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„Effects of the oestrus cycle and gestation on resting metabolic rates in female guinea pigs (*Cavia porcellus*)"

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Abstract

Reproduction is one of the most demanding processes in female mammals and is always linked to fluctuations in hormone concentrations. Different hormone concentrations have an influence on the energy balance and therefore maybe also on the resting metabolic rate (RMR). In this study, RMR was measured in 11 female guinea pigs during the stages of oestrus, dioestrus, and gestation and linked to the steroid hormones oestrogen, progesterone, and cortisol as well as body mass, which usually characterize these reproductive stages.

Oestrus was characterized by lower body mass but higher oestrogen and cortisol concentrations compared to dioestrus. A higher RMR in oestrus compared to dioestrus was found when body mass was considered in the analysis. The hormones oestrogen, progesterone, and cortisol increased significantly with the gestation day, as well as body mass. RMR did not change during gestation and did also not differ compared to dioestrus, but with increasing body mass, RMR was rising more steeply in gestation.

The results indicate that oestrus is energetically more demanding than dioestrus. In addition, RMR increased more during gestation than in dioestrus referred to same body mass. Therefore, different reproductive states seem to be characterized by different metabolic scaling, but more investigations are needed.

Keywords: guinea pigs, metabolic rate, reproduction, oestrus, dioestrus, gestation, oxygen analyser, oxygen consumption

1. Introduction

Reproduction is essential in creating variation through genetic recombination and is one of the most energetically demanding biological process in female mammals (Schneider, 2004). Complex endocrine processes determine the ovarian cycle as well as pregnancy, parturition, and lactation. Oestrus lasts only a few hours or days, until ovulation takes place (for a short overview see e.g. Olson & Blumstein, 2009). This endogenous process is controlled by the hypothalamic-pituitary-gonadal (HPG)-axis. The developing follicle in combination with increasing release of LH (luteinising hormone) and FSH (follicle-stimulating hormone) by the pituitary produces oestrogens, which peak shortly before ovulation. Thereafter oestrogen levels decrease and stimulated by LH, the corpus luteum develops and starts to produce progesterone. If no fertilisation took place, the phase ends with the degeneration of the corpus luteum, a decrease of progesterone, and the onset of a new cycle. In case of fertilisation progesterone levels remain elevated throughout pregnancy (Fortune, 1994).

Although these processes may differ across species, reproduction is always accompanied by changes in hormone concentrations and this is obviously linked to an individual's energy balance (Schneider, 2004). Therefore, the metabolic rate (MR) of each species and each individual may differ not only in relation to body mass and size, with heavier/larger individuals showing higher MRs, but also due to its physiological and reproductive state (Speakman, 2008). The MR is defined as the energy expenditure of an organism per unit of time. It is not a fixed value, but is subject to fluctuations and depends on age, sex, activity, but also on the time of day and season or food composition as well as metabolizable nutrients (Hildebrandt et al., 2005). The resting metabolic rate (RMR) in particular reflects the energy expenditure of an adult animal at rest in a temperature-neutral environment in a fasting state and is required to maintain physiological functions (Hulbert & Else 2004).

RMR can vary with the steroid hormone balance, whereby especially glucocorticoid (cortisol or corticosterone) levels correlate positively with resting metabolism as shown in an interspecific analysis in mammals (Haase et al., 2016). An intraspecific study in zebra finches by Jimeno et al. (2018) showed that corticosterone concentrations increased with increasing MR under stressed and non-stressed conditions. However, only a few studies directly investigated the relationship between MR and steroid hormone concentrations during reproductive cycles in mammals. Among those, Parker et al. (2001) collected data over four complete ovarian cycles in rats. They showed that body mass decreased in oestrus but that energy expenditure was 21 % higher than in dioestrus. This effect would definitely argue against "metabolic scaling", which describes the linear dependency of the

logarithms of the metabolic intensity on the logarithm of body mass, but suggests a hormonal influence.

Based on the results of other studies regarding changes in energy expenditure associated with oestrus in female rats (Brobeck et al., 1947) ferrets (Donovan, 1985), hamsters (Moline & Albers, 1988), or cows (Arney et al., 1994), Giles et al. (2010) tested the activity and energy balance of lean and obese rats in different oestrus cycle stages. In both groups, activity varied across the oestrous cycle. Activity in lean rats was 14 % and in obese rats 20 % higher during oestrus than during dioestrus or proestrus. Furthermore, their data demonstrated, that a decrease in food intake was connected to rising oestrogen levels. These results also suggest direct effects of hormone concentrations on energy balance, but MR was not investigated in this study.

Brzęk et al. (2014) tested if MR differs before, during and after reproduction in two mouse linetypes selected for high and low MR, respectively. Body-mass-corrected basal metabolic rate (BMR) of reproducing females differed consistently between both line types before and after first reproduction and was higher after first reproduction in both lines. However, Selman et al. (2001) found that a difference in RMR between pre-breeding and lactating females was only a function of their difference in body mass. Furthermore, in long brown-war bats there was a significant effect of the stage of pregnancy on BMR (McLean & Speakman, 2000). The mean whole-animal BMR increased from 60 mW during days 20–16 to 100 mW during days 5–1 before parturition. Moreover, the BMR in pregnant bats was significantly higher than in non-reproductive females with similar body mass. This result matches with those from Herreid (1967) in long tailed bats. Although all these studies demonstrated effects of different reproductive stages on MR and energy balance, direct relationships to altered steroid hormone concentrations accompanied with these stages, remain understudied.

Our model species, the domestic guinea pig, shows a classic oestrus cycle of 16-18 days, with oestrus lasting 1-6 days (Ishii, 1920). Oestrogen and cortisol concentrations are highest on the days before vaginal opening (day 0 corresponds to oestrus). Afterwards progesterone increases and stays on a plateau from day 5 to day 13 of the cycle (Nemeth et al., 2018). Nevertheless, body mass decreases during oestrus, which was shown to be caused by a negative effect of oestradiol on food intake (Czaja & Goy, 1975). During gestation, body mass of guinea pigs strongly increases (Nemeth et al., 2017). A long gestation period of 66 – 69 days requires a high investment from the mothers and the pubs are born very highly developed (Kunkele, 2000). In Kunkele's (2000) experiment, body mass of pregnant females with a natural litter size (3 pups) increased by about 38 to 60 %. Although

the body mass gain is so enormous, the daily energy consumption of the mothers is relatively low in relation to the efficiency of energy allocation into offspring production compared to other rodents (compare e.g., Kunkele, 2000; Speakman, 2008).

The guinea pig is very well studied with regard to its behavioural, social and physiological responses (Sachser, 1986; Künkele, 2000; Schöpper et al., 2011), but knowledge on energy expenditure (e.g. RMR) associated with different reproductive states and hormone concentrations is understudied. Therefore, the aim of this study was to find out whether individual RMR changes during the stages of oestrus, dioestrus, and gestation. Further we considered effects of steroid hormones (cortisol, oestrogens, progesterone) and body mass on RMR during each stage. Due to this first attempt to use a portable oxygen analyser (the OxBox 4.1) for analysing RMRs in guinea pigs, another point of this study was to determine relations between an individual's behaviour and oxygen consumption during the measurements as indicator of the measurement's validity.

2. Methods

2.1 Ethical statement

Experiments were performed in accordance with EU Directive 2010/63/EU and approved and permitted by the institutional board on animal ethics and experimentation (Faculty of Life Sciences, University of Vienna; no. 2018-026) and the Austrian Federal Ministry of Science and Research (BMBWF-66.006/0010-V/3b/2019).

2.2 Animals and housing conditions

For this study, 11 domesticated virgin female guinea pigs with a mean age of 18.3 ± 1 months were used. All guinea pigs were bred at the Department of Behavioral and Cognitive Biology of the University of Vienna and were used to human contact. The individuals were distinguished and identified by their natural fur markings.

The animals lived in single-sex groups, composed of 6 and 5 animals, respectively. Each group was kept in a rectangular shaped enclosure of 3.2 m^2 equipped with two shelters each and standard woodchip bedding material covering the floor, which was renewed once a week. Each group was daily fed by 25g standard diet for guinea pigs (ssniff V2233, ssniff Spezialdiäten GmbH, Soest, Germany) and 10 g hay per animal, which was supplemented once a week by some vegetables. The photoperiod was a constant 12/12h light-dark cycle, lights on at 7:00 a.m., the temperature was 20°C ± 2°C, and the humidity 50% ± 5% throughout.

2.3 Experimental procedure

The experiments and measurements of steroid hormone concentrations and RMR were repeatedly performed for each female in oestrus, dioestrus and during gestation. During gestation, this was planned to be carried out three times, once for each trimester of pregnancy. However, due to technical problems, measurements of RMR are only available for the first and second trimester.

The determination whether a female was in oestrus or dioestrus was based on the fact, that the vagina is usually covered by a membrane which ruptures at the onset of physiological oestrus and regenerates 1-6 days afterwards (Young, 1937). The whole oestrus cycle lasts approximately 16 days (Ishii, 1920). Whenever a female was in oestrus on a testing day, as indicated by the first day of an open vagina, this female was subjected to the measurements. If no female was in oestrus on a testing day, one of the females in dioestrus was tested, which was ten days after oestrus. Based

on the daily vaginal inspections before the experiment, six females were tested first in dioestrus and five first in oestrus. The number of days between measurements during oestrus and dioestrus within individuals ranged from 6 to 23 days. After obtaining data from each female in oestrus and dioestrus, three males were added to each female group for four weeks of mating. This time span ensured that at least 2 cycles per female were covered by the presence of males. Afterwards, males were removed and returned to their single sex male groups. Based on continued vaginal inspections during mating, females were further subjected to the measurements at the same time to ensure measurements for each trimester of gestation. After an order of possible conception dates was determined, this particular order was continued in three-week intervals for further measurements during gestation. The duration of pregnancy in guinea lasts about 68 days (Rowlands, 1949). The three-week measurements should have, therefore, covered each trimester.

Throughout the experiment, successful measurements could be performed for 9 females in oestrus, 10 females in dioestrus, and 9 females during gestation.

2.4 Individual measurements

After the daily vaginal inspections at 09:00 a.m. for determining if a female was in oestrus, the focus animal was weighed on a common kitchen scale and a first saliva sample was collected with a cotton swab for later cortisol evaluations. Therefore, the cotton swab containing the saliva sample was put into a 1.5 ml micro centrifuge tube, centrifuged (10000 rpm, 5 min) and frozen at -20 °C for later analysis. Afterwards, the guinea pig was placed in the respiratory chamber (8L) and the oxygen consumption was measured by the Oxbox 4.1 oxygen analyser (more detailed information see Measurement of oxygen consumption) for 150 minutes. The animal was observed for the whole duration and video recorded for later behavioural analyses. Afterwards, another saliva sample was collected and the animal was placed in a separated area ($1m^2$) for 10 minutes to collect faecal samples for later analyses of oestrogen and progesterone concentrations. The faecal samples were taken directly from the bedding material without any urine contamination and stored at -20°C until further analyses. The animal was then returned into its enclosure and all animals were fed.

2.5 Measurement of oxygen consumption

RMR was measured as VO_2 (volume of oxygen consumption) using the Oxbox 4.1. This portable oxygen analyser was constructed by Thomas Ruf and Thomas Paumann at the Research Institute of Wildlife Ecology, University of Veterinary Medicine Vienna, Austria. At the beginning of the measurement, the animal was placed in a rectangular-shaped transparent respiratory chamber (volume 8l). This chamber was airtight, except two little holes (1 cm in diameter) across from each other on the shorter sides of the box. The ambient air was pulled continuously through the respiratory chamber and flowed through a tube with silica gel for air drying into the Oxbox. We adjusted a constant flow rate of 80 l/h which was maintained by an external pump controlled by the Oxbox and the integrated flowmeter. The air within the chamber was, therefore, fully renewed 10 times per hour, which corresponds to every 6 minutes. Mean oxygen consumption in ml O_2/h was recorded in 30 s intervals and a reference measurement of 120 s of the ambient air was performed every 30 min. The oxygen consumption of each individual was measured for 150 min. The chemo-electric oxygen sensor of the Oxbox was calibrated before the study, a zero calibration to remove difference in pressure was performed before each measurement. The measurements were carried out under normal environmental indoor conditions (20 ± 2°C temperature and 50 ± 5% humidity).

2.6 Extracting the resting metabolic rate (RMR)

For calculation of RMR, the first 10 minutes of measurements were deleted. This included the 2 minutes of reference readings in the beginning, the first two minutes after the reference reading deleted by the Oxbox itself, and the following 6 minutes because this is the time it takes that the Oxbox gets fully flushed with fresh air initially. Oxygen consumption by the animal was considered to be fully reflected by the measurement after this time span. For RMR extraction, the lowest mean values for 3- and 6-minute intervals with the lowest coefficients of variation from the VO_2 were considered (for an example see Fig. 1). As it turned out that the 3- and 6-minute values strongly correlated with each other (Fig. 2), but the variation of 6-minute values was in general higher, 3-minute values were considered to reflect the RMR.

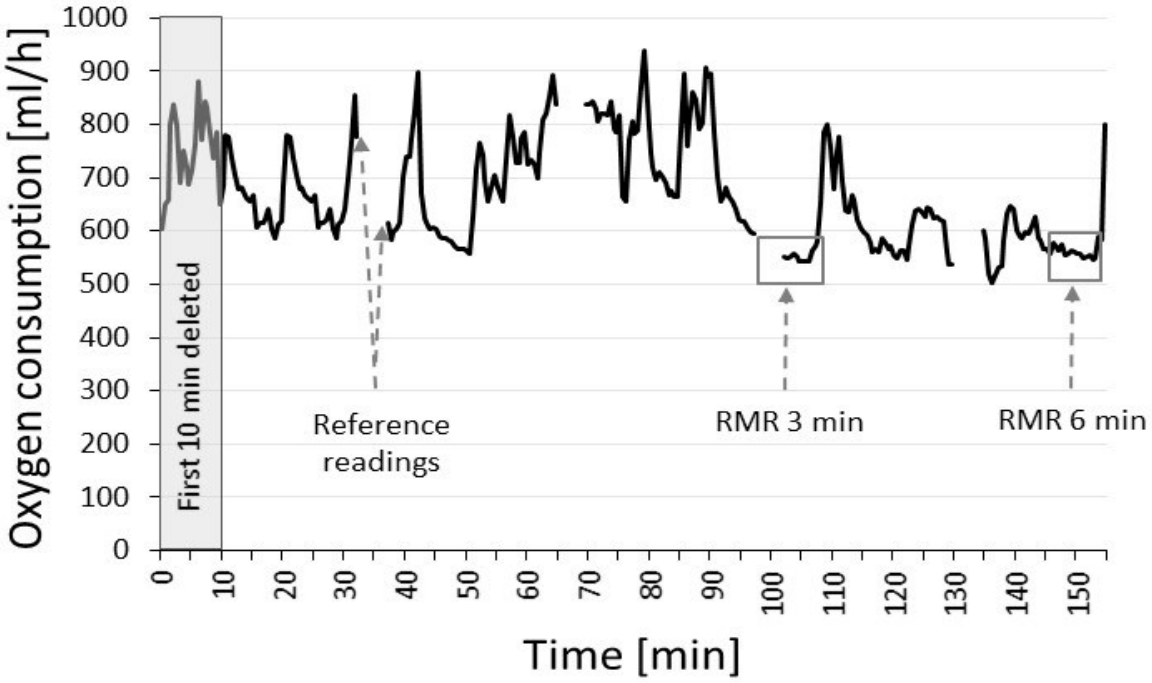

Figure 1: *Example of oxygen consumption and systematical process in one individual over 150 minutes. The first 10 minutes were deleted because of the settings. Reference readings for 2 min were performed every 30 min. At minute 105, the oxygen consumption [ml/h] for the resting metabolic rate based on 3 minutes is shown. At minute 145 the oxygen consumption [ml/h] for the resting metabolic rate based on 6 minutes is shown.*

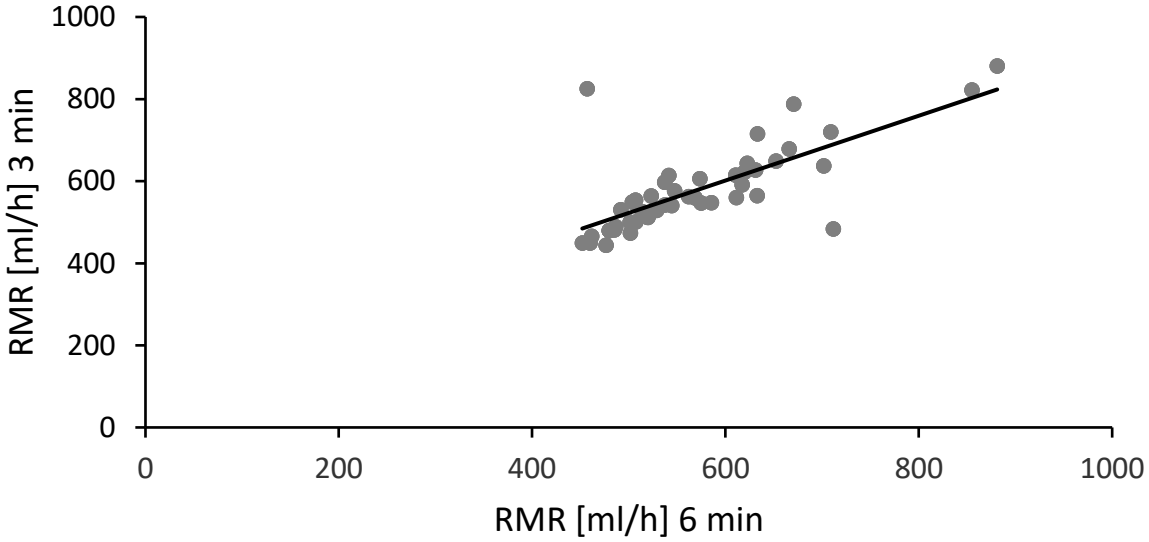

Figure 2: *Correlation of RMR (ml/h) calculated from 3- and 6-minute intervals.*

2.7 Hormone analyses

Hormone analyses were carried out using biotin-streptavidin enzyme-immunoassays (Palme & Moestl, 1997; Palme & Möstl, 1994). The enzymes and antibodies were obtained from the University of Veterinary Medicine, Vienna, Austria. All hormone analyses described in the following have been validated biologically for the use in guinea pigs (Bauer et al., 2008; Fenske, 1997; Nemeth et al., 2016).

For analysing saliva cortisol concentrations, samples were diluted 1:50 (assay buffer, pH 7.5) and cortisol concentrations measured in 10-µL aliquots using a cortisol specific antibody (Palme & Moestl, 1997). Intra- and inter coefficients of variance were 15.86 % and 22.08 %, respectively.

The hormones in the faecal samples were extracted by dissolving 0.2 g dehumidified (75°C) faeces in 2 ml of 80% methanol and centrifugation (15 min, 3700rpm). Afterwards, the supernatant was divided in equal parts for oestrogen and progesterone analyses. The dilution for faecal oestrogens was 1:30 and for faecal progesterone 1:50 (assay buffer, pH 7.5). Faecal oestrogens were measured in 20-µL aliquots, using a total oestrogen antibody (Palme & Möstl, 1994). The coefficient of variation for the intraassay was 2.40% and 8.46% for the interassay. Faecal progesterone concentrations were measured in 10 µL aliquots and a progesterone specific antibody was used (Schwarzenberger et al., 1996). For faecal progesterone the intraassay variation coefficient was 5.89 % and the interassay coefficient 1.42 %.

2.8 Behavioural analysis

The animals' behaviour in the respiratory chamber during measurements of oxygen consumption was recorded by a Go Pro camera. The aim of the behavioural analysis was to find indications if the oxygen consumption during the measurement was influenced by the behaviour of the animal. Considering the fact, that it should take 6 min until the air in the box is completely renewed, another issue was to get information on the time delay for such an effect. Therefore, the animal's active behaviour during each 30-s oxygen measurement interval, as recorded by the OxBox, was analysed. For each 30s-interval, the frequency of the following behaviours was recorded: sniffing, scratching, shaking, head moving, body moving, jumping, chewing, trying to drink, grooming. For further analysis, these behaviours were summed up to the total number of behaviours during a 30 s-interval and the total number of behaviours during a complete 150min-measurement.

2.9 Statistical analyses

For statistical analyses R version 3.4.1 (R Core Team, 2018) and the implemented package “nlme” (Pinheiro et al., 2017) was used. After checking the normal distribution with the Shapiro-Wilk Test and the homogeneity of variance with the Levene’s test, linear mixed models (LMM’s) were used to investigate body mass, hormone concentrations, and RMR, effects of body mass and hormone concentrations on RMR, and behaviour. In all analyses, cycle stage (oestrus, dioestrus) or the gestation day (continuous variable, quadratic term) were fixed effects and the individuals were included as random effect, to correct for repeated measurements. To analyse the effects of behaviour on O_2 consumption, the 30 s measurements were manually delayed in 30 s steps for overall 660 s to determine the time lag with regard to the highest explained variance. All post-hoc analyses using package “phia” (De Rosario-Martinez, 2015) were Bonferroni corrected at the relevant level of analysis. Some of the response variables (e.g. cortisol) had to be transformed by a natural logarithm, but untransformed data were used for visualization of the results. Model statistics are based on type three sum of squares. Significance level was set at $p \leq 0.05$. All results are expressed as means ± standard errors.

3. Results

3.1 Effects of behaviour on O_2 consumption

Most of the variance in oxygen consumption during the 30 s measurement intervals was explained by the shown behaviours when the time lag was 330 s (9.28%). There was an increase in R^2 during the first 300 s, a peak at 330 s, and a decrease after 360 s (Fig. 3).

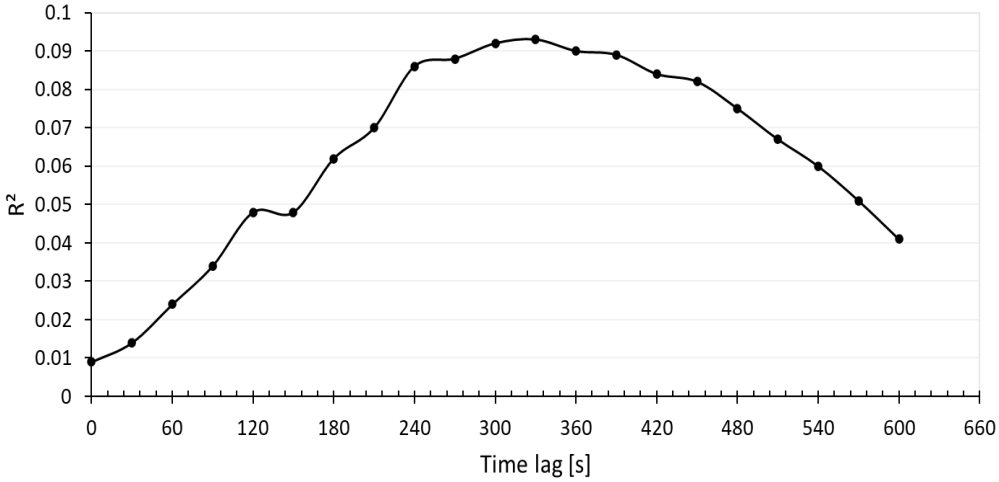

Figure 3 *Time lag of the explained variance in oxygen consumption due to the shown behaviour during 30 s intervals of Oxbox measurement. The R^2 value describes the percentage of the explained variance. Most of the variance in MR was explained with a time lag of 330 s.*

Chewing, turning the head, sniffing and grooming had significant effects on the O_2 consumption (Table 1). In sum, effects of behaviours did not differ when comparing oestrus, dioestrus and gestation (p=0.481).

Table 1 *Effects of single behaviours on the O_2 consumption during the Oxbox measurement.*

Behaviour	Mean (±s.e.m.)	p-value
Chewing	3.331 (± 0.177)	<0.001
Turning head	9.334 (± 0.845)	<0.001
Drinking	3.503 (± 1.648)	0.034
Turning	8.425 (± 5.376)	0.117
Sniffing	5.331 (± 0.281)	<0.001
Grooming	5.021 (± 0.534)	<0.001
Jumping	3.059 (± 4.140)	0.460
Shaking	-4.967 (± 10.037)	0.620

3.2 Oestrus cycle

Body mass (Fig. 4A) was significantly lower in oestrus than in dioestrus ($F_{1,7}$ = 5.286; p < 0.001). Saliva cortisol concentrations (Fig. 4B) were significantly higher in oestrus than in dioestrus ($F_{1,7}$ = 5.633; p = 0.049). Faecal oestrogen concentrations (Fig. 4C) were higher in oestrus than in dioestrus ($F_{1,6}$ = 8.838; p = 0.025), but faecal progesterone concentrations (Fig. 4D) did not differ ($F_{1,7}$ = 1.447; p = 0.268).

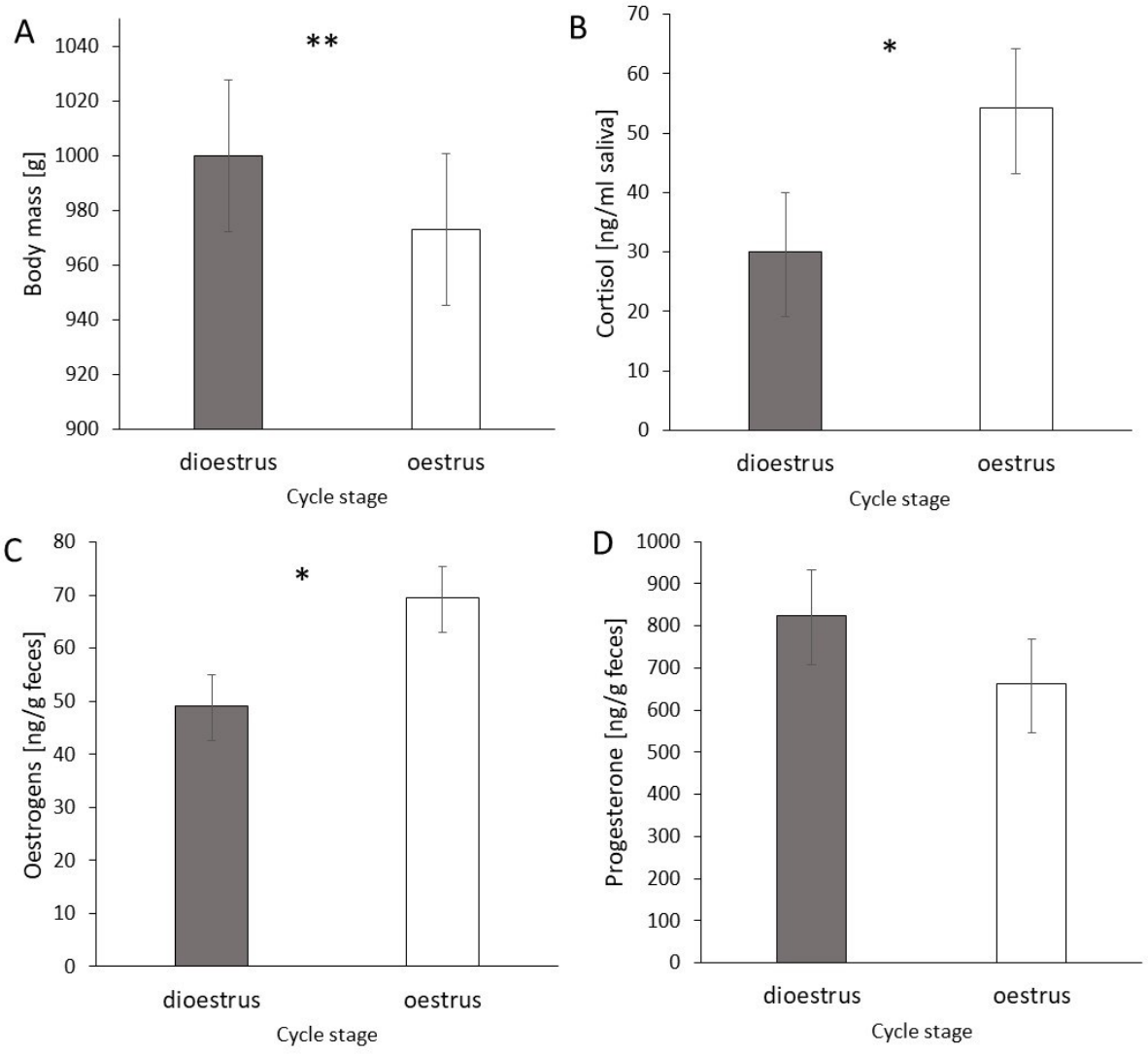

Figure 4 *General cycle characteristics. (A) Body mass [g], (B) Saliva cortisol concentrations [ng/ml], (C) faecal oestrogen metabolite concentrations [ng/g], and (D) faecal progesterone metabolite concentrations [ng/g] in oestrus and dioestrus (n=11, means ± SD). * p ≤ 0.05, ** p ≤ 0.01.*

O_2 consumption as an indicator for RMR was in average about 50 ml/h higher during oestrus than dioestrus but remained non-significant ($F_{1,7}$ = 3.78; p = 0.093; Fig. 5A). When body mass was taken into account, RMR in ml/h/kg was significantly higher in oestrus than in dioestrus ($F_{1,7}$ = 7.35; p = 0.030; Fig. 5B). When including body mass as covariate in the analysis, RMR in oestrus was also higher than in dioestrus ($F_{1,5}$= 6.676; p = 0.049; Fig. 5C). Moreover, an interaction of body mass with the cycle stage missed the criterion of significance marginally ($F_{1,5}$ = 5.569; p = 0.065). In dioestrus, RMR was positively affected by body mass, but no correlation was detected during oestrus (Fig. 6).

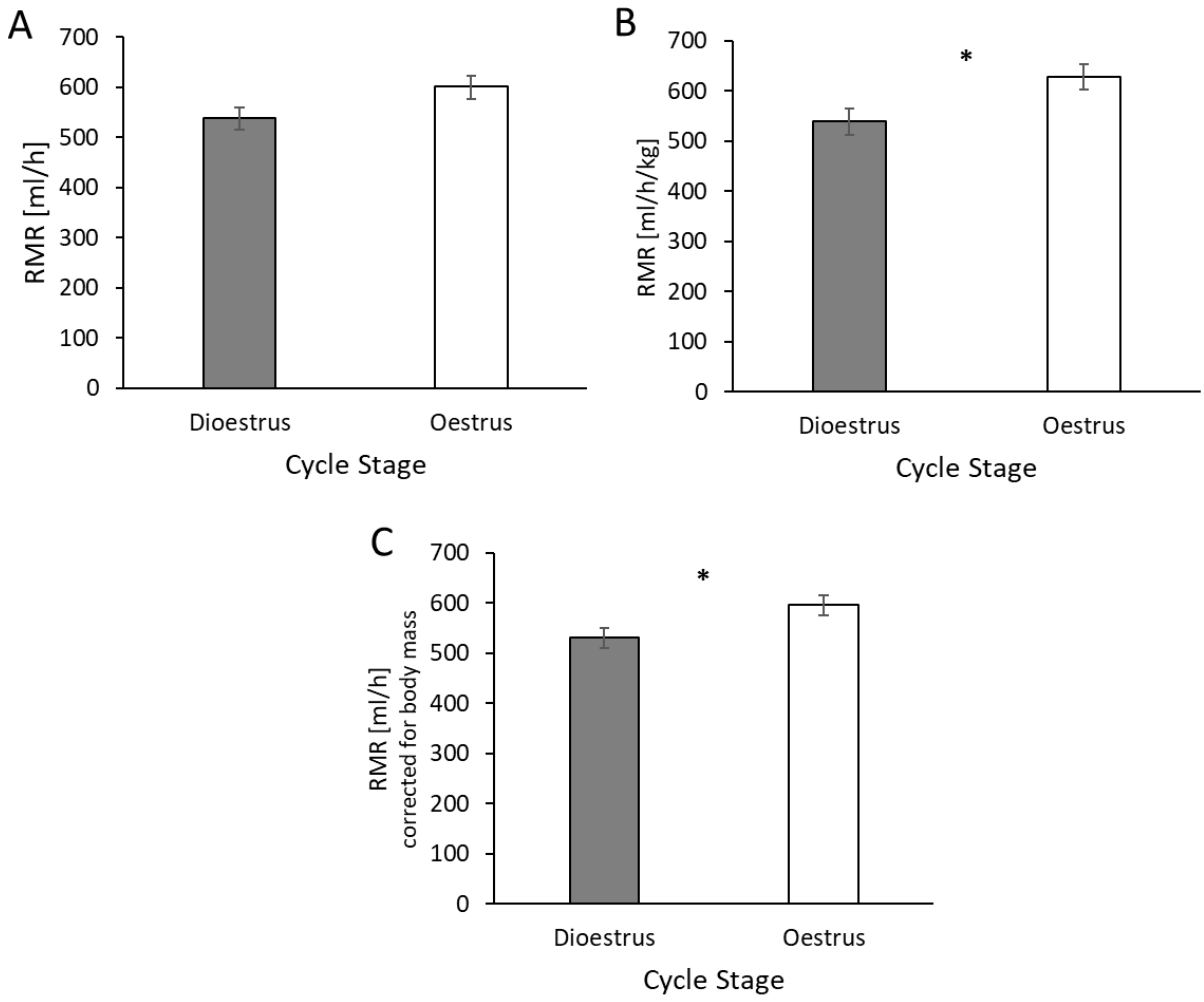

Figure 5 *Resting metabolic rate (RMR) in oestrus (n=7) and dioestrus (n=11) in (A) ml/h, (B) ml/h/kg, and (C) ml/h with body mass as covariate. * p ≤ 0.05.*

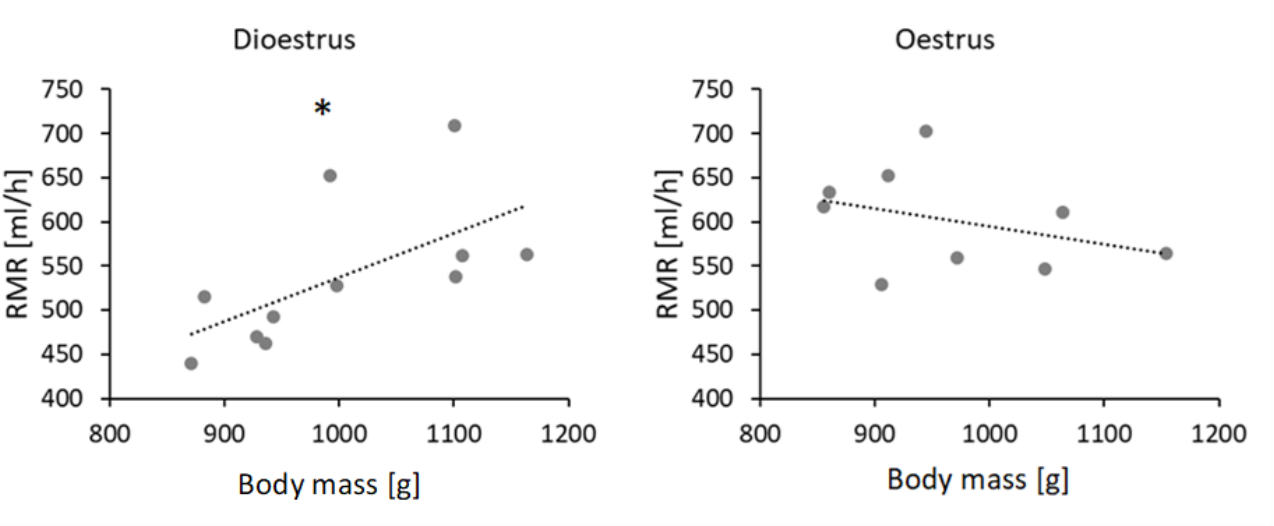

Figure 6 *Effects of body mass on RMR in ml/h during dioestrus (n=11) and oestrus (n=9). Oestrus vs dioestrus: p = 0.065. * p ≤ 0.05.*

In table 2, full model statistics for the influence of cortisol, oestrogen, and progesterone concentrations on RMR are shown. None of these hormones had a significant influence on RMR.

Table 2 *Influence of the hormones cortisol, oestrogen and progesterone on the resting metabolic rate (RMR).*

Hormone	Response (RMR)	Predictor	F-value	p-value
Cortisol	ml/h/kg	Cycle stage	0.100	0.764
		Cortisol	0.957	0.373
		Cycle stage : Cortisol	0.599	0.474
	ml/h	Cycle stage	3.833	0.145
		Body mass	4.993	0.112
		Cortisol	1.445	0.316
		Cycle stage : Body mass	5.054	0.110
		Cycle stage : Cortisol	0.014	0.912
Oestrogen	ml/h/kg	Cycle stage	1.144	0.345
		Oestrogen	1.382	0.305
		Cycle stage : Oestrogen	0.269	0.632
	ml/h	Cycle stage	10.036	0.087
		Body mass	6.759	0.122
		Oestrogen	3.513	0.202
		Cycle stage : Body mass	7.564	0.111
		Cycle stage : Oestrogen	2.473	0.256
Progeste-rone	ml/h/kg	Cycle stage	0.074	0.796
		Progesterone	0.023	0.884
		Cycle stage : Progesterone	2.149	0.203
	ml/h	Cycle stage	3.978	0.14
		Body mass	5.867	0.094
		Progesterone	0.013	0.917
		Cycle stage : Body mass	4.229	0.132
		Cycle stage : Progesterone	0.701	0.469

3.3 Gestation

During gestation, body mass increased from 1000 to 1350 g on average ($F_{2,59}$ = 253.71; $p < 0.001$; Fig. 7A). In the first trimester the body mass increased slowly, in the second and third trimester the body mass gain was faster. Cortisol concentrations also increased throughout gestation process (Fig. 7B). The result was significant ($F_{2,39}$ = 10.77; $p < 0.001$), even when taking the outliers into account which were all caused by the same individual showing increased cortisol concentrations from the beginning of gestation. Faecal oestrogen ($F_{2,23}$ = 14.467; $p < 0.001$; Fig. 7C) and progesterone concentrations ($F_{2,22}$ = 18.575; $p < 0.001$; Fig. 7D) also increased significantly during gestation. Litter size was included in all these analyses as covariate (only as main effect possible due to low sample sizes), but never had a significant effect (always $p > 0.161$).

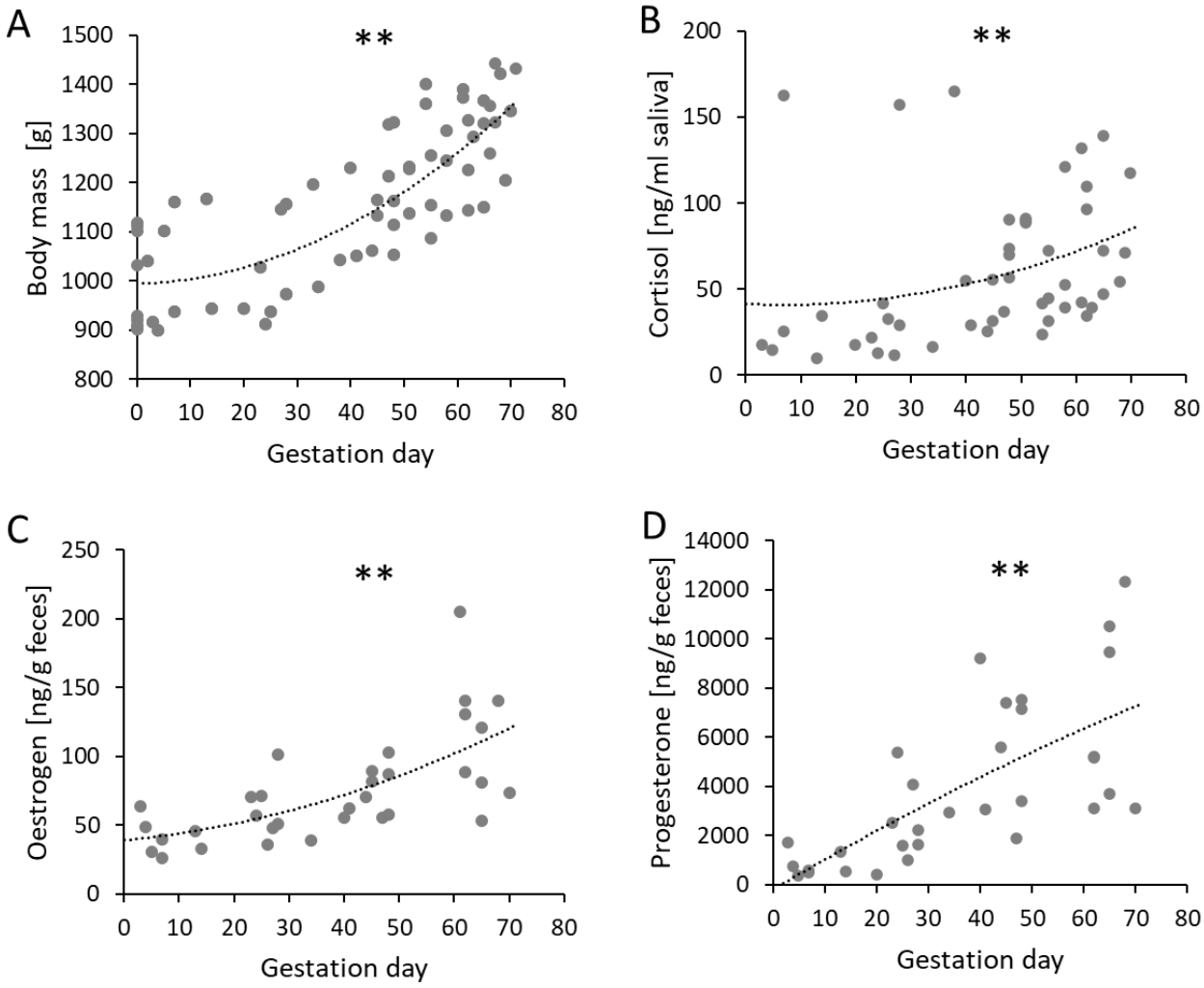

Figure 7 *General gestation characteristics. (A) Body mass [g], (B) saliva cortisol concentrations [ng/ml], (C) faecal oestrogen metabolite concentrations [ng/g faeces], and (D) faecal progesterone metabolite concentrations [ng/g faeces] during gestation. (n=11) ** p ≤ 0.01*

None of the RMR measurements showed any changes during gestation (ml/h: $F_{2,6} = 0.066$; $p = 0.937$; Fig. 8A; ml/h/kg: $F_{2,6} = 2.015$; $p = 0.215$; Fig. 8B; ml/h with body mass as covariate: $F_{2,5} = 4.237$; $p = 0.084$). However, RMR in ml/h was positively affected by body mass ($F_{1,5} = 16.130$; $p = 0.010$; Fig. 9). No effects of litter size were detected (always $p > 0.537$).

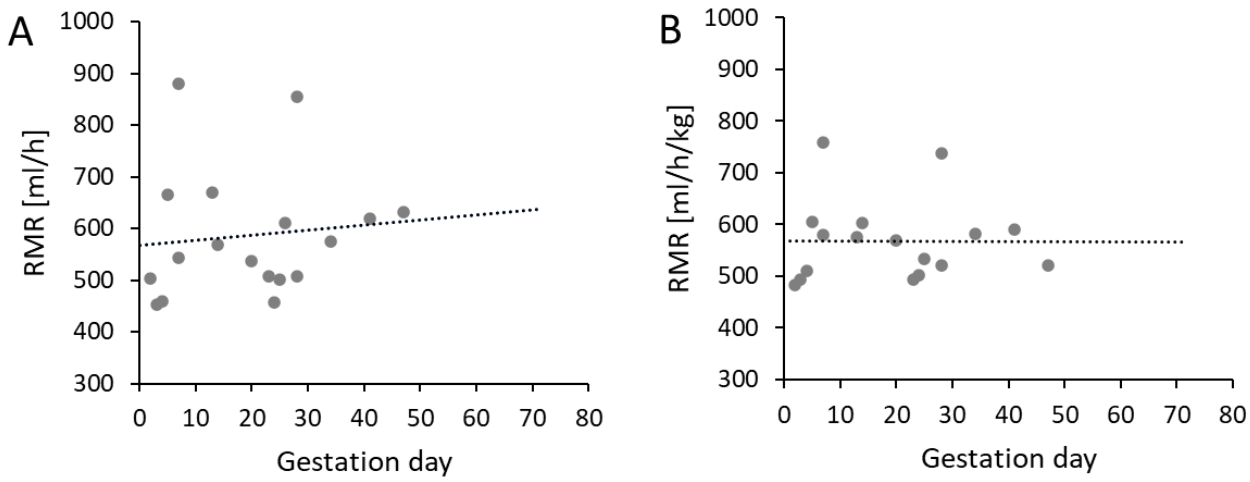

Figure 8 *Resting metabolic rate (RMR) in (A) ml/h and (B) ml/h/kg during gestation.*

In table 3, full model statistics for the influence of cortisol, oestrogen, and progesterone concentrations on RMR are shown. None of these hormones had a significant influence on RMR during gestation.

Table 3 *Full model statistics for the influence of the hormones cortisol, oestrogen and progesterone on the resting metabolic rate (RMR) during gestation.*

Hormone	Response (RMR)	Predictor	F-value	p-value
Cortisol	ml/h/kg	Gestation day	1.83264	0.2723
		Cortisol	0.00431	0.9508
	ml/h	Gestation day	3.334070	0.1728
		Body mass	13.614642	0.0345
		Cortisol	0.109972	0.7620
Oestrogen	ml/h/kg	Gestation day	6.12968	0.0605
		Oestrogen	0.05181	0.8311
	ml/h	Gestation day	5.795303	0.0932
		Body mass	11.481299	0.0428
		Oestrogen	0.000000	1.0000
Progesterone	ml/h/kg	Gestation day	3.02547	0.1377
		Progesterone	1.47458	0.2788
	ml/h	Cycle stage	7.884593	0.0409
		Body mass	4.085988	0.1133
		Progesterone	19.774610	0.0113

3.4 Metabolic scaling

Comparing the effects of body mass on RMR in m/h between dioestrus and gestation revealed a significantly stronger effect during gestation ($F_{1,15}$ = 5.040; p = 0.040; Fig. 9). At low body mass of approximately 900 g, RMR did not differ between dioestrus and gestation, but with increasing body mass, RMR was rising more steeply in pregnant females.

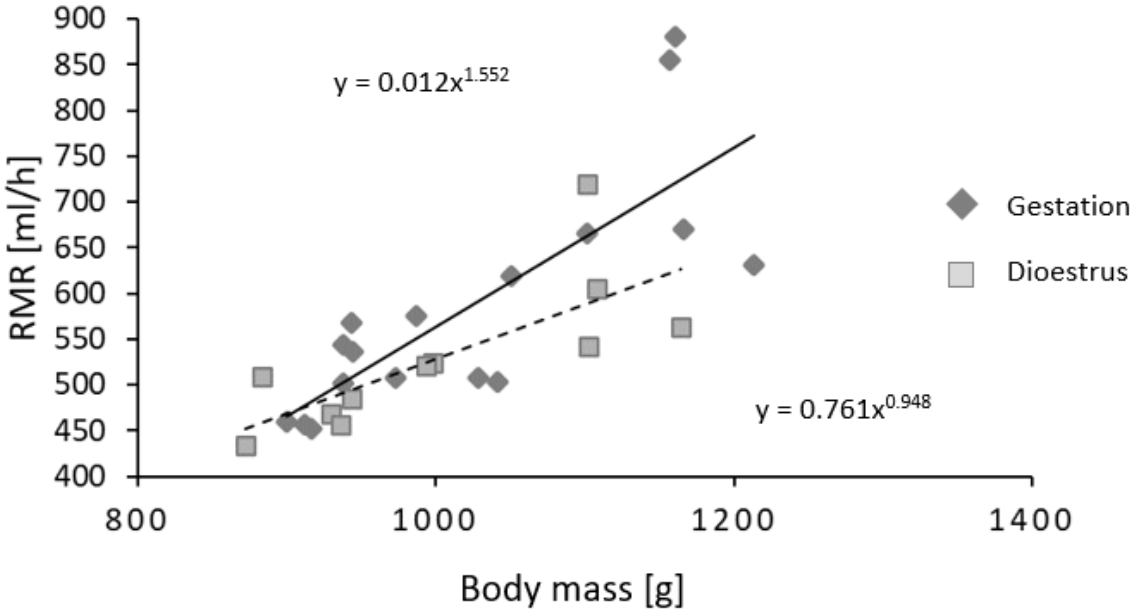

Figure 9 *Different effects of body mass [g] on RMR [ml/h] during dioestrus (n=11) and gestation (n=9).*

4. Discussion

This study investigated RMRs in female guinea pigs during different reproductive states. In order to get reliable results on how an individual's RMR may change during its oestrus cycle and gestation, the study was based on repeated measurements. If RMRs would have been measured in different individuals during e.g. different cycle stages, the risk of distorted results would have been too high, because individual differences in body mass, hormone concentrations, or activity could have influenced RMR considerably (Hulbert & Else, 2004). Especially a consideration of body mass could be of particular importance when measuring RMRs in guinea pigs, because body mass strongly changes during oestrus and gestation (Nemeth et al., 2017, 2018), which was corroborated by the results of this study. Body mass was the major modulator of RMR, but as all measurements were performed within individuals during the different reproductive states, the effects on RMR can definitely be attributed to the respective physiological state than to individual differences in body mass. However, not only body mass can influence MR, but also different hormones, which change during the oestrus cycle and gestation, could have pronounced effects. Especially cortisol is one of the hormones which should strongly affect MR as well as the mass-specific MR (Haase et al., 2016). In this context, measuring hormones with non-invasive methods should have caused lower or even no stress responses in the animals but are still very effective for measuring hormonal states in guinea pigs (Nemeth et al., 2018). Also for animal welfare and ethical reasons, it is more justifiable to take non- or minimally invasive saliva and faecal samples than to take blood samples. These non-invasive measurements yielded common hormone profiles in this study, including higher oestrogen and cortisol levels in oestrus and an increase in all hormones during gestation, thus it is ensured that the RMR measurements were performed in the corresponding reproductive states.

At first sight there was only a minor, yet not significant, difference in the actual RMR measured in ml/h when comparing dioestrus and oestrus. The RMR [ml/h] was marginally higher in oestrus than in dioestrus. As body mass is highly recommended to be considered in RMR analyses (Speakman, 2013), RMR measurements were further corrected for body mass by division and by including it as

covariate. Both analyses revealed a significant difference in RMR between the cycle stages, and higher values in oestrus. The fact that females had a lower body mass, but a higher RMR in oestrus than in dioestrus strongly implies that oestrus is energetically demanding. Similarly to previous studies (Nemeth et al., 2018) it was found that not only oestrogen but also cortisol concentrations were significantly higher in oestrus than in dioestrus. This could indicate that the animals had higher allostatic loads because of hormone production and the variety of physiological changes associated with oestrus and ovulation (Espey, 1980; Richards et al., 2002), and therefore, the energy they got by food intake had to be used for other procedures than fat storage in the body. Eckel (2011) described in her study, that oestradiol has a negative influence on the food intake in female rats. A logical conclusion would be, that females should consume more food in oestrus because of the higher energetic demand, but also in guinea pigs food consumption is higher in dioestrus (Czaja & Goy, 1975). In female rats, plasma oestradiol concentrations start to rise during dioestrus, peak during the afternoon of proestrus, and then fall rapidly to basal levels at the onset of oestrus (Eckel, 2011). If oestradiol has a negative influence on food intake, the logical conclusion is, that if there is less oestradiol produced, the food intake should rise. In oestrus, oestradiol starts to decrease to a basal level again, so one could conclude that food intake must increase, unlike in dioestrus, where oestradiol rises slowly and food intake decreases. An assumption could be, that the oestrus stage in precocial guinea pigs is energetically more demanding and characterized by a much more pronounced body mass loss than in altricial rats, therefore, these effects could be stronger reflected in body mass and are still visible afterwards. Furthermore, in rats, no spontaneous active luteal phase exists, therefore progesterone in dioestrus is not increased in long term. Oestrus cycles of rats last only 4-5 days and the hormone processes differ from those of guinea pigs (Westwood, 2008).

Cortisol also has a negative influence on food intake in rodents (Calvez et al., 2011). Thus food intake could also have increased because of the lower hormonal stress and cortisol levels after oestrus (Calvez et al., 2011). Cortisol is known to interact with body mass and RMR, so if cortisol concentrations increase, body mass decreases (Nemeth et al., 2014) and MR usually increases (Haase

et al., 2016), highlighting the catabolic effects of this steroid hormone. However, the data were also analysed for direct correlations between hormone concentrations and RMR, but no significant correlation was detected at all. Our opinion is, that the sample size in this study was too low to find direct relationships between these parameters. Another reason for not finding any direct effect could be, that other hormones and physiological parameters not measured here had a stronger influence on MR than cortisol, progesterone, and oestrogen. This may also be supported by the result that body mass had a positive effect on RMR in dioestrus, but not in oestrus. This indicates, that when hormone concentrations stay on the same level, as shown for dioestrus, body mass shows the strongest influence on RMR (Selman et al., 2001). However, in oestrus, when hormone concentrations, body mass, and other physiological processes change (Espey, 1980; Richards et al., 2002), RMR is no longer affected by body mass, but it is assumed, that the sum of all the other physiological changes influence the RMR.

In addition, also a behavioural change could have an influence. Birke (1981) described, that the behaviour of female guinea pigs changes during the oestrous cycle and they show increased locomotion and higher social activity around oestrous. This may affect energetic demands and MRs too. In this study, the animals' behaviour in their social groups was not investigated, but the behaviour during the MR measurements was observed. Obviously, some behaviours influenced the metabolic rate more than others. When the animal was more active the oxygen consumption *per se* was higher, but this cannot be translated to their RMR, because this was calculated while the individuals were inactive. Nevertheless, for further investigations it should be considered, that also an individual's behaviour during measurements can influence MRs or at least its interpretation. However, a time lag of approximately six minutes was detected between an increased activity in the metabolic chamber and an increase in oxygen consumption. This corroborates not only that the system used here generated adequate data (see calculation from box size and flow rate in the methods), but also that effects of specific behaviours on MRs may be measured in future studies.

Unfortunately, due to technical problems, it was only possible to measure RMR during the first and second trimesters of gestation. As mentioned, body mass increased extremely during gestation in guinea pigs, in this study up to 1400 g, starting from an initial weight of 900 g. A strong increase in RMR could have been expected, but due to the disfunction of the Oxbox after the second trimester, it was not possible to make a reliable assertion regarding any correlation. With regard to hormonal changes during gestation, progesterone increased very fast, but cortisol and oestrogen concentrations started to increase at the end of the second trimester of gestation. Therefore, one may assume, that because these hormones increased stronger as gestation progressed also RMR should have increased at this time. However, due to the disfunction of the Oxbox, there is no data for the third trimester, and it is only speculation that the RMR could have increased in the third trimester. Such an increase would have been expected because of the results from other MR studies on pregnant rodents, like the study by Speakman & McQueenie (1996), which showed that basal metabolic rate (BMR) increased during gestation in mice or Künkele's (2000) study with guinea pigs, which showed that BMR was 2,4 times higher in gestation than before. Moreover, until the second trimester, there is no evidence for a direct correlation between hormones and RMR. Further investigations of RMR in pregnant guinea pigs would therefore be highly desired.

An increased RMR during gestation, however, was at least indicated when RMR in ml/h with body mass as covariate was analysed and compared between dioestrus and (the available data from) gestation. When body mass increases during gestation, RMR is higher than in dioestrus, which would argue for a different metabolic scaling and hence different energetic demands per body mass in different reproductive states. This would definitely make sense, because developing foetuses should cause higher overall energetic demands, but also hormonal and other physiological influences could play an essential role during gestation compared to dioestrus. Although body mass strongly increases during gestation, it is known from other studies (Künkele, 2000) that the energy turnover is not as high as during lactation, although body mass is usually similar to pre-gestation levels during lactation. Künkele (2000) described in his study, that pregnant females gained up to

60 % body mass during gestation, but RMR during gestation and lactation was similar. Furthermore, the MEI (metabolizable energy intake) peaked at a level of 2,4 times BMR during gestation, but during lactation the MEI peak reached a level of 3,7 times BMR. Thus, BMR was lower despite higher body mass during gestation compared to lactation. In general, also litter size had an effect on the MEI in pregnant females, but not during lactation, which makes the high energetic demands during lactation even more interesting. In this study, however, litter size had no effect on RMR, but it is recommended to investigate this topic in more detail.

5. Conclusion

This study indicated that oestrus is obviously more demanding than dioestrus, which may be explained by higher oestrogen and cortisol concentrations, but also other physiological influences (e.g. on body mass) could play an important role. Further investigations are definitely necessary to understand the effects on RMR during oestrus. However, with this study the first RMR data during different oestrus phases and gestation in guinea pigs are compared. These results could serve as reference values for further studies on MRs in guinea pigs and other rodents. Guinea pigs are special among rodents, because of their long oestrus cycle and gestation period and a clear and visible distinction between oestrus and dioestrus. Due to these special properties of female guinea pigs, they are a suitable model organism for higher mammals like primates and humans and should be considered in future studies on MRs.

6. References

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Zusammenfassung

Reproduktion ist einer der energetisch aufwendigsten Prozesse bei weiblichen Wirbeltieren und steht immer in Verbindung mit wechselnden Hormonkonzentrationen. Diese Änderungen der Hormonsekretion haben Einfluss auf den Energiehaushalt und daher ebenfalls auf die Ruhestoffwechselrate (RMR). In dieser Studie wurde die Ruhestoffwechselrate von 11 weiblichen Meerschweinchen (*Cavia porcellus domestica*) während des Ovarialzyklus in den Phasen Östrus und Diöstrus sowie während der Trächtigkeit gemessen und Einflüsse der Steroidhormone Östrogen, Progesteron und Kortisol sowie des Körpergewichts untersucht.

Der Östrus war im Vergleich zum Diöstrus durch ein niedrigeres Gewicht, aber höhere Östrogen- und Kortisolkonzentrationen charakterisiert. Unter Berücksichtigung des Körpergewichtes wurde eine höhere RMR im Östrus verglichen zum Diöstrus gefunden. Sowohl die Hormone Östrogen, Progesteron und Kortisol als auch das Körpergewicht stiegen im Verlauf der Trächtigkeit signifikant an. Die RMR veränderte sich weder während der Trächtigkeit noch unterschied sie sich zu jener im Diöstrus. Mit ansteigendem Körpergewicht, stieg die RMR jedoch auch während der Trächtigkeit immer stärker an.

Die Ergebnisse zeigen, dass die Östrusphase energetisch aufwendiger ist, als die Diöstrusphase. Außerdem kann man erkennen, dass bei gleichem Gewicht die RMR während der Trächtigkeit höher ist als im Diöstrus, Um diesbezüglich eine genauere Aussage treffen zu können, sind jedoch weitere Untersuchungen erforderlich.

Schlagwörter: Meerschweinchen, Stoffwechselrate, Reproduktion, Östrus, Diöstrus, Trächtigkeit, Sauerstoffanalysegerät, Sauerstoffverbrauch