Daily Rhythms in Metabolic and Locomotor Behaviour of Prematurely Ageing PolgA Mice

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Abstract

Ageing is an inherent and intricate biological process that takes place in living organisms as time progresses. It involves the decline of multiple physiological functions, leading to body structure and overall performance modifications. The ageing process differs among individuals and is influenced by various factors, including lifestyle, environment, and genetic makeup. Metabolic changes and reduced locomotor activity are common hallmarks of ageing. In the present study, we investigated the metabolic and activity phenotype in prematurely ageing PolgA$^{(D257A/D257A)}$ mice at 41-42 weeks by assessing parameters such as oxygen consumption (VO2), carbon dioxide production (VCO2), respiratory exchange ratio (RER), and locomotor activity using a metabolic cage for four days. Our findings revealed that VO2, VCO2, RER, locomotor activities, water intake, and feeding behaviour show a daily rhythm, aligning with roughly a 24-hour cycle. Female PolgA mice showed a significant reduction in locomotor activity compared to male PolgA and their age-matched wild-type littermates (WT), indicating an early sign of ageing. In addition, female PolgA mice displayed a distinct phenotype with reduced walking speed, body weight and grip strength in comparison to both male PolgA and WT mice. Taken together, our findings highlight the influence of sex on the manifestation of ageing traits in the PolgA mice and further suggest that PolgA mice could serve as a valuable model for investigating the intricate mechanisms underlying the ageing process.

Keywords: **PolgA mice, Ageing, Daily rhythm, Phenotyping, Phenomaster, Metabolic behaviour, Locomotor activity**

Introduction

Over the past decade, laboratory mice have emerged as a commonly used and invaluable model system to study the underlying mechanism of biological ageing in humans (Yuan, Peters, and Paigen 2011). Several biological functions, including signalling pathways, cellular mechanisms, metabolism, and body composition, decline with ageing (Ramos et al. 2003; Kraan, Matta, and Mobasheri 2017; Silva and Schumacher 2021; Palmer and Jensen 2022; St-Onge and Gallagher

2010). These age-related changes at the tissue and organ levels were also observed in old mice compared to young mice (Singh et al. 2019; Chen et al. 2021; Rodrigues et al. 2022; Shoji and Miyakawa 2019). Many physiological parameters oscillate throughout the day, and the circadian clock system governs the physiological and behavioural rhythms in a 24-hour cycle. These include rest-activity cycles, metabolic rate, body temperature, food, and water intake (Skene and Arendt, 2006; Maywood et al., 2007; Vollmers et al., 2009; Noh et al., 2014). At the cellular level, circadian clocks play a pivotal role in regulating daily rhythms in mitochondrial oxidative metabolism (Peek et al. 2013; Neufeld-Cohen et al. 2016).

The mitochondria use nutrients such as lipids and carbohydrates and convert them to energy through oxidative phosphorylation, where oxygen is consumed in mitochondria, and carbon dioxide is the product of the citric acid cycle that displays daily oscillations (Adamovich et al., 2017, 2019; Emans et al., 2017). The energy source used by the body in which oxygen is consumed (VO2) and carbon dioxide is produced (VCO2) is measured by calorimetry (Brandi et al., 1997; Battezzati and Vigano`, 2001). The ratio of VCO2/VO2 calculates the RER, which estimates the respective contributions of fats and carbohydrates to energy production, enabling the calculation of total energy expenditure (Rothschild et al., 2022). The circadian rhythms, which directly influence tissue homeostasis, sleep regulation, behaviour, and metabolism, are closely linked to the ageing process (Basso et al. 2016; Sato et al. 2017, 2022; Kuintzle et al. 2017; Welz and Benitah 2020). The ageing process is marked by a reduction in mitochondrial function, which is intimately tied to energy expenditure and contributes to cellular metabolic alterations (Yen et al. 1989; Bratic and Trifunovic, 2010; Porter et al., 2015; Amorim et al., 2022). Previous studies have indicated that mitochondrial mutations and dysfunction are associated with ageing (Kazachkova et al. 2013; Kennedy et al. 2013; Copeland and Longley 2014; Lee and Wei 2012; Sun, Youle, and Finkel 2016). It is shown that the PolgA (D257A/D257A) mice (also known as PolgA) display a premature ageing phenotype due to extensive mitochondrial dysfunction and point mutations on mitochondrial DNA (Kujoth et al. 2005; Scheuren et al. 2020a; Scheuren et al. 2020b). In earlier studies, PolgA mice displayed diverse age-related phenotypes, including kyphosis and alopecia at ~ 40-45 weeks (Trifunovic et al. 2004). At 40 and 46 weeks, these mice showed progressive musculoskeletal deterioration characterized by reduced bone and muscle mass and impaired function (Kujoth et al. 2005; Scheuren et al. 2020a). Additionally, dopaminergic dysfunction and behavioural and metabolic characterization were assessed in PolgA mice at 3 and 14 months, showing mitochondrial electron transport chain functions and experiencing a notable decline in activity as individuals grow older (Dai et al. 2013). Moreover, PolgA mice have exhibited shifts in the metabolism of amino acids and lipids during 3 to 11 months of age. These alterations were examined using dynamic labelling techniques with 13C and 15N (Lim et al. 2021). PolgA mice show distinctive indicators of musculoskeletal ageing. These mice demonstrate premature frailty and a notable bone density and muscle mass reduction, as observed in human musculoskeletal ageing processes. This resemblance makes PolgA mice a valuable model for studying and understanding the intricate mechanisms underlying age-related changes in the musculoskeletal system (Yılmaz

et al. 2023). However, locomotor activity and metabolic behaviour indicators of premature ageing have not been thoroughly explored in these mice. Considering the crucial role of these parameters in selecting inbred strains for age-related research, this study aimed to comprehensively understand and provide normative data on locomotor activity and metabolic behaviour during the light-dark cycle. Therefore, the primary objective of this study is to evaluate metabolic patterns, encompassing variables such as VO2, VCO2, RER, locomotor activity, and feeding behaviour in 41–42-week-old PolgA and WT mice. Further, this study involves a comparative examination between PolgA and WT mice, focusing on quantifying and measuring biological ageing by tracking the specified parameters within a metabolic cage. We used PhenoMaster metabolic cages (Brown et al. 2018) and monitored over four days and nights. Our study provides a detailed assessment of the metabolic and locomotor behaviour of PolgA and WT mice, revealing that both groups show daily rhythms over parameters such as VO2, VCO2, RER, water intake, and feeding behaviour with a periodic length of ~ 24 hours. We found that the PolgA (female) mice show the higher peak-to-trough ratio in RER compared to the WT mice. Moreover, female PolgA mice displayed reduced body weight, walking speed, locomotor movement, and muscle grip strength compared to male PolgA and their WT counterparts. These changes were observed to be more pronounced in 41-42-week-old female PolgA mice compared to males, which have not been previously described. Therefore, PolgA mice can serve as a valuable model for investigating the mechanisms underlying the ageing process. This model allows researchers to study age-related processes and interventions within a shorter time frame, providing insights into potential therapeutic strategies for age-related disorders.

Results

Daily rhythms in oxygen consumption and carbon dioxide release

To examine and track the metabolic and locomotor behaviours, along with their daily changes, in both PolgA and WT mice (41-42 weeks old), we used PhenoMaster metabolic cages. The experimental setup is illustrated in Figure 1A-E. The PolgA and WT mice, with eight males and females in each group, were individually housed in PhenoMaster metabolic cages. We recorded the levels of VO2, VCO2, RER, locomotor activity, water intake, and feeding patterns in 10-minute intervals over four days. Consistent with the behaviour of nocturnal animals, we observed a significant increase in VCO2 (Figure 2A) and VO2 (Figure 2B) during the night-time (12h) of the light-dark cycle. Further, the RER increased in both PolgA and WT mice at night-time (Figure 3A). We observed an approximately 1.5 times increase in the VO2 and VCO2 levels at night-time compared to the daytime (12h). Female mice showed increased total VO2 and VCO2 in PolgA and WT mice (Figure 3B, C). Notably, PolgA mice (female and male) demonstrated slightly higher RER than WT mice at night (Figure 3D). Higher oxygen consumption is typically associated with elevated energy expenditure (Ando et al. 2019), and animals with smaller body sizes tend to have high metabolic rates, ultimately associated with high energy expenditure

(Healy et al. 2013). The RER is a widely used informative index to assess substrate utilisation in tissues, specifically the oxidation of fatty acids and carbohydrates. The RER values can vary between approximately 0.7, indicating a predominant reliance on fat as an energy source, and 1.0, indicating a preference for carbohydrates as a fuel source (Brand 2005; Fraipont and Preiser 2013; Kopp Lugli et al. 2019). In our study, we observed that the average RER was significantly increased during the night-time of the light-dark cycle for both male and female mice, as depicted in (Figure 3D). This finding suggests a shift towards a higher reliance on carbohydrate metabolism due to more food intake during the active phase. Moreover, when comparing the RER values between the PolgA and WT mice under both light and dark conditions, we observed a statistically significant distinction in which PolgA mice displayed elevated RER values compared to WT mice, suggesting distinct metabolic profiles between the two mouse groups during different day and night-time of the light-dark cycle. Notably, the PolgA mice show higher RER than the WT mice, signifying a shift in their fuel substrate utilisation towards a higher reliance on carbohydrates (approaching 1.0) and reduced reliance on fats as their predominant energy sources. Next, we employed a harmonic regression model to analyse the daily rhythm of the RER, as illustrated in (Figure 4A). In constructing the model, we used the average data from the last 48 hours of measurements, ensuring a robust representation of the cyclic pattern. The model analysis provided valuable insights into the temporal patterns of RER, showing that female PolgA mice displayed a delayed peak and peak-to-trough ratio compared to male PolgA and female WT mice (Figure 4B). Additionally, the RER showed daily rhythmicity, contributing to the synchronised metabolic processes occurring within the 24-hour cycle. Additionally, we calculated energy expenditure during the night using the Weir equation and found that PolgA mice spend (female=6908.504, male=6529.177 Kcal/d) energy, whereas WT mice spend (female=6968.872, male=6403.492 Kcal is/d) energy. Our observation shows that female mice in WT and PolgA have higher energy expenditure than their male counterparts.

Daily fluctuations in food consumption, water intake, and body weight

Next, we monitored these mice's water intake and feeding habits. Assessing the patterns of food and water consumption is crucial as these behaviours serve as vital biomarkers of the mice's overall well-being. We examined the feeding habits of these mice. Both male and female PolgA mice tended to consume more food than the WT mice at night (Figure 5A, Figure 5B).

In contrast, male and female PolgA mice displayed similar water intake patterns (Figure 5C). Subsequently, In the next phase of our investigation, we turned our attention to the body weight of the mice, recognizing it as a crucial metabolic parameter that can have significant implications for ageing and overall health (St-Onge 2005; Heilbronn et al. 2005). We observed a notable reduction in the body weight of the PolgA mice in comparison to the WT mice, as illustrated in Figure 5D.

Daily locomotor activity

Investigating the locomotor activity of these mice during both day and night-time is crucial in comprehending their complete activity patterns and potential variations in their behaviour. Monitoring total movement is particularly important in the ageing process, as it fosters functional independence, maintains muscle mass and strength, and enhances cardiovascular health. In metabolic phenotyping cages, the total movement was recorded and detected through horizontal walking with beam breaks in the x and y directions. As expected, locomotor activity is elevated in the night-time of the light-dark cycle. Our findings revealed that female WT and PolgA mice show significantly higher total locomotor activity than male mice (Figure 6A, 6B) in the night-time of the light-dark cycle. Nevertheless, female PolgA mice showed reduced walking distance compared to WT mice in the light-dark cycle (Figure 6C). Furthermore, the daily variations in walking speed in the PolgA mice show a substantially reduced walking speed compared to their WT counterparts (Figure 6D).

Grip strength

Next, we assessed the grip strength of the mice, as it is a valuable measure for evaluating muscle strength. Grip strength is an essential indicator of disability, morbidity, and mortality (Bellantuono et al. 2020). We performed the grip strength at 41–42-week-old PolgA and WT mice. Our analysis revealed decreased grip strength in the PolgA compared to the WT mice (Figure 6E). The observed decrease in grip strength in the female PolgA mice suggests a more pronounced premature ageing phenotype than the WT mice.

Discussion

Ageing is associated with complex phenotypical and functional changes affecting different organs, skeletal muscle strength, energy balance, and physiological activity across the organism's life span (Heilbronn et al., 2005; Rondanelli et al., 2016; Solomon et al., 2008). In recent years, ageing research has become a focal point of scientific investigation, with a particular emphasis on gaining a deeper understanding of the intricate interplay between chronological and biological ageing phenotypes. Longitudinal studies are vital for investigating ageing and pinpointing risk factors linked to age-related diseases. These studies are meticulously conducted within controlled environments to guarantee the accuracy and reliability of the findings without experimental bias across different laboratories (Chen et al., 2022; Evans et al., 2021; Santin et al., 2021). In this conjuncture, we monitored oxygen consumption, carbon dioxide release, food intake, and spontaneous locomotor activity in 41–42-week-old PolgA and WT mice.

Our results indicated an elevation in VO2, RER, and VCO2 levels at the night-time of the light-dark cycle (Figure 3B-D). This is consistent with the fact that mice and rats are nocturnal animals and are most active at night-time (Hawkins and Golledge, 2018). On the other hand, circadian studies on humans or fungi show diurnal activities and are most active during the day-time (Duffy and Czeisler, 2009; Singh et al., 2022). We found a slight increase in the average RER mean value during the night-time in PolgA (female 0.90, male 0.92) due to increased food intake in comparison to WT mice (female 0.88, male 0.89) (Figure 3D). In the context of ageing, a previous study conducted with male mice unveiled a significant decrease in their RER as they progressed in age. This decline signifies a transition towards a greater emphasis on fat metabolism (Petr et al., 2021). An alternate study revealed that elderly mice exhibited a reduced RER compared to their younger counterparts, suggesting that as mice age, there is a corresponding increase in the proportion of fat oxidation (Houtkooper et al., 2011). Another study found that, under resting conditions, RER remained comparable between older and younger individuals (Grevendonk et al., 2021). It is important to note that RER is just one of many ageing biomarkers that can be used to study biological ageing. RER is influenced by many different factors, such as diet type (Spriet and Peters, 1998; Carey et al., 2001), food intake (Bergman and Brooks, 1999), diet restriction (Keim and Horn, 2004), extreme ambient temperature (Layden et al., 2002), and energy balance (Weyer et al., 1999). In our study we found that PolgA mice show higher food intake (Figure 5B) compared to their WT counterparts. One of the key nutrients they might be consuming more of is carbohydrates. When carbohydrates are consumed in excess, the body has more readily available energy substrates, leading to increased utilization of carbohydrates as a fuel source. This increased food intake could contribute to the higher RER observed in PolgA mice. Further, we observed a notch (sharp decline, followed by an increase) in RER in the late active phase in WT mice due to a sharp decrease in food intake (Figure 5A). In our present study, we did not explore different parameters that influenced the RER index linked to ageing, such as total oxidative metabolism assessment, the association of the RER to physical fitness or endurance, fasting under a controlled temperature, etc. Furthermore, we did not conduct experiments such as constant-dark measurements or feeding-fasting cycles, which could have provided valuable insights into this behaviour within the context of circadian clock regulation. Previous research has demonstrated that food and water intake is influenced more by strain rather than sex differences (König et al., 2020). We observed that PolgA mice, regardless of sex, displayed a higher propensity for increased food consumption than WT mice (Figure 5A, Figure 5B). A rise in feeding patterns among mice could show shifts in appetite regulation, metabolic shifts, or age-related physiological transformations. Ageing can influence the control of appetite, energy disbursement, and the use of nutrients. These changes may increase feeding habits, where mice consume more food. Further, increased feeding habits might be due to the PolgA mice showing osteosarcopenia (Scheuren et al., 2020a), and sarcopenia can induce obesity, diabetes, and other metabolic-associated diseases (Yanai, 2015). Next, we examined the water consumption patterns of these mice, as maintaining adequate hydration can significantly impact bodily functions such

as digestion, temperature regulation, and cognitive performance (Jequier and Constant, 2010; Schlanger et al., 2010). Our results reveal comparable water intake levels in both PolgA and WT mice (Figure 5C). Next, a decrease in body weight was observed in female PolgA mice compared to their WT counterparts (Figure 5D). Both humans and mice commonly experience a decline in body weight with age (Seidell and Visscher, 2000). The reduction in body weight is predominantly attributed to a decrease in lean muscle tissue rather than a fat mass loss. Since muscle is denser than fat, substituting muscle with fat plays a significant role in the overall decrease in body weight. Strikingly, previous studies have demonstrated the presence of osteosarcopenia in aged PolgA mice (Scheuren et al., 2020a), indicating that physical dysfunction and age-related complications may be contributing factors to the weight loss in these mice during the ageing process. Our findings emphasize the significance of body weight as an essential metabolic parameter to consider when studying the effects of ageing and potential genetic influences. We evaluate the locomotor activities of these mice, as the investigation into age-related alterations in memory, cognitive functions, and motor abilities has been extensively studied in prior research (Forster et al., 1996; Marshall and Berrios, 1979; Gunning-Dixon and Raz, 2003; Ownby, 2010). Investigations have shown decreased locomotory behaviour and overall activity in ageing populations (Fahlström et al., 2011; Shoji et al., 2016; Baker and Tang, 2010). These changes are often accompanied by underlying physiological shifts, including sarcopenia or dynapenia in skeletal muscles (Mitchell et al., 2012; Scheuren et al., 2020a,b).

Our analysis unveiled a notable sex difference in total movement, with female WT mice exhibiting higher locomotor activity than their male counterparts (Figure 6A, Figure 6B). However, in contrast, female PolgA mice demonstrated diminished walking distance compared to the WT group (Figure 6C) and walking speed (Figure 6D). These findings are consistent with previous reports that suggest a decline in locomotor activity in aged animals. Further, we observed that female WT and PolgA exhibit higher energy expenditure than their male counterparts, with female WT demonstrating more energy expenditure than PolgA mice. This outcome implies a tendency for walking speed to diminish in ageing PolgA mice compared to WT mice, aligning with earlier research that demonstrated age-related declines in walking speed (Weber, 2016). Our analysis also revealed a daily rhythmic pattern in total movement among these mice (Figure 6A, Supplementary Figure S1) as well as the walking speed (Supplementary Figure S2).

Grip strength is frequently referred to as a biomarker of aging, indicative of various factors linked to the decline in strength commonly observed with age. These factors encompass alterations in muscle structure and composition due to aging, leading to reductions in muscle fiber size and density. Our study showed diminished grip strength in female PolgA mice compared to the wild-type counterparts (Figure 6E). The reduced grip strength observed in PolgA mice corresponds with their decreased body weight, as indicated in Figure 5D. It's worth noting that weight loss or reduction in body fat can potentially impact grip strength.

Given this context, the current findings suggest that female PolgA mice have reached a point where their mobility becomes compromised. Taken together, all our results indicate that PolgA

mice exhibit an accelerated ageing phenotype at 41-42 weeks compared to their WT counterparts of the same age. Further, female PolgA mice exhibit a more noticeable ageing compared to their male counterparts. Therefore, PolgA mice can be used as a model to study ageing-related research work efficiently in the future. The home-cage-like platform can be replicated to characterize behaviour, physiology, and intervention testing in different laboratory model organisms. Such study design aligns with Directive 2010/63/EU and the 3R principles (replacement, reduction, refinement). One of the limitations of the present study is that the precise mechanism underlying the ageing effect through metabolic parameters was not explored, and further detailed studies are needed to explore the signalling molecules involved in the metabolic effects. Additionally, the finding relies on metabolic cage measurements of oxygen consumption, carbon dioxide release, locomotor activity, and feeding behaviour in freely moving animals. Therefore, further experiments will be required to explore how ageing mice impact the phase resetting of circadian clocks by changing the light-dark cycle and feeding patterns.

Materials and Methods

Animals

The local authority approved all animal experiments (Veterinäramt des Kantons Zürich, License Nr ZH35/2019 A colony of the mouse strain expressing an exonuclease deficient version of the mitochondrial DNA polymerase γ (PolgAD257A, B6.129S7(Cg)-Polgtm1Prol/J, JAX stock 017341, The Jackson Laboratory) was bred and maintained at the ETH Phenomics Center (12h:12h light-dark cycle, maintenance food, and water ad libitum, 3-5 animals/cage). The “Kliba Nafag 3437” (Kliba Nafag, Kaiseraugst, Switzerland) maintenance diet was employed consistently throughout the study. Eight mice per sex PolgA and WT were selected for the experiment. Animals had a general health check before the experiment.

Metabolic cage and Data collection

At the ETH Phenomics Center, the PhenoMaster system (TSE PhenoMaster, Bad Homburg, Germany) was used to examine the metabolic and behavioural characteristics of PolgA and WT mice. Each mouse was kept in a single house in a cage with ad libitum access to food and water for four days. The PhenoMaster system consists of two environmental chambers, each capable of accommodating up to eight cages and monitoring 16 mice in parallel. Each cage records physiological and behavioural measurements of parameters through sensor systems, creating a stress-free home cage environment. A calorimetry system measures carbon dioxide production and oxygen consumption in cages. The carbon dioxide and oxygen sensors were calibrated with calibration gas mixtures following the manufacturer’s recommendations.

Additionally, the cages contain weight and feeding basket sensors to measure body weight and food intake accurately. The TSE system is equipped with a multi-dimensional infrared beam system that provides real-time measurement of locomotor activity in a 3D direction. The following crucial parameters related to metabolic, physiological, and behavioural parameters are

recorded: i) Drinking and feeding amount, ii) Walking speed, all movement, and locomotor activities (Total and walking distance), iii) Metabolic performance VO2 consumption, VCO2 production, and RER at 10 min intervals for four days. The experiment was performed in WT (male and female) and PolgA mice (male and female) with a sample size of eight individuals per sex. All recorded parameters are provided in Supplementary Table 1.

Forelimb grip strength

The study assessed the forelimb grip strength of PolgA and their WT littermates at 41-42 weeks-old mice using a force tension apparatus (Grip Strength Meter, model 47200, Ugo Basile). Mice were positioned horizontally on a grid and pulled from their tails until they lost their grip. The force exerted was recorded, and the test was repeated five times for each mouse. The average force value (gram force) of the best three trials was used for analysis.

Data analysis

Each experimental run was treated as an independent event, and the average values of each feature over time were calculated for each mouse (e.g., WT male/female or PolgA male/female). The first 48 hours of data were excluded from the analysis to mitigate potential stress from acclimatisation to the metabolic cage. The last 48 hours of measurements were consolidated into a single light-dark cycle by computing the average within a 24-hour window and used for the visualization. All measured metabolic and locomotory parameters are reported in Supplementary Table 1. The data were further smoothed using Locally Estimated Scatterplot Smoothing (LOESS). Comparative analyses were conducted using a t-test, such as comparing males to females in the WT or males to females in the PolgA mice in the light-dark cycle. The effect size was assessed using Cohen's d calculation. Further, multiple comparisons and *p*-values were corrected using the Bonferroni procedure. The effect size interpretation of the conventional t-test proposed by Cohen's d is 0.2 (small effect), 0.5 (moderate effect), and 0.8 (larger effect). A value greater than 0.5 suggests a greater effect in our analysis. All statistical result, such as *p*-value and Cohen's d, are reported in the figures. The basic formula for calculating energy expenditure is typically expressed in terms of oxygen consumption (VO2) and carbon dioxide production (VCO2). Here, we used the Weir equation to find the expenditure EE=(3.941×VO2) +(1.106×VCO2) kcal/d. We used the dark period time (12h) average value of VO2 and VCO2 for this calculation.

To study the daily rhythmic behaviour, we fit the following harmonic function to the RER data of the last 48 hours of measurement to estimate the values of the parameters β_0, β_k, α_k. By analyzing the fitted sine curve, we calculated the periodic length of the daily rhythm, corresponding to the duration of one complete oscillation cycle.

$$[y(t) = \beta_0 + \beta_k \cos(2\pi kt) + \alpha_k \sin(2\pi kt)]$$

Where y(t) is the response variable at time t, β_0 is the intercept term, β_k are the coefficients for the cosine terms, α_k are the coefficients for the sine terms, and $2\pi kt$ is the angular frequency (w) of the harmonic terms. Given the consistent and smooth nature of the RER data (VCO2/VO2), a harmonic function was applied to analyse the data. Meaningful conclusions were drawn based on the RER data, such as the periodic length of the daily rhythm and the peak in the night-time. Data input, cleaning, and statistical analysis were conducted using the R programming language. Further, MATLAB was used for data smoothing, fitting the harmonic model, and generating visualizations.

Figure legend

Figure 1. Experimental study design.
(A) The study used PolgA and WT mice, with a sample size of 8 males and females per group. (B) The experimental setup included using the PhenoMaster system and an environmental chamber for automated phenotyping, which was captured in pictures. (C) The experimental measurement time for each animal was monitored for four days and nights. (D) Data were collected for each animal, where each row represented the time, and each column represented a specific measurement. (E) The collected data underwent statistical data analysis.

Figure 2. Daily rhythms in oxygen consumption and carbon dioxide release.

(A) The study recorded carbon dioxide release. (B) Oxygen consumption using metabolic cages for four days, as described in the methods section. The solid line (blue) is the average of eight mice recorded at 10-minute intervals, while the blue shaded areas represent the standard deviation (SD). The solid line (red) is the Loess smoothing in the left panel for PolgA mice. The right panel showed the results for the WT mice. The yellow vertical boxes indicated the 12-hour day-time, while the grey areas corresponded to the 12-hour night-time.

Figure 3. Daily rhythms in respiratory exchange ratio.

(A) RER measurements from the metabolic cage: the solid blue line represents the average value of eight mice, and the blue shaded areas correspond to the standard deviation (SD). The solid red line in the left panel indicates Loess smoothing for PolgA mice. The right panel displays results for WT mice, with the solid blue line representing the average of eight mice and the solid red line representing Loess smoothing and red-shaded areas corresponding to the standard deviation (SD). Yellow vertical boxes indicate the 12-hour day-time, while the grey areas correspond to the 12-hour night-time. (B) The box plot of VCO2 in the light-dark cycle. (C) The box plot of VO2 during the light-dark cycle. (D) The box plot represents the total measurement of RER. (B-D) t-tests were performed to compare PolgA and WT mice in light and dark conditions, with the left panel corresponding to females and the right panel corresponding to males. Yellow vertical boxes mark the day-time period, and the gray vertical boxes represent the dark period. The figure provides the p-value and Cohen’s d-value for each comparison test.

Figure 4. The harmonic regression models.

(A) The harmonic regression model analysis was applied to RER data. The solid red line represents the model prediction, and the blue dotted line represents experimental data. The left panel of the graph shows the results for the PolgA mice, while the right panel shows the results for the WT mice. Yellow shading in the graph represents the daytime, and grey shading in the graph represents the nighttime. The model coefficients (parameters) are displayed on the plot. (B) The fitted model is represented by the blue line for PolgA and the red line for WT females (left) and males (right).

Figure 5. Body weight, water, and food intake.

(A) The food intake during the light-dark cycle in PolgA mice and WT mice. The cumulative food intake difference of WT mice during the light-dark cycle. The term 'no food intake' is represented by the red circle during the time intervals between 20-22h. (B) The box plot represents food intake in the light-dark cycle. (C) The box plot represents drinking water intake in the light-dark cycle. (D) Body weight of PolgA and WT mice. (B-D) t-tests were conducted to compare PolgA and WT mice in light and dark conditions. The left panel corresponds to females, and the right panel corresponds to males. The daytime period is marked by yellow vertical boxes, and the grey vertical boxes represent the dark period. The figure provides the p-value and Cohen’s d-value for each comparison test.

Figure 6. Daily rhythms in locomotor activity and grip strength measurement.

(A) The daily rhythm of the total movement of both PolgA and WT mice. The solid line (blue) averages eight mice recorded at 10-minute intervals. The solid line (red) is the Loess smoothing in the left panel for PolgA mice, and the right panel is for WT mice. (B) The box plot illustrates total movements in the x and y direction of the metabolic cage. (C) The box plot displays the total walking distance during the light-dark cycle. (D) The box plot displays the walking speed during the light-dark cycle. (B-D) t-tests were conducted to compare PolgA and WT mice. The left panel corresponds to females and the right one to males. The figure provides the p-value and Cohen’s d-value for each comparison test. The grey vertical boxes represent the dark period. The figure provides the p-value and Cohen’s d-value for each comparison test. (E) Grip strength comparison between PolgA and WT mice.

Supplementary Figure S1: Daily cycle of locomotor activity of PolgA and WT mice.

The data depict the total walking movements of PolgA (left panel) and WT (right panel) mice, and the measurements were recorded at 10-minute intervals over four consecutive days and nights.

Supplementary Figure S2: Daily cycle of walking speed.

The data illustrate the walking speed of PolgA mice (left panel) and WT mice (right panel) measured in the metabolic cage over four consecutive days and nights.

Supplementary Table 1: The table contains the mean and standard deviation of metabolic and locomotory parameters of PolgA and WT mice.

Author Contributions

A.S., G.K. and R.M. contributed to the design of the study. A.S. wrote the paper and performed data analysis. D.Y. analyzed the grip strength. All the authors revised the manuscript collaboratively, and they unanimously approved the final version for submission.

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Conflict of interest

The authors declare that they have no conflict of interest.

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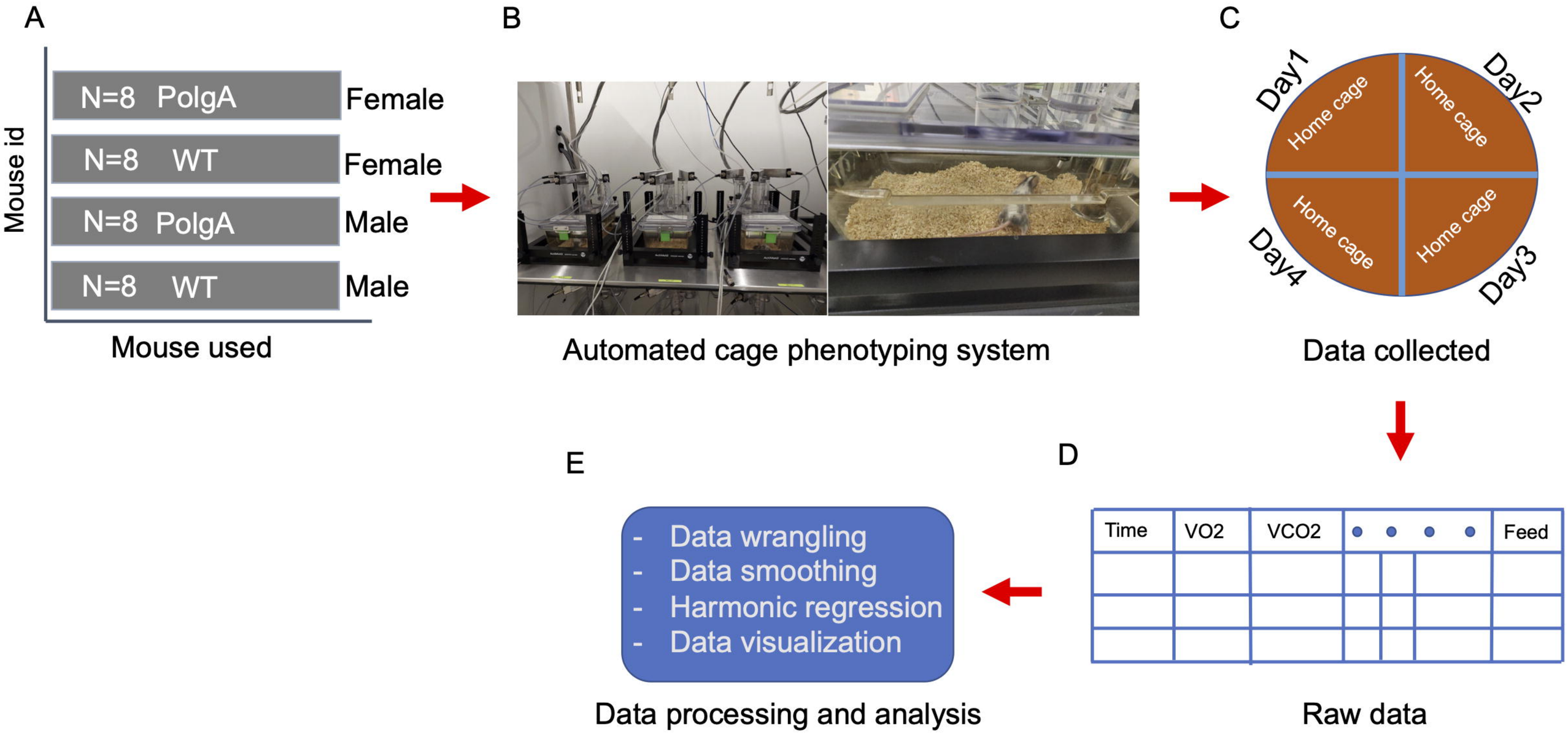
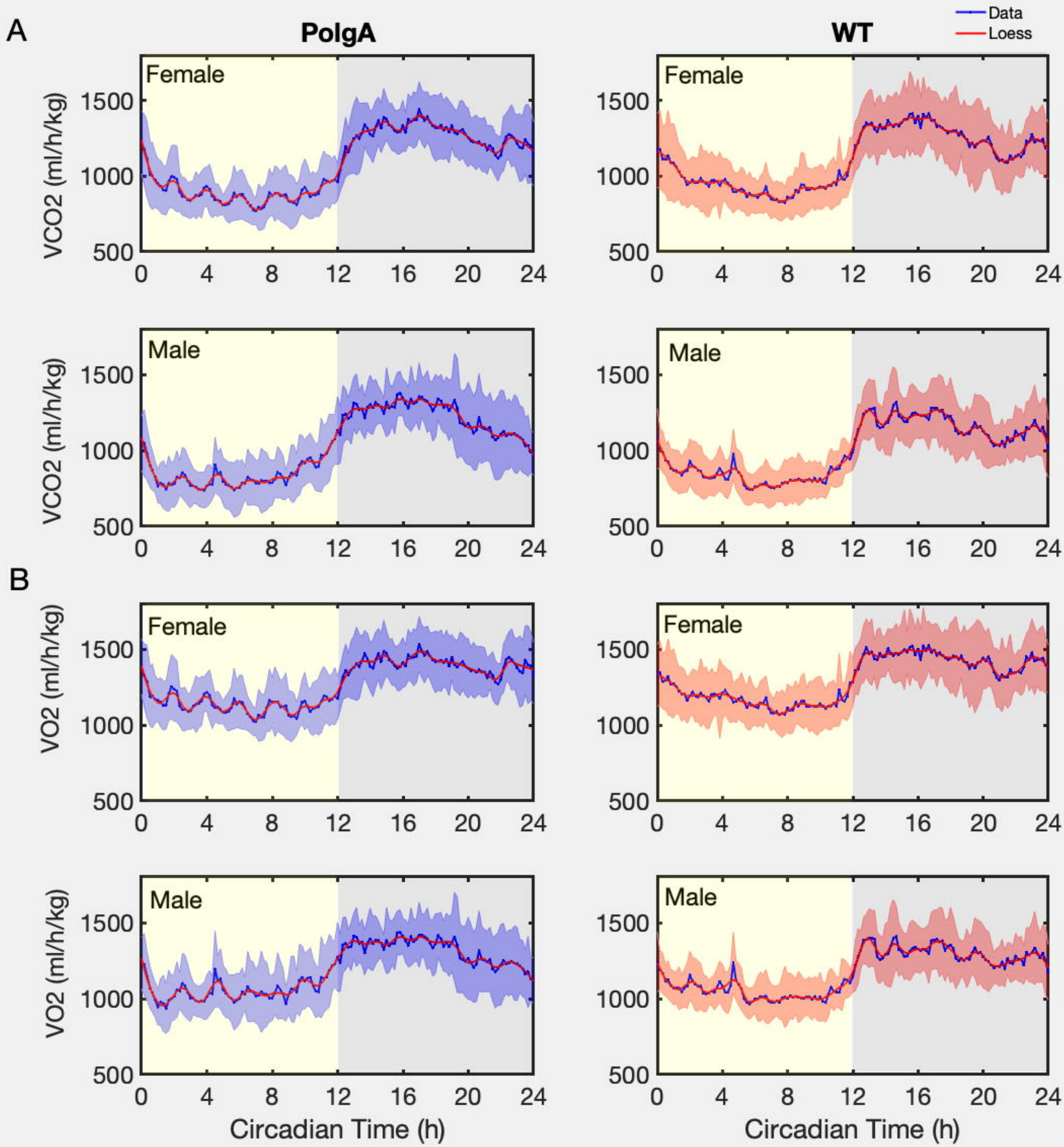
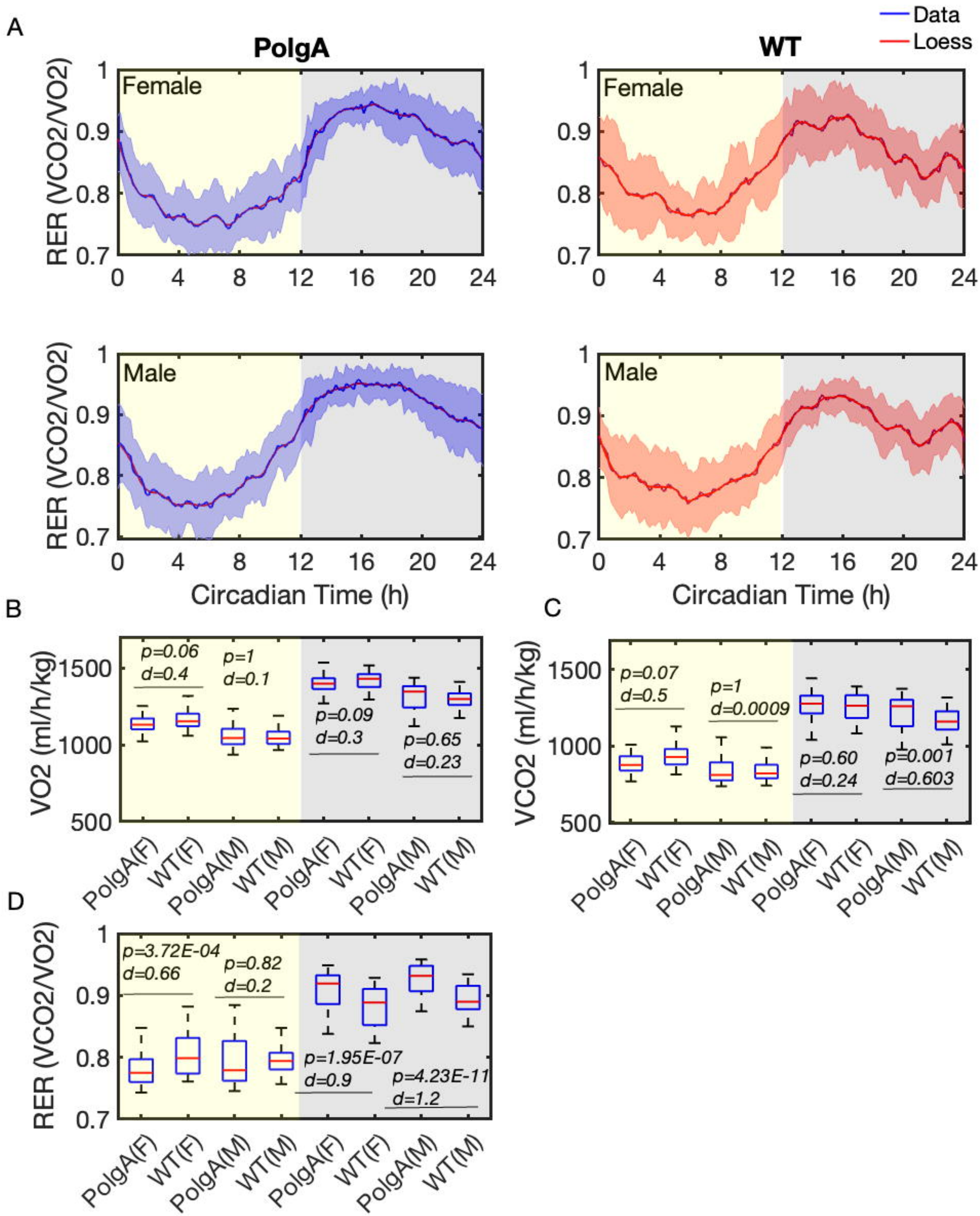
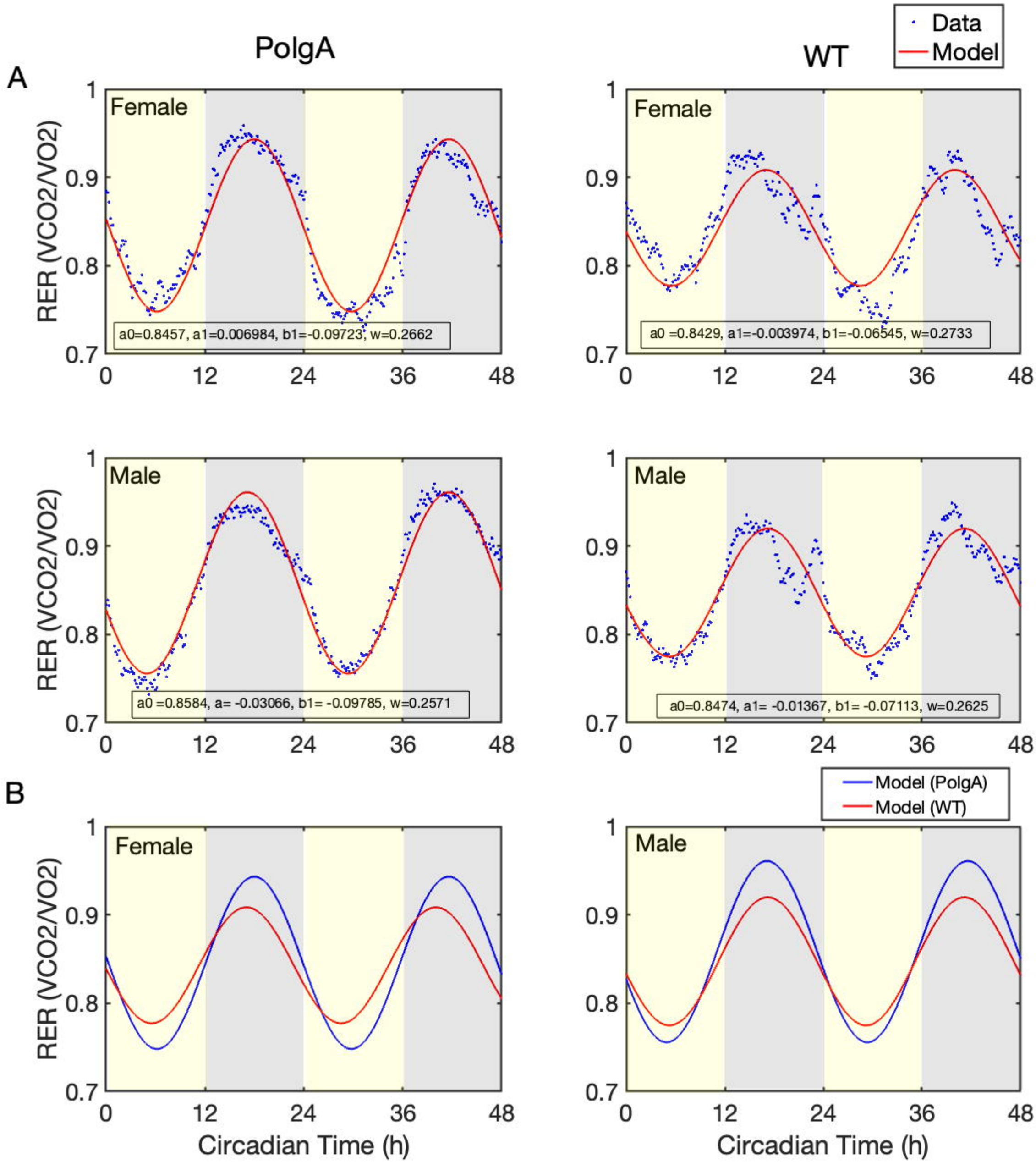
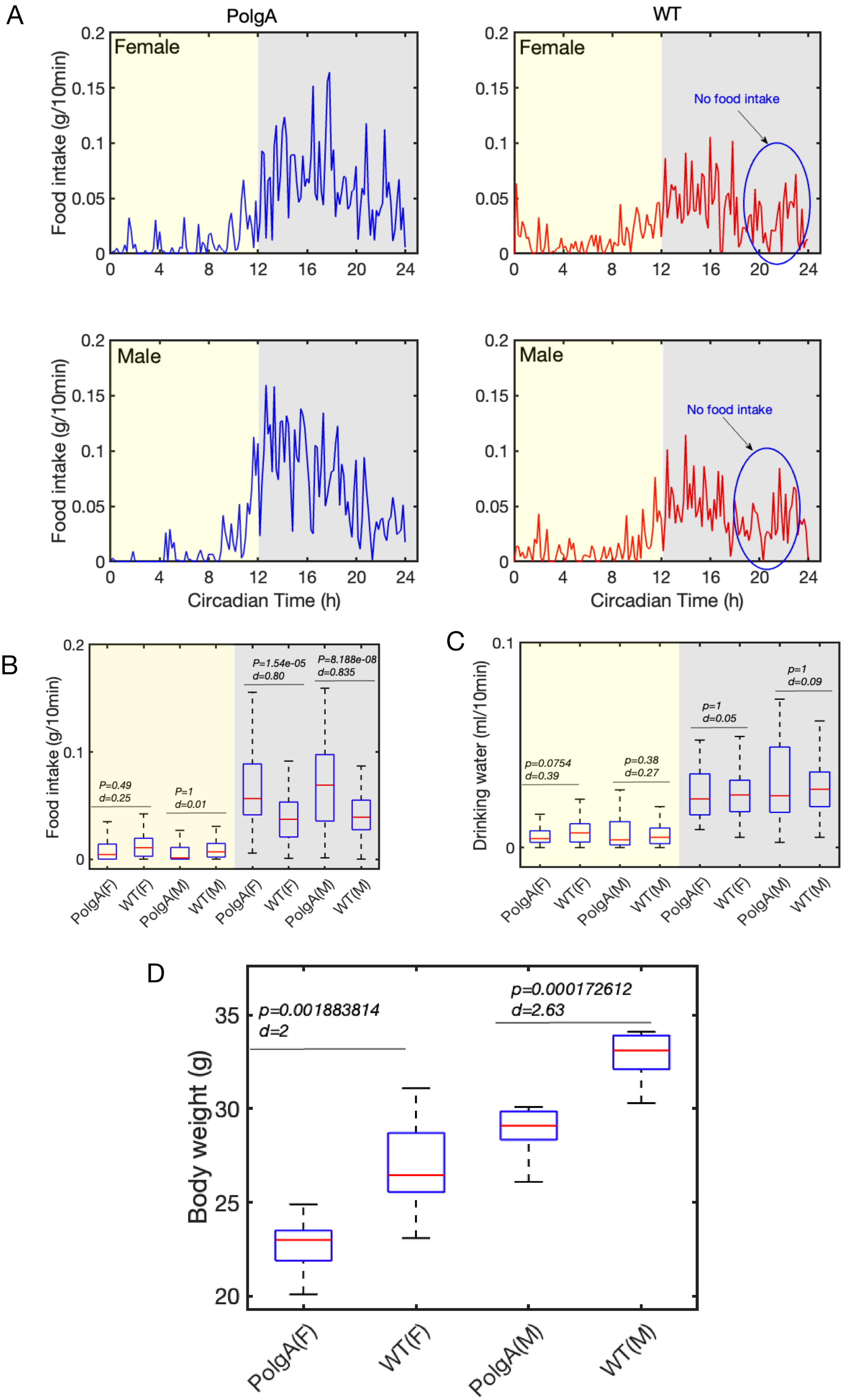
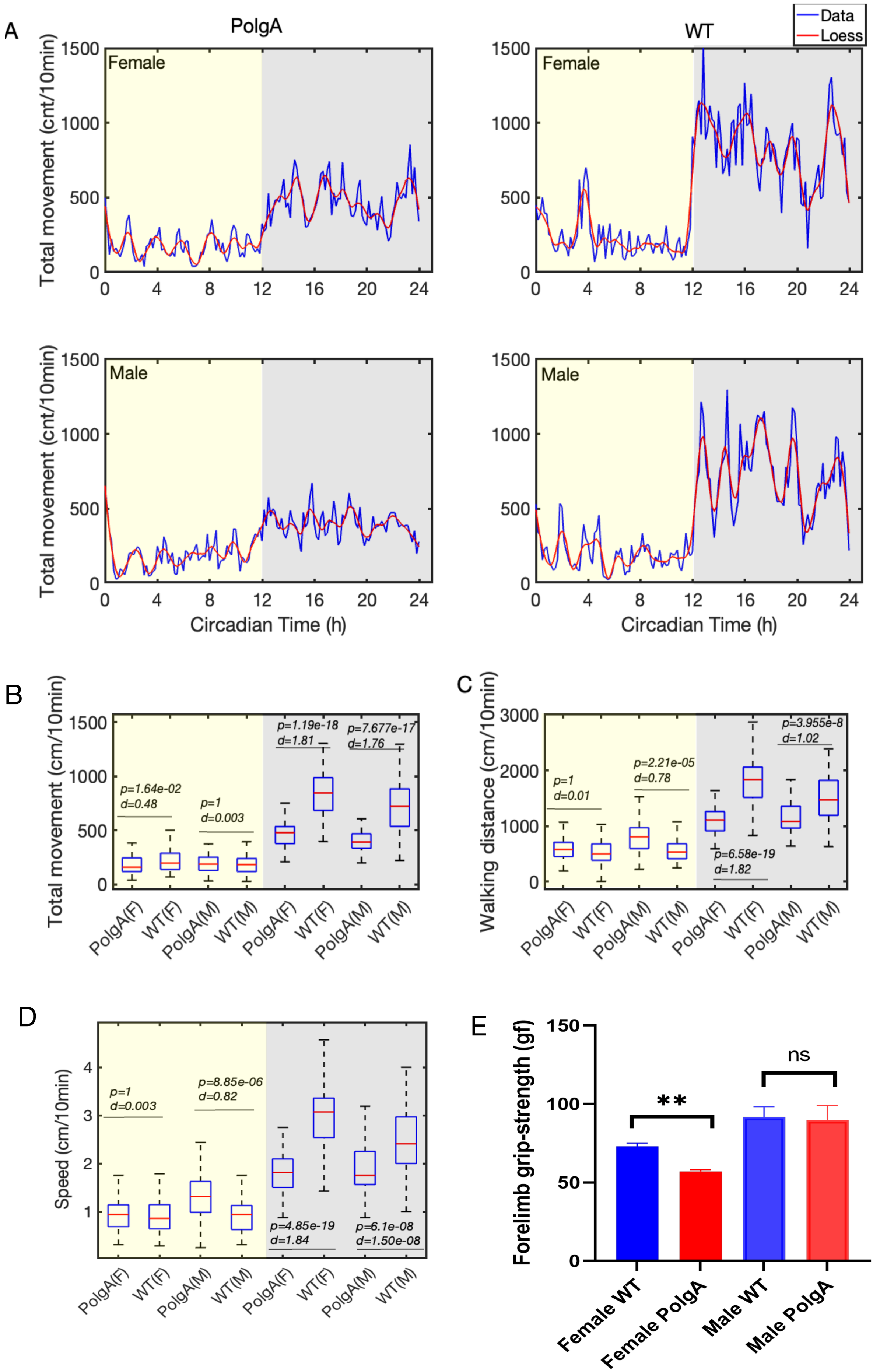

A
Mouse id
N=8 PolgA
Female
N=8 WT
Female
N=8 PolgA
Male
N=8 WT
Male
Mouse used
B
Automated cage phenotyping system
C
Day1
Day2
Day3
Day4
Home cage
Home cage
Home cage
Home cage
Data collected
D
Time
VO2
VCO2
Feed
Raw data
E
- Data wrangling
- Data smoothing
- Harmonic regression
- Data visualization
Data processing and analysis


A
PolgA
WT
Data
Loess
Female
Male
VCO2 (ml/h/kg)
B
VO2 (ml/h/kg)
1500
1000
500
0
4
8
12
16
20
24
Circadian Time (h)


A
PolgA
WT
Data
Loess
Female
Male
RER (VCO2/VO2)
Circadian Time (h)
B
VO2 (ml/h/kg)
p=0.06
d=0.4
p=1
d=0.1
p=0.09
d=0.3
p=0.65
d=0.23
C
VCO2 (ml/h/kg)
p=0.07
d=0.5
p=1
d=0.0009
p=0.60
d=0.24
p=0.001
d=0.603
D
p=3.72E-04
d=0.66
p=0.82
d=0.2
p=1.95E-07
d=0.9
p=4.23E-11
d=1.2
PolgA(F)
WT(F)
PolgA(M)
WT(M)


A
PolgA
WT
Data
Model
Female
Male
RER (VCO2/VO2)
a0=0.8457, a1=0.006984, b1=-0.09723, w=0.2662
a0 =0.8429, a1=-0.003974, b1=-0.06545, w=0.2733
a0 =0.8584, a= -0.03066, b1= -0.09785, w=0.2571
a0=0.8474, a1= -0.01367, b1= -0.07113, w=0.2625
B
Model (PolgA)
Model (WT)
Circadian Time (h)


A
PolgA
WT
Female
Male
No food intake
Food intake (g/10min)
Circadian Time (h)
B
P=0.49
d=0.25
P=1
d=0.01
P=1.54e-05
d=0.80
P=8.188e-08
d=0.835
C
Drinking water (ml/10min)
p=0.0754
d=0.39
p=0.38
d=0.27
p=1
d=0.05
p=1
d=0.09
D
Body weight (g)
p=0.001883814
d=2
p=0.000172612
d=2.63
PolgA(F)
WT(F)
PolgA(M)
WT(M)


A
PolgA
WT
Data
Loess
Female
Male
Total movement (cnt/10min)
Circadian Time (h)
B
Total movement (cm/10min)
p=1.64e-02
d=0.48
p=1
d=0.003
p=1.19e-18
d=1.81
p=7.677e-17
d=1.76
C
Walking distance (cm/10min)
p=1
d=0.01
p=2.21e-05
d=0.78
p=6.58e-19
d=1.82
p=3.955e-8
d=1.02
PolgA(F)
WT(F)
PolgA(M)
WT(M)
D
Speed (cm/10min)
p=1
d=0.003
p=8.85e-06
d=0.82
p=4.85e-19
d=1.84
p=6.1e-08
d=1.50e-08
E
Forelimb grip-strength (gf)
**
ns
Female WT
Female PolgA
Male WT
Male PolgA