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INTRODUCTION

With the growing use of genetically engineered mice in **phenotyping studies**, a wealth of functional genetic information is being generated. Consistent phenotyping data within and across labs require **standardization** of protocols but also of **housing conditions**, well known to influence mouse phenotype characteristics such as behavior. Individually Ventilated Cage (IVC) housing is popular among animal facilities because of its sanitary and animal welfare advantages. Nowadays, various IVC systems with their own specific features are commercially available. So far however it is unknown how different IVC systems may influence mouse phenotypes.

The present study was designed to **evaluate the impact of 3 different IVC systems on mouse phenotypes**. To this end, C57BL/6N mice were submitted to batteries of tests allowing evaluation of Behavioral and Physiological phenotyping and Sensitivity to Diet-Induced Obesity (DIO).

Housing and Animals

Housing conditions: IVC M.I.C.E® (Animal Care System), IVC SealSafe® Plus (Tecniplast) and Innocage® (Innovive Inc), (respectively named here for simplification as **IVC1**, **IVC2** and **IVC3**) were evaluated with C57BL6/NTac mice in housing rooms under controlled temperature (21-22°C), 12-12 hours light-dark cycle and food and water available *ad libitum*, except for specific metabolic and biochemical analyses (fasting for OGTT and IPIST). Bedding: poplar in IVC1 and IVC2; corn cob in IVC3.

Phenotyping : based on the procedures used in EMPRESS, the European standardized phenotype screen (<http://empress.har.mrc.ac.uk/>);

Behavior: 72 mice fed with chow diet (n=12 per group)

Physiology and DIO model: 9 male and 9 female mice per IVC system (72 mice). Here, for logistical reasons, IVC 2 and IVC3 system comparisons were performed distinctly, with IVC1 system as our reference.

Diet Induced Obesity model

Age (weeks)	5	6	7	8	9	...	19	20	21	22	23	24	25
Diet	Chow Diet					High Fat High Carbohydrate Diet (HFHCD)							
Tests	Weekly Body Weight												
	Acclimation	Body temperature and Body composition by qNMR	Blood pressure and heart rate measurement				Body composition by qNMR	Body temperature and OGTT		IPIST		Blood collection; Necropsy and histology	

Table 2 : Physiology and DIO model procedures

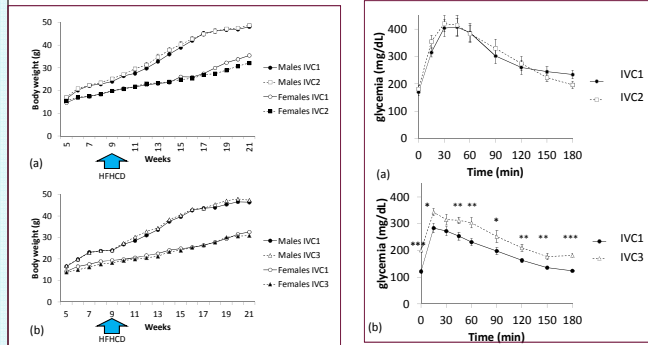


Fig. 2: Body weight gain, male and female mice in (a) IVC1 vs IVC2, (b) IVC1 vs IVC3.

Fig. 3: OGTT in male mice, (a) IVC1 vs IVC2; (b) IVC1 vs IVC3. (*:p<0.05, **:p<0.01, ***:p<0.001)

- Whatever the IVC system, HFHCD induce significant increase in body weight reproducing well the DIO model.
- IVC3 housed males showed increased glycaemia in OGTT, due to an incomplete fasting with the corn cob bedding.
- All other results belong to reference values observed in our laboratory*

Physiology

Blood parameters:

- Hematology** : White blood cells, red blood cells, platelets, hemoglobin, hematocrit and Mean cell volume. There was no significant difference between caging systems.
- Biochemistry** : Glucose, urea, creatinine, K, Cl, Ca, P, Mg, Total proteins, albumin, amylase, lactate dehydrogenase, alanine aminotransferase, alkaline phosphatase, HDL-cholesterol, LDL-cholesterol, total cholesterol, triglycerides, insulin, adiponectin and leptin. Few results presented differences. However, all data belong to reference values observed in our laboratory*.

Cardiology: no difference have been found for blood pressure and heart rate.

Necropsies and histology:

- Necropsies** : males in IVC2 had heavier hearts than Males in IVC1 and females in IVC3 had lighter livers than females in IVC1. However, all data belong to reference values observed in our laboratory.
- Histology**: as expected, male livers presented hepatic steatosis in all cages systems showing the DIO model is well reproduced.

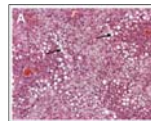


Figure 1: Liver (H&E x50) in male mice from IVC1; moderate to severe hepatic steatosis; fat vacuoles (arrows).

- Plasma chemistry and necropsies: few differences between cages systems.
- Results belong to our reference values*

	Males				Females				Males				Females			
	IVC1	IVC2	IVC1	IVC2	IVC1	IVC2	IVC1	IVC2	IVC1	IVC3	IVC1	IVC3	IVC1	IVC3		
Glucose	16.9 ± 0.81	16.6 ± 0.96	18.6 ± 0.57	17.6 ± 0.66	22.2 ± 1.33	20.4 ± 0.98	16.1 ± 0.78	16.4 ± 0.63	5.57 ± 0.27	5.43 ± 0.18	3.31 ± 0.21	3.04 ± 0.16	1.37 ± 0.15	1.24 ± 0.16		
Cholesterol	6.68 ± 0.12	5.66* ± 0.17	3.74 ± 0.16	3.33 ± 0.21	5.57 ± 0.27	5.43 ± 0.18	3.31 ± 0.21	3.04 ± 0.16	1.37 ± 0.15	1.24 ± 0.16	0.83 ± 0.15	0.65 ± 0.06	3.40 ± 0.75	3.09 ± 0.99		
Triglyceride	1.49 ± 0.07	1.35 ± 0.08	1.06 ± 0.13	1.13 ± 0.16	21.7 ± 2.03	18.9 ± 0.36	13.6 ± 2.86	12.5 ± 0.6	21.7 ± 2.03	18.9 ± 0.36	13.6 ± 2.86	12.5 ± 0.6	6.91 ± 0.44	8.05* ± 0.24		
Insulin	6.66 ± 1.04	7.62 ± 1.07	4.65 ± 1.39	3.32 ± 0.83	21.7 ± 2.03	18.9 ± 0.36	13.6 ± 2.86	12.5 ± 0.6	21.7 ± 2.03	18.9 ± 0.36	13.6 ± 2.86	12.5 ± 0.6	6.91 ± 0.44	8.05* ± 0.24		
Leptin	35.5 ± 3.75	29.7 ± 1.95	26.5 ± 1.91	26.7 ± 4.88	6.91 ± 0.44	8.05* ± 0.24	11.5 ± 0.29	13.3* ± 0.4	6.91 ± 0.44	8.05* ± 0.24	11.5 ± 0.29	13.3* ± 0.4	6.91 ± 0.44	8.05* ± 0.24		
Adiponectin	6.95 ± 0.29	6.53 ± 0.28	11 ± 0.56	8.8 ± 0.89	6.91 ± 0.44	8.05* ± 0.24	11.5 ± 0.29	13.3* ± 0.4	6.91 ± 0.44	8.05* ± 0.24	11.5 ± 0.29	13.3* ± 0.4	6.91 ± 0.44	8.05* ± 0.24		

Table 1: Plasma chemistry in different cages. (*:p<0.05, **:p<0.01, ***:p<0.001).

* Reference values in our laboratory were observed in IVC1 (data not shown).

Behavior

Table 3: Behavioral phenotyping tests performed after 2 weeks acclimation:

Age (weeks)	10	10	11	12	12	12	13	14	14
Tests	Open field	Light-dark test	Grip test	Rotarod test	Tail suspension	Auditory startle reflex and Pre-Pulse Inhibition	Fear conditioning	Hot plate	Tail Flick
	Anxiety		Motor function		Susceptibility to despair	Sensorimotor gating	Learning and memory	Pain sensitivity	

Results:

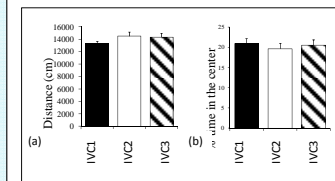


Fig. 4: Open field test: (a) total distance in the arena; (b) time spent in the center for females

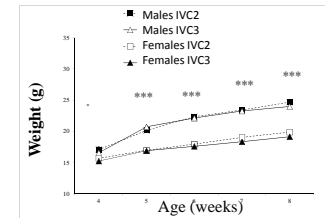


Fig. 5: Evolution of body weight from weaning to adulthood in male and female mice housed in IVC2 and IVC3. * p<0.05, ** p<0.01 vs age- and cage-matched females

Gender	Males			Females		
IVC system	IVC1	IVC2	IVC3	IVC1	IVC2	IVC3
Body temperature (°C)	36.7 ± 0.2	36.7 ± 0.2	36.4 ± 0.2	36.9 ± 0.1	37.0 ± 0.1	36.9 ± 0.1
Rotarod- 4 to 40 rpm in 5 min (s)	130.7 ± 14.5	116.2 ± 13.2	111.7 ± 18.5	150.1 ± 12.4	161.8 ± 17.4	148.1 ± 8.9
Grip strength	7.6 ± 0.2	7.9 ± 0.2	8.1 ± 0.2	9.9 ± 0.3	10.5 ± 0.3	9.3 ± 0.2s
Tail flick (s)	3.4 ± 0.1	3.3 ± 0.2	3.3 ± 0.2	4.1 ± 0.2	3.9 ± 0.3	4.1 ± 0.2
Hot plate (s)	10.2 ± 0.4	11.0 ± 0.7	9.7 ± 0.4	12.1 ± 0.9	13.2 ± 0.6	10.0 ± 0.5**ss

Table 4: Neurological evaluation and pain sensitivity

- IVC3 housed females, but not males, showed reduced muscle strength when compared with IVC2 housed females
- Hot plate sensitivity is increased in IVC3 females.
- Anxiety-related behavior was not affected by caging systems in the open field.
- Motor coordination, susceptibility to despair and cognitive abilities were not affected by Caging systems

CONCLUSION

Our results reveal that most **parameters** recorded were **not affected by the caging system**.

Nevertheless depending on the caging system used, some differences were observed. However, changes observed remain within the physiological range and thus, **are not likely to occlude potential phenotypes** associated with genetic manipulations.

These results also argue that phenotyping data obtained under **standardized protocol using various existing IVC housing system** may be **confidently shared or replicated** across or within laboratories.