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Original Research Article

EFFECT OF METABOLIC CAGE HOUSING ON METABOLIC CHANGES IN THE LIVER OF YOUNG MALE LABORATORY RATS

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Abstract: The aim of this study is to investigate if a metabolic cage housing is appropriate for studies of metabolic changes in rat liver. Metabolic cages are intended for separately collecting animal urine and feces and for measuring of feed and water consumption. They are often used in biomedical research, especially in studies involving metabolism and nutrition, where it is necessary to control the total intake of feed and water. In numerous studies, researchers have found that housing in a condition similar to housing in metabolic cages i.e. grid floor, lack of bedding, lack of movement, and single housing, could cause stress and thus alter the results and influence the research. Two months old male Wistar rats were randomly assigned to two different housing systems for a three week period. One group of rats was housed individually in metabolic cages and rats in the other group were paired and housed in polycarbonate type IV cages with wood shavings bedding material. The body mass and feed consumption were monitored daily. At the end of the study, four rats from each group were anesthetized and the liver and blood samples were taken. The rest of the rats were used for primary hepatocytes cell culture to determinate glucose production. Metabolic cage housing conditions in our study did not cause stress of sufficient impact to alter the test parameters.

Key words: metabolic cage; hepatocytes; insulin resistance; plasma lipids

Introduction

The metabolic cage has been designed for efficient separate collection of urine and feces samples. It is important in biomedical as well as in nutritional experiments where it is imperative to closely monitor total intake of feed and water and to collect urine or feces samples separately. Typical metabolic cage is constructed with a polycarbonate upper chamber with a grid floor and a funnel in the lower chamber. The feeder is placed outside the cage (1). In studies on nutrition and metabolism, there is a strong need to control feeding of the animals and to obtain samples and perform measuring by avoiding stress in animals, which is known to interfere with metabolic status. Stress can lead to fatty liver and other metabolic changes linked with a metabolic syndrome (2). Metabolic cages are also known for not allowing the natural behavior of laboratory animals. Cage is rather small, with no bedding on grid bottom and no hiding places for the animal. Usually, cage holds a single animal. In a normal environment rats and mice are very social animals that live in groups. There are numerous studies on the effect of housing conditions on animal welfare and stress. The lack of bedding seems to be a major problem and grid cages flooring can cause injuries. There is also a strong concern about the

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lack of movement and socialization. In research on single or group housing, female rats demonstrated significant variation in feed intake and triglyceride levels, but not in total cholesterol or body mass gain (3). It was observed that rats housed alone had stress-like responses to common procedures such as cage change, restraint, and subcutaneous or tail vein injection. Rats housed individually had higher heart rate and mean arterial pressure than rats housed in groups, but rats housed in pairs had similar results as rats housed individually (4). Alternating housing conditions in male rats between a standard housing and metabolic cages on grid floor showed reduction in body mass gain and increased excretion of feces, indicating mild stress in animals when housed in metabolic cages. Cortisol levels did not significantly differ between housing conditions (5). In another study, rats were housed on solid bottom or grid floor over a two weeks period and then crossed over to alternate condition for another two weeks. Grid bottom or solid bottom bedded cages had no effect on corticosterone levels, growth, energy expenditure and behavior (6). Considering all possible stressful conditions of a metabolic cage (individual housing, grid floor, no bedding) which can alter metabolic status in rats, control animals were housed in pairs, on a standard solid floor with wood shavings for bedding.

Materials and methods

Animals and housing

Twelve male Wistar rats, two months old, were randomly divided into two groups. The control group, Standard Cage Group (SCG), was kept in standard 425x266x185mm polycarbonate cages, floor area 800 cm^2 (Techniplast, Buguggiate, Italy) with bedding of wood shavings. To reduce social isolation the animals were kept in pairs. The animals in a Metabolic Cage Group (MCG) were housed in metabolic cages with a floor area of 320 cm^2 (Techniplast, Buguggiate, Italy) and kept individually, with no bedding or any enrichment. The room in which the animals were kept was at a constant temperature of 21 °C with twelve hours light and dark cycle.

Principles of animal care (Croatian Animal Welfare Regulation Acts NN135/06; NN 37/13; NN55/13) were followed.

Feed consumption and body mass

Animals were given a standard rodent diet (Mucedola, Milano, Italy), but for the group in a metabolic cage, the pellets were ground. Feed consumption and body mass were monitored daily and the monitoring was held by the same trained handler at the end of the light cycle. The process lasted 3-5 minutes per animal. Animals had access to feed and water *ad libitum*.

Sampling and analysis

After three weeks of monitoring mass gain and feed consumption, four animals from the each group were fasted overnight and were anesthetized by an intraperitoneal injection of sodium thiopental (Rotexmedica GmbH, Trittau, Germany), 10 µg/100 g body mass. The skin of the tail tip was cut for the glucose sampling with the test strip. Three mL of blood from *v. cava inferior* and a liver were taken. The liver was washed in a cold saline, weighed and cut in small sections for histological analysis. The rest of the animals were used to obtain primary liver cell culture. Those animals had not have fasted overnight.

Biochemical analysis

After taking three mL of venous blood in EDTA test tubes, plasma was removed after centrifugation at 3500*g* for 30 min and stored at 4 °C or frozen at -80 °C until evacuation. The serum concentrations of triacylglycerols (TG), total cholesterol (CH), and HDL cholesterol (HDL-CH), were measured enzymatically using commercial kits (Herbos Dijagnostika plc., Sisak, Croatia).

Fasting glucose was measured with a drop of blood from the tip of the tail and was picked on a test strip and measured using Accu Chek glucose meter (Roche Diagnostic, Mannheim, Germany).

Histology

Immediately after cutting the liver into small sections, samples were put in a Carnoy fixative. After appropriate time in fixative, the samples were set in paraffin blocks, cut and stained with hematoxylin and eosin stain.

Primary cell culture

Hepatocytes were isolated by a modified collagen perfusion technique (7) from two animals of each group. Animals were anesthetized with sodium thiopental (10 µg/100 g body mass) via intraperitoneal injection. For the liver perfusion, calcium-free Swim's S-77 medium was used with the addition of collagenase (0.5 g/L). After washing twice with the same media but without added collagenase and insulin, the cells were suspended in M-199 medium containing in addition 2 g bovine albumin, 900 µg L-glutamine and 2.2 g $NaHCO_3$ per liter, to the concentration of one million cells per mL of media. The viability of the cells was greater than 95 % as determined by trypan blue dye exclusion. Three mL of suspension was placed in collagen-coated Petri dishes of 60 mm. The plates were kept in an incubator at 37 °C and 5 % CO_2 gas and 95 % air mixture. After four hours, the media was replaced.

Collagen was purchased from Serva Electrophoresis GmbH (Heidelberg, Germany), collagenase, bovine albumin, L-glutamine and M-199 medium from Sigma-Aldrich (St Luis, MO, USA).

Glucose production in cultured hepatocytes

After 24 hours of incubation, the medium was removed and the hepatocytes were incubated in Hans-Hepes medium without glucose with pyruvate (10 mmol /L). After 0, 60, 120 and 180 minutes of incubation 100 µL samples were collected for determination of glucose. The GOD-PAP method was used using Glucose Liquicolor set. After three hours of incubation, the medium was removed from dishes and the cultures were frozen in liquid nitrogen. Hepatocytes were digested in 0.2 N NaOH to obtain samples needed for the determination of protein by Lowry's method (8).

Insulin and pyruvate were purchased from Sigma-Aldrich (St Luis, MO, USA), Glucose Liquicolor from Human GmbH (Wiesbaden, Germany)

Statistics

For all statistical analyses, two-group comparisons were carried out using Student's t-test. Results were shown as mean SEM and values of $p \leq 0.05$ were considered statistically significant.

Results

Mass gain and feed consumption

Animals had an almost linear body mass gain in both groups. The initial body mass was 253 ± 6.1 g and the final body mass was 310 ± 16.1 for SCG. For MCG the initial mass was 260 ± 7.7 g, and the final was 316 ± 8.2 g (Figure 1). There were no statistical differences in the final body mass gain and feed consumption between groups (Figure 2). Liver mass and liver mass/body mass ratio was the same in SCG and MCG group (Figure 3).

Histology

There were no visible differences in livers between groups. Macroscopically there was no evidence of liver fat accumulation. Liver histology slides have shown no fat accumulation in hepatocytes or other histopathological signs in either of the groups (Figure 4).

Blood lipids and fasting glucose

Results for blood lipids and glucose are shown in Table 1. There were no statistically significant differences between groups. Metabolic cage did not influence on lipids or fasting glucose in blood.

Glucose production

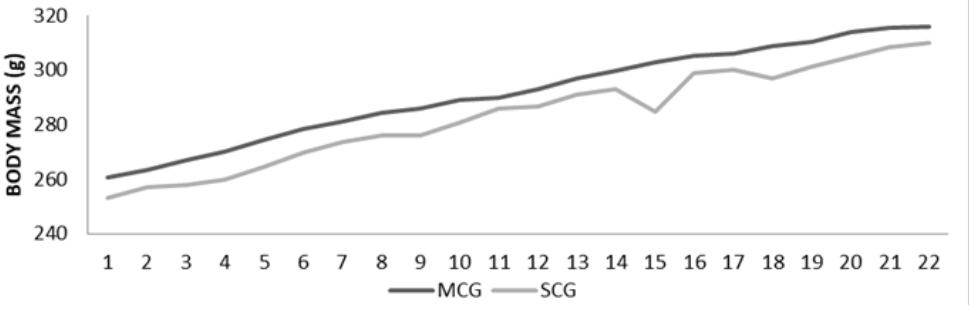

Figure 1: Body mass gain

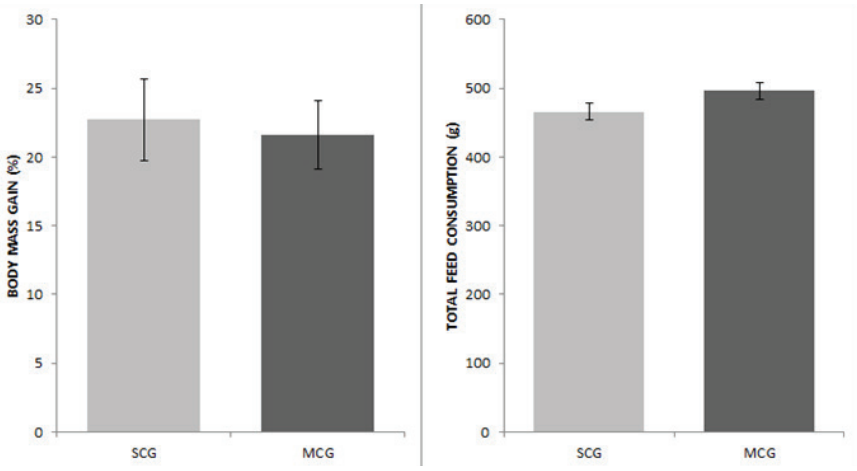

Figure 2: Total mass gain in % of initial mass and total feed consummation in grams. Each point value is a mean ± *SEM*

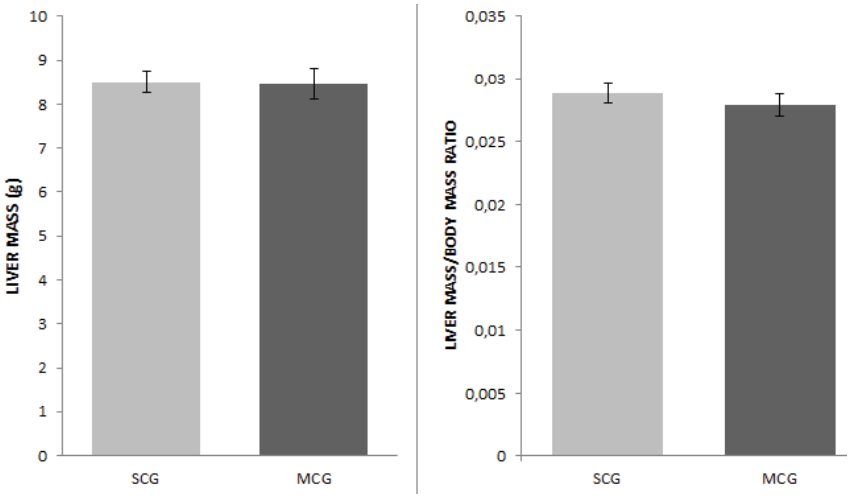

Figure 3: Liver mass and liver mass/ body mass ratio. Each point value is a mean ± *SEM*

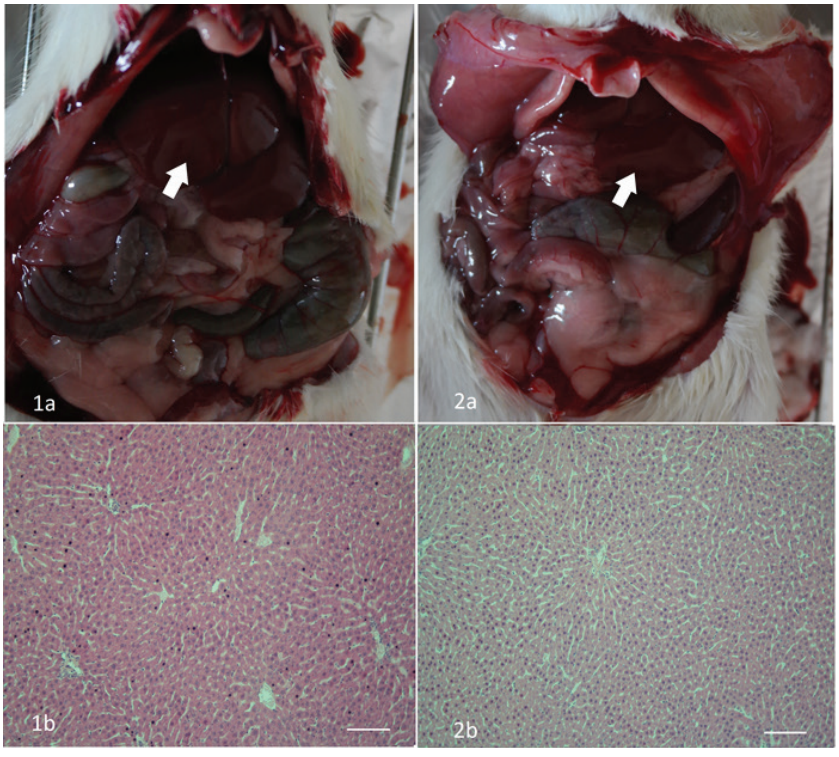

Figure 4: The viscera organs and the liver (arrow) in SCG (1a) and MCG (2a); Histological liver slide H&E, 100x magnifications, scale 100μm in SCG (1b) or MCG (2b)

Table 1: Blood lipids and glucose. Each point value is a mean ± *SEM*

	SCG	MCG
Fasting glucose (mmol/L)	6.45 ± 0.30	5.62 ± 0.77
Triacylglycerols (mmol/L)	0.88 ± 0.19	0.54 ± 0.05
Total cholesterol (mmol/L)	0.89 ± 0.04	0.95 ± 0.06
HDL-cholesterol (mmol/L)	0.58 ± 0.17	0.51 ± 0.07

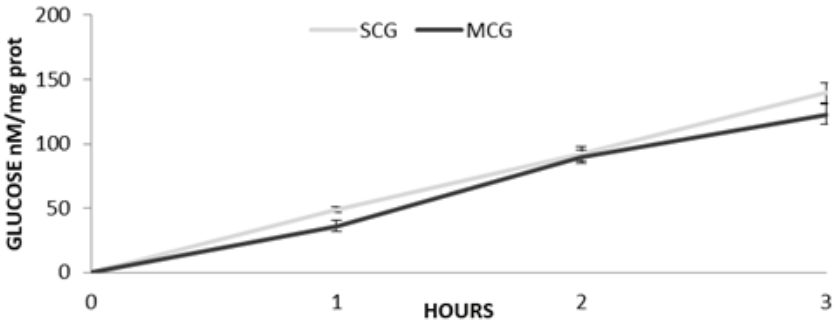

Figure 5: Glucose production (nmol/mg protein) in cultured hepatocytes in glucose-free Hanks-Hepes medium in presence of 10 mmol/L pyruvate. Each point value is a mean ± *SEM*

In the cultures from SCG and MCG groups, gluconeogenesis from pyruvate was constant (Figure 5). There were no statistically significant differences in hepatocyte glucose production isolated from animals in different housing.

Discussion

Housing an animal in a metabolic cage may result in metabolic syndrome as a consequence of restricted physical activity combined with *ad libitum* feeding and stress. In the study on housing activity, it was found that rats kept individually in smaller cages gained more body mass without the differences in feed consumption in comparison with the rats housed in groups using large pens (9). In our study, animals in an MCG and SCG group had the same mass gain and total feed intake, indicating that the housing conditions in our experiment had similar effects on both groups on energy intake and expenditure. It was suggested that elements of housing in metabolic cages such as grit flooring can be associated with an increase in corticosterone levels and injury. Excessive cortisol as a response to environmental and psychological stressors leads to the formation of fatty liver and central accumulation of fat, which in turn leads to insulin resistance (2). Stress can cause a liver enlargement (10). In that study, rats were put into two stressful situations, forced swimming or restraint, 90 minutes every day for two weeks period. Both stress groups had elevated liver mass, but only in forced swimming group this elevation was statistically relevant. Chronic stress study on rats indicated that chronic stress that had lasted three weeks caused dyslipidemia, evident two weeks after stressful conditions (11). A murine model of acute stress demonstrated the development of insulin intolerance and hepatic insulin resistance(12). Also, it has been shown that stress can cause profound changes in hepatic gene expression leading to the development of metabolic syndrome (13). In our research, there were no signs of central or liver fat accumulation. There was no accumulation of fatty droplets in hepatocytes as both groups had a normal histology of liver. Liver mass and liver mass to body mass ratio were the same in both groups. There were no differences in the concentration of blood lipids. Liver glucose production and fasting glucose in blood were not elevated in MCG group compared to SCG group. Animals, who were housed three weeks in metabolic cage housing condition, had the same results of the tested parameters as those housed in standard cage conditions. Modified conditions of the metabolic cage did not lead to dyslipidemia, hyperglycemia or accumulation of fatty droplets in the liver and did not alter liver glucose production in young male rats.

In conclusion, although there was a very different

environment of housing the rats in the metabolic cages compared to their natural surroundings and social behavior, our results show no indication of acute or chronic stress through observed parameters which are altered in these states.

Housing a young male rat for three weeks in metabolic cage had no effect on tested parameters.

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VPLIV BIVANJA V PRESNOVNI KLETKI NA SPREMEMBE V PRESNOVI JETER PRI MLADIH PODGANJIH SAMCIH

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Povzetek: Cilj študije je bil raziskati ali je bivanje v presnovnih kletkah primerno za proučevanje presnovnih sprememb v podganjih jetrih. Presnovne kletke so namenjene ločenemu zbiranju živalskega urina in blata ter merjenju porabe krme in vode. Pogosto se uporabljajo v biomedicinskih raziskavah, zlasti v študijah presnove in prehrane, kjer je potrebno nadzorovati skupni vnos krme in vode. V številnih študijah so raziskovalci ugotovili, da lahko bivanje v pogojih, ki so podobni metabolnim kletkam tj. na tleh, ki so sestavljena iz mrež, pomanjkanju nastilja, pomanjkanju gibanja in posamičnem bivanju živali povzroča stres in lahko vpliva na rezultate raziskav. Dva meseca stare samce podgan Wistar smo naključno razporedili v dva različna sistema za nastanitev za obdobje treh tednov. Ena skupina podgan je bila nameščena posamično v presnovnih kletkah, podgane druge skupine pa so bile nastanjene v parih v klasičnih polikarbonatnih kletkah tipa IV s steljo iz lesnih ostružkov. Dnevno smo spremljali njihovo telesno maso in porabo krme. Na koncu študije smo štiri podgane iz vsake skupine anestezirali in odvzeli vzorce jeter in krvi. Preostale podgane smo uporabili za pridobivanje primarnih jetrnih celic z namenom določanja proizvodnje glukoze. Pogoji bivanja v presnovnih kletkah v študiji niso povzročili stresa, ki bi imel značilen vpliv na proučevane spremenljivke.

Ključne besede: presnovna kletka; jetrne celice; odpornost na inzulin; plazemske maščobe