Türk Bilimsel Derlemeler Dergisi
E-ISSN: 2146-0132 15(1): 115-120, 2022
Derleme Makalesi

METABOLIC CAGES CAN BE USED FOR SOCIAL ISOLATION STRESS RATHER THAN METABOLISM STUDIES WITH THEIR CURRENT FORM

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(Received 18th April 2022; accepted 07th June 2022)

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ABSTRACT

Metabolic cages are frequently used in metabolism or pharmacological experiments for measurement of total intake of food and water as well as excretion of urine and feces in small rodents. Therefore, the grid floor and single housing are a necessity for separated collection and measurement of these samples. However, there are several reports on the unfavorable effects of metabolic cage housing on animal welfare and physiological parameters mainly due to individual housing and grid flooring. In our previous experiments, we observed that long-term housing in metabolic cages caused social isolation stress in male and female rats. We concluded that housing of a rat or mouse in a metabolic cage causes a more effective and painful social isolation stress rather than single housing in a standard cage due to the effect of grid flooring. Therefore, in the present review, it is discussed that metabolic cages in their current form may be a useful option for social isolation stress paradigm in small rodents outside of metabolism studies.

Keywords: *Metabolic cage, animal welfare, social isolation, stress*

ÖZET

Metabolik kafesler, küçük kemirgenlerde günlük toplam gıda alımı ve su alımının yanı sıra idrar ve dışkı atılımının ölçülmesi için metabolizma veya farmakolojik deneylerde sıklıkla kullanılmaktadır. Bu nedenle, ızgara zemin ve tekli barındırmaya uygun gövde, bu numunelerin ayrı toplanması ve ölçülmesi için kullanılmaktadır. Bununla birlikte, metabolik kafeste ızgara zeminde ve tekli barındırmaya bağlı olarak hayvan refahı ve fizyolojik parametrelerde olumsuz etkiler oluştuğuna dair raporlar mevcuttur. Daha önceki deneylerimizde, metabolik kafeslerde uzun süreli barınmanın erkek ve dişi sıçanlarda sosyal izolasyon stresine neden olduğu belirlenmiştir. Elde ettiğimiz verilerimiz ışığında, sıçan veya farenin metabolik kafeste barındırılmasının, ızgara döşemenin etkisiyle standart bir kafeste tek başına barındırmaktan daha etkili ve acılı bir sosyal izolasyon stresine neden olduğu kanısına varılmıştır. Bu nedenle, derlememizde metabolik kafeslerin mevcut haliyle metabolizma çalışmaları dışında küçük kemirgenlerde sosyal izolasyon stres paradigması için daha uygun bir seçenek olabileceği hususu ele alınmaktadır.

Anahtar Kelimeler*: Metabolik kafes, hayvan refahı, sosyal izolasyon, stres*

INTRODUCTION

Determination of the change in energy balance is a fundamental goal in metabolism studies. This is also true for pharmacokinetic studies. For this reason, food and water intake and fecal and urinary excretion must be precisely measured. Metabolic cages are important laboratory materials designed for this process [1]. Therefore, these special cages are equipped with a unique system including grid flooring, absence of bedding substrate and enrichment, as well as individual housing and small living area suitable for single housing [2]. Single housing, wire mesh floor and other features are important for evaluating individual food intake and body excretion [1,2]. A grid floor also allows preventing contamination of urine and effective separation of faces and urine into collection tubes outside the cage [3]. All of these features that are important for metabolism studies have become questionable over time due to their significant negative effects on the experimental animals. For the last twenty years, there have been considerable reports on the negative effects of metabolic cages on animal welfare and physiological parameters due to their characteristic structures [2, 4-8].

Mice and especially rats are social animals [1]. Therefore, long-term individual housing of rats causes physio-psychological abnormalities [9-11]. Moreover, there are many reports on the relationship between metabolic cage housing and social isolation stress in rodents [10,12,13]. Recently, we reported that long-term metabolic cage housing increases anxiety and depression-like behaviors with elevated serum corticosterone levels in adult male rats [1].

In light of current reports and our experience with the effects of metabolic cage housing on animal welfare and physiology, it can be used as a modeling option of metabolic cages for social isolation stress, rather than conducting long-term metabolism studies in these cages due to single housing on grid flooring.

DISADVANTAGES OF METABOLIC CAGES IN TERMS OF ANIMAL WELFARE

Firstly, according to the current EU Directive [14], metabolic cage housing for more than one day should be accepted as mild distress or pain, and more than five days should be accepted as moderate distress or pain factor. Furthermore, the Animal Research Review Panel (ARRP) [15] recommends that wire mesh floors not be used for rat caging unless the treatment has the express permission of the institution's Animal Ethics Committee and there is compelling evidence for the need to use such flooring. In such cases, a solid floor section and nesting material are preferable.

Secondly, it is widely accepted that allowing rodents to habituate and acclimate to changes in location and caging is a common best practice for obtaining reliable data from subsequent experiments [16]. Therefore, ARRP suggests that rats should be acclimatized to metabolic cage before studies commence [15]. Hunt and Hambly [17] proposed that singly housed rodents be separated from their group for at least two weeks before being measured. However, according to Kalliokoski et al. [18], male mice do not become accustomed to metabolic cage housing in three weeks. In this case, how long is the appropriate housing period for metabolism studies in these cages?

Finally, in a metabolism study, determination of daily fecal and urinary output or analysis of urine and/or feces may be one of the essential steps of experiments. Therefore, grid flooring is a result of necessity for experiments that need a separated collection process for urine and feces [4,5] without contamination of the urine samples [3]. However, housing an animal on grid flooring can cause foot hypersensitivity and pain, as well as sleeplessness. As a result, psychophysiological and metabolic parameters may

deteriorate over time [1]. Indeed, there are many reports that housing rodents on grid flooring leads to elevated blood corticosterone levels, as well as blood pressure, high heart rates and body temperature [1, 4, 5, 19,20]. These results indicate that metabolic parameters are affected by housing on wire mesh floors in metabolic cages.

In our previous experiments, we observed that both 10-day and long-term (30 days) housing in metabolic cages caused some psychophysiological alterations in rats [1,21]. 10-day housing in these cages caused an increase in depression-like behaviors in female rats [21]. In male rats, increased anxiety and depression-related behaviors were determined after 30-day housing in these cages [1]. As a remarkable common feature in both studies, the scores of the total distance in the open field test were lower in the metabolic cage groups [1,21]. A reduction in the locomotor activity in the open field test may be interpreted as a sign of reduced exploration behavior due to emotional disturbance induced by high anxiety or fear [12]. We also considered that decreased locomotor activity could be due to anxiety, as well as foot hypersensitivity and pain caused by the grid floor of metabolic cages [1]. Indeed, the scores of the total distance after 10-day housing were lower in the metabolic cages housed female rats, but these did not differ between the metabolic cage group and the control group after 10 days of rest period in normal cages [21]. Important disorders that may occur in animals due to metabolic cage housing are summarized in Table 1.

Figure 1: General view of the metabolic cage (A), having to live single in the metabolic cage (B) and forced feet contact with the grid floor (C).

Table 1. Disorders due to the disadvantages of the metabolic cage	
Disorder	**Reason**
Social isolation stress	Single housing
Neuropsychiatric disorders (anxiety or depression)	Single housing, Grid floor
Foot pain	Grid floor
Sleeplessness	Grid floor
Convective heat loss	Grid floor
High corticosterone	Single housing, Grid floor
High heart rates	Single housing, Grid floor
High blood pressure	Single housing, Grid floor

OPINIONS AND CONCLUSIONS

Our opinion mainly indicates that metabolic cages may be an option for modelling social isolation stress due to single housing on the grid floor (Figure 1 A, B and C). Individual housing of mice and rats is not a suitable living condition due to deprivation of these animals of their social needs [1]. It is known that especially the absence of olfactory interaction is an essential source of stress for animals. The ARRP guideline emphasizes that, when metabolic cages are used to house rodents individually, the degree of physical isolation is greater than that in the case of individual housing in standard cages, because the design of these cages will restrict olfactory, visual and auditory contact of the animal with others [15]. Nevertheless, metabolic cages do not fully prevent this contact due to their air flow areas. Additionally, the restrictive effect of metabolic cages on olfactory, auditory and visual contact may be greater than single housing in standard cages, but these effects are valid for individual housing conditions. In our opinion, the effect of housing on grid flooring in metabolic cages is the major factor for inducing a greater social isolation stress than individual housing in standard cages because all standard cages include a bottom floor.

In animals and humans, hypersensitive or lesioned feet may be a reason of pain. A chronic painful condition in the body can induce the occurrence of psychological disorders such as anxiety and depression [22-24]. A study suggested that there is a high ratio of major depression and especially anxiety disorder in patients with chronic pain [25]. Regarding food pain, it was found that symptoms of depression and anxiety were considerably higher in the patients who suffered from foot pain [26]. There is an interesting study that reported an association between plantar heel pain and symptoms of stress, depression and anxiety [27]. Therefore, wire mesh housing contributes to chronic foot pain and psychophysiological abnormalities. Moreover, long-term housing on grid floors can cause sleeplessness in rats [1]. Sleeplessness is a considerable risk factor for psychological disorders. It is known that sleeping on a grid floor also leads to increased convective heat loss in animals [28]. Thus, metabolic parameters may be adversely affected in the animals due to their altered energy expenditure. Therefore, the ARRP Guideline recommends that, if rats are kept in wire-bottomed cages without a solid resting area and nesting material (as in metabolic cages), the room temperature be in the 24-26°C range [15]. On the other hand, it may be assumed that the majority of metabolism or pharmacological studies in grid-flooring cages are performed below this mentioned temperature range. Each of these factors may be individually assessed for damage in terms of the negative effects on behavioral and physiological responses in small rodents. However, housing in these cages includes a result of the combination of all these stressful and painful factors [2].

A decrease in the well-being of these animals may affect the validity of scientific data [29]. In the current form of the metabolic cages, housing the animals on grid floors in metabolism studies represents a negative effect on the well-being of laboratory animals. If an experiment is being conducted in laboratory animals to assess biological activity in humans, animal welfare should be considered. This is a very important aspect of standardization of experiments by eliminating the influence of non-experimental factors on research data. Thus, it may be possible to achieve the universal nature of knowledge. We suggest that some modifications such as a resting platform or new constrictions should be performed for metabolism or pharmacology studies in these cages. Indeed, there is a study which reported that the well-being of rodents was similar between metabolic cage housing with resting platform and solid-bottom cage housing [30]. In their current form, metabolic cages may be a useful option for social isolation stress paradigm in small rodents, except for metabolism studies.

Conflict of interest: Author declares that there is no conflict of interest.

Financial conflict of interest: Author declares that he did not receive any financial support in this study.

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