Blood pressure

Complete data of all rats from every session of blood pressure recording could not be obtained because of motion disturbances or problems to register the caudal artery pulsations. The blood pressure recording-time varied between 1 and 16 minutes with a mean of 5.9 ± 0.2 minutes per rat. The tail-cuff was inflated with a mean of 5.3 ± 0.2 times per rat. Individual and mean systolic blood pressure recordings of the SD and SH rats are shown in figure 12. During week nine of the study there was an indication ($p=0.056$) that SD rats kept in NOV-cages and ERC-system had an increased systolic blood pressure (156 ± 6 mmHg and 155 ± 4 mmHg respectively) compared to SD rats housed in ST-cages (136 ± 3 mmHg). During week ten, the SD rats kept in NOV-cages had an increased systolic blood pressure (155 ± 5 mmHg) compared to SD rats held in ST-cages (138 ± 4 mmHg) ($p<0.05$). There were no significant differences in blood pressure among the SH rats in the various housing systems during week nine and ten.

## Insulin and corticosterone analyses

As expected the SH rats had a significantly higher plasma insulin concentration ($p<0.001$) than the SD rats. There were no significant differences in plasma insulin and corticosterone concentrations within each strain when comparing housing systems (table 4). There was a significant positive correlation ($p<0.001$) between plasma insulin concentration and body weight among the SH rats ($r=0.88$) (figure 10). No other correlations were found between body weight and insulin or corticosterone concentration.

*Table 4. Insulin-, and corticosterone-concentration in plasma of male Sprague Dawley (SD) rats and Spontaneously Hypertensive (SH) rats housed in Standard Makrolon type IV cages (ST), Scantainer$^{NOVO}$ (NOV) or Enriched Rat Cage (ERC). Blood samples taken at euthanization. Data are presented as mean ± SEM*

| | N | Insulin (µg/l) | Corticosterone (ng/l) |
|---|---|---|---|
| ST-SD | 8 | 2.5 ± 0.4 | 231.5 ± 28.7 |
| NOV-SD | 8 | 3.1 ± 1.2 | 201.0 ± 17.2 |
| ERC-SD | 8 | 2.4 ± 0.6 | 201.3 ± 17.0 |
| ST-SH | 8 | 6.4 ± 1.9 | 213.8 ± 21.6 |
| NOV-SH | 8 | 9.2 ± 1.5 | 247.5 ± 34.1 |
| ERC-SH | 8 | 7.4 ± 1.6 | 283.1 ± 20.5 |

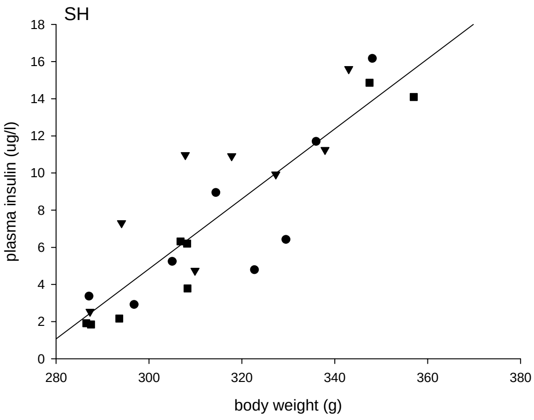


*Figure10. Correlation between plasma insulin concentration and body weight (r=0.88) in male Spontaneously Hypertensive (SH) rats. Squares (■) indicates rats kept in Standard Makrolon type IV cages (ST), triangles down (▼) indicates rats kept in Scantainer$^{NOVO}$ (NOV) and dots (●) indicates rats kept in the Enriched Rat Cage (ERC).*

## Inclined plane test

No significant differences could be seen comparing the different housing systems and rat strains in the maximum inclination test (table 5) or in the endurance test on the inclined plane (figure 11A-B). Seven out of 16 rats in the ST-caged rats did not succeed in the endurance test for 60 s at the inclination of 55°. Two out of 16 rats kept in NOV-cages and in the ERC-system, respectively, did not succeed in the inclined plane endurance part for 60 s at the inclination of 55°.

*Table 5. Inclined plane test in male Sprague Dawley (SD) rats and Spontaneously Hypertensive (SH) rats housed in standard Makrolon type IV cages (ST), Scantainer$^{NOVO}$ (NOV) and Enriched Rat Cage (ERC). The maximal inclination ($^o$) of the rats on the inclined plane. Data are presented as mean ± SEM*

| | Inclined plane |
|---|---|
| | Maximal inclination ($^o$) |
| ST-SD | 65 ± 1 |
| NOV-SD | 67 ± 0 |
| ERC-SD | 66 ± 1 |
| ST-SHR | 66 ± 1 |
| NOV-SHR | 68 ± 1 |
| ERC-SHR | 67 ± 1 |

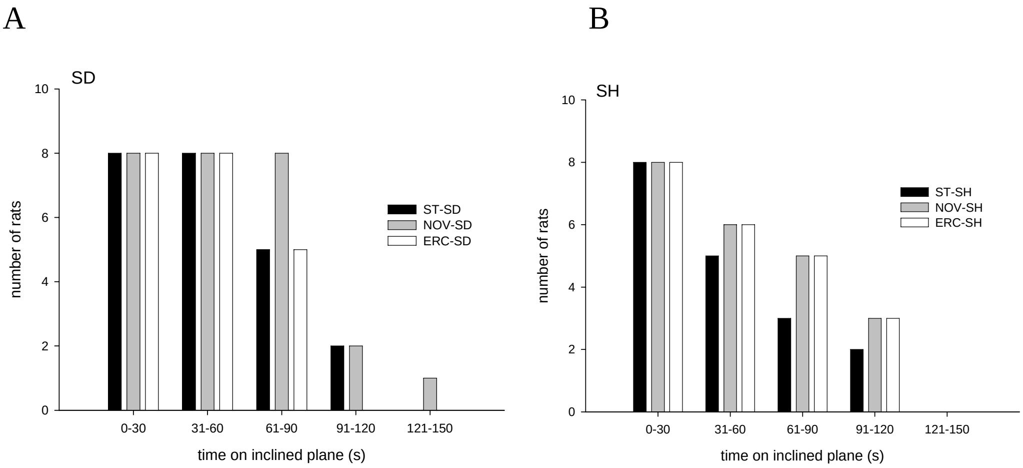


*Figure 11A-B. Endurance test (s) in the inclined plane test performed by Sprague Dawley (SD) rats (A) and Spontaneously Hypertensive (SH) rats (B) housed in standard Makrolon type IV cages (ST), Scantainer$^{NOVO}$ (NOV) and Enriched Rat Cage (ERC). The inclination was 55° for the first 60 s and thereafter increased with 5° for each following minute.*

## Body and extremity measurements

There were no significant differences in either foreleg or hind leg circumference in the SD rats between the different housing systems. The SH rats housed in the ERC-system had an increased front leg circumference (4.8 ± 0.1 cm) compared to the ST-cages (4.4 ± 0.1 cm) ($p<0.05$). There was no difference in hind leg circumference in the SH rats.

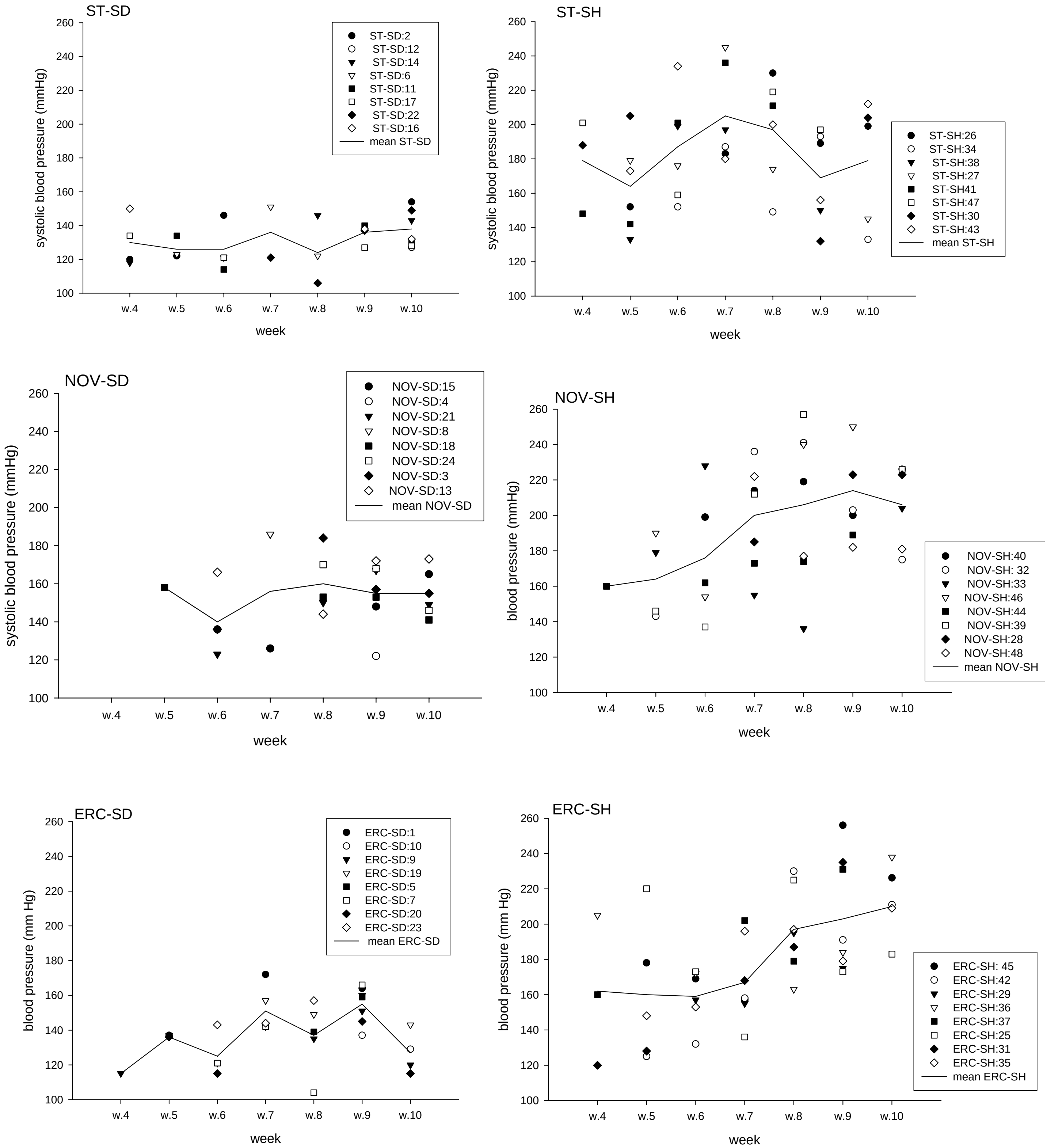


*Figure 12. Individual and mean systolic blood pressure (mmHg) recorded weekly in male Sprague Dawley (SD) rats and Spontaneously Hypertensive (SH) rats kept in Standard Makrolon type IV cages (ST), Scantainer$^{NOVO}$ (NOV)-cages and Enriched Rat Cage (ERC).*

## DISCUSSION

The aim of this study was to investigate whether two different commercially available housing systems would increase home cage activity and thereby affect physiological parameters in male Sprague Dawley and Spontaneously Hypertensive rats. The rats kept in the NOV-cages increased their endurance capacity and had lower lactate levels in the exercise test. The SH rats showed an increased systolic blood pressure and insulin resistance despite being housed in different cage types. The physiological parameters body weight, heart weight, blood pressure and insulin concentration were not changed by cage type in the SH rats, which proves that this animal model is unaffected by improvements in the housing.

The body weight is a parameter that usually is affected by physical activity. Studies have shown a reduced body weight gain in rats trained on treadmill (James *et al,* 1984; Zendrian-Piotrowska *et al*, 1993; Wisløff *et al,* 2001; Spangenberg*,* unpublished data), in running wheels (Hoffman *et al*, 1978; Lambert *et al* 1996; Morimoto *et al,* 2000; Narath *et al,* 2001; Rodnick *et al*, 1989; Rodnick *et al,* 1990) or on a swimming schedule (Evenwel *et al*, 1979). In addition, it has been shown that rats kept in large pens also had a reduced body weight gain (Spangenberg *et al,* 2005). There was a temporary reduction in body weight gain among the SD rats kept in NOV-cages during the second week in this study. The rats in the NOV-cages were probably more physically active than those kept in ST-cages. The reason for not reaching a permanent reduction in body weight gain in this study can depend on several factors. One factor is the of amount physical activity. Rodnick *et al.* (1989) found a significant reduction in body weight only in rats that had been running for more than approximately 7 km/day. The rats that had been running on an average of 3 km/ day had no significant reduction in body weight. Although the rats in the present study increased their physical activity in the larger cages, it is likely that this increase still did not reach levels that would permanently lower body weight gain. An increase in workload intensity is required in order to produce a continued training effect (Wisloff *et al*, 2001). The cage sizes were constant during the entire study, with the proportional cage size per rat becoming smaller as the rats grew in size. The difference in body weight range between cage types was most pronounced in SD rats; at the end of the study it was 94 g in ST-cages and only 67 g in ERC-system. This variation in body weight among the rats could affect research results since it is important to have animals that are as similar as possible. The SH rats housed in ERC-system had an increased front leg circumference compared to SH rats housed in the ST-cages. This indicates increased climbing activity among the ERC-housed rats. The ERC-system has large cage doors with grid, and it was noted that the rats often climbed on these. Wild rats are good climbers, and the laboratory rats take the opportunity to climb when given the chance, which probably increases their muscle strength.

The design of the exercise test protocol was successful, since the majority of the rats succeeded to complete both exercise tests. With the laddermill it is possible to have a larger amount of rats that can succeed in an exercise test, which is often difficult when using the treadmill (Norton *et al*, 1990; Cvek *et al*, 2007) There are studies that only select rats that are willing to run (Narath *et al*, 2001; Norton *et al,* 1990; White-Welkley *et al*, 1996), with the risk of obtaining biased results. The lower lactate levels in exercise test 2 in SD rats from the NOV-cages, compared to ST-housed rats, is in agreement with a previous study where rats kept in groups of four or eight performed better than pair-housed rats (Spangenberg *et al,* unpublished data). This indicates a higher home-cage activity in NOV-cages, which is supported by the results from the endurance test. When comparing the first and second exercise tests, we found that both rat strains housed in ST-cages had an increased blood lactate concentration during the second exercise test at the intensity 7 m/min, with at tendency for the same increase at the highest intensity in SH rats kept in ST-cages. This indicates that rats kept in standard cages did not perform physical activity enough to decrease blood lactate concentrations in an exercise test. In contrast, this seems to have been the case in NOV and ERC cages. However, the SH rats kept in the ERC-system had an increased blood lactate concentration at the intensity 9 m/min in the second exercise test when comparing the two tests. The explanation for this could be that the SH rats are hyper-responsive to stressful stimuli (McDougall *et al*, 2004) and probably increased their blood lactate concentration due to the stress of performing in a exercise test. According to Pilis *et al. (*1993) the anaerobic threshold in untrained rats is approximately at the level of 4 mmol/l. The lactate concentration in the rested state in the rats in the present study were approximately around this level, which is higher compared to another study (Eydoux *et al*, 2000) This could affect the results of the rats in the exercise test and endurance test in this study, with the rats having a muscle oxygen deficit before the actual start of their performance. In spite of the negative effect of an increased blood lactate concentration in the rested state, there is an individual motivation to run in rats (Lambert *et al,* 1996) that can affect the results. An increase in workload intensity is also required when the performance capacity is improved in the rat in order to produce a continued training effect (Wisløff *et al*, 2001). When comparing the first and second exercise test all of the SH rats in this study had an increased endurance capacity, which was also true for the SD rats kept in the NOV-cages. Lambert *et al.* (1996) hypothesized that an improved running economy in combination with an increased training level of the rats could make them run for longer periods without reaching exhaustion in an endurance test. This could be a reason for the improved endurance capacity in the second exercise test in the present study. The strain difference could also influence the present results, since SH rats have a more pronounced stress reaction compared to SD rats when exposed to a stressful event (McDougall *et al,* 2004), in this case the exercise test.

In this study the SD rats housed in the NOV-cage had an increased systolic blood pressure during the last week of the study. The probability of the rats being active prior to blood pressure recordings in these cages is higher than that of the rats in the ST-cages. It has been stated that it is necessary to warm the rat prior to blood-pressure recordings using a tail-cuff due to intermittent pulsations in the caudal artery, thereby achieving a heat-induced vasodilatation (National Research Council, 1996). However, Kubota *et al.* (2006) successfully recorded systolic blood pressure using a tail-cuff on non pre-heated animals at a lower systolic blood pressure compared to pre-heated rats. The blood pressure values in the study by Kubota *et al.* (2006) were similar to data from telemetry-operated rats. In this study we obtained data from non pre-heated rats. During the present study, there were some troubles in recording pulsations in the tail-artery, and also in the restraining of the rats. We noted that the SD rats were increasingly more difficult to restrain in a towel or on a lap as the study proceeded. An explanation could be differences in activity-level of the rats prior to the blood pressure recording. Many studies on the blood pressure lowering effect of physical exercise have demanding exercise protocols. Véras-Silva *et al.* (1997) had the rats running on a treadmill for 18 weeks, five times per week for 60 minutes at 16-20 m/min. Lajoie *et al.* (2004), found no effect on blood pressure in SH rats after long–term exercise, 120 min/ day for five weeks at 18 m/min. They did however notice a significantly reduced blood pressure 3 and 12 h following a single bout of treadmill running in those rats. Comparing the present results with the previously mentioned studies, it appears likely that the amount of physical activity in the rats have not been enough to give a blood pressure-lowering effect in either of the rat strains. Different blood-pressure recording methods in rats can be used; invasive techniques such as telemetry or carotid catheter or the non-invasive tail-cuff technique. Ibrahim *et al*. (2006) found that systolic blood pressure measurement using a tail-cuff is a reliable method for long-term blood pressure recording. The amount of data on the systolic blood pressure in this study was rather limited, and with many factors affecting blood pressure it is difficult to make any strong conclusions on these data. During the restraining of the rats, it is important for the rats to be given time to acclimatize to the recording procedure, in order to reduce the stress-induced increase of blood pressure in a handling or restraining situation (McDougall *et al*, 2000). We chose not to restrain the rats in standard plastic tubes often used in research (Kubota *et al,* 2006). The tail-cuff equipment is extremely sensitive to movements; even the very small movement of a rat´s sniffing nose will cause disturbances in the recording. By choosing to manually restrain the animal it is possible to be aware of every tiny movement the rat does. This will produce more reliable recordings because it minimizes the risk of recording a movement disturbance as a true pulsation, and blood pressure.

In the inclined plane test, there were individual variations in the willingness of the rats to hold on to the rubber mat. This probably also affected the performance of the rats and could explain the lack of differences between cage types. Former studies have shown a large variability also in rats' willingness to voluntarily run in running wheels (Lambert *et al,* 1996). The varying conformation of the housing systems in this study gave the rats´ different opportunities to improve their climbing skills and that should establish differences in the capacity to climb and perform on the inclined plane. The rats kept in ST-cages had the lowest capacity in the endurance part in the inclined plane test (56% succeeded to hold on to the rubber mat in an inclination exceeding 55°) indicating that these rats had lower muscular strength because of the lack of climbing opportunity in the cage. Among the rats kept in the NOV-cages, on the other hand, 88% succeeded to hold on to the rubber mat at 55° of inclination, and 75% of the rats from the ERC-system.

There were no differences in relative heart weight of the SD rats kept in the different cage types in this study, which would have indicated a cardiac hypertrophy. The absence of cardiac hypertrophy following a training schedule in rats was also seen by Rodnick *et al.* (1989), where the rats were voluntary running in running wheels. Wisløff *et al.* (2001) recorded an increase in relative heart weight during a more intense training protocol with the rats running on treadmill, which is more than the rat's own level of spontaneous physical activity. Hence, the rats in the present study did probably not have an activity level that was high enough to create a cardiac hypertrophy. There were no significant differences in absolute and relative adrenal weights between the different housing systems in this study. Neither where there any significant differences in the corticosterone concentration, which indicates that none of the cage types were more or less aversive for the rats.

The Spontaneously Hypertensive rats, developed hypertension, cardiac hypertrophy and a hyper-insulinemic state even when housed in the larger cages (NOV and ERC). There was a significant correlation between body weight and insulin concentration, explained by the SH rats´ gradual development of a hyper-insulinemic state. These important findings show that housing types that allow for more physical activity do not affect the hypertensive model. Therefore it is possible to house them in larger, better cages, and improve their welfare, without changing the model or influencing scientific results.

In conclusion, we found that the NOV-cages had the highest effect on home cage activity in the rats as shown by the results in the exercise tests. The physiological features of the SH rat model was not influenced by housing type. The non-invasive tail-cuff method was successfully used without pre-heating the rats. The results from this study indicate that rats increase their spontaneous activity in a larger cage, however not enough to create a distinct training effect. It is still important to emphasize the value of an increased home cage activity for an improved animal welfare and that this can be achieved without jeopardizing scientific results.

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