ORIGINAL ARTICLE

Endothelial specific prolyl hydroxylase domain-containing protein 2 deficiency attenuates aging-related obesity and exercise intolerance

Lihong Pan · Xiaochen He · Rui Xu · Umesh Bhattarai · Ziru Niu · Jussara do Carmo · Yuxiang Sun · Heng Zeng · John S. Clemmer · Jian-Xiong Chen · Yingjie Chen

Abstract Obesity and exercise intolerance greatly reduce the life quality of older people. Prolyl hydroxylase domain-containing protein 2 (PHD2) is an important enzyme in modulating hypoxia-inducible factor-alpha (HIF) protein. Using vascular endothelial cell-specific PHD2 gene knockout (PHD2 ECKO) mice, we investigated the role of endothelial PHD2 in aging-related obesity and exercise capacity. Briefly, PHD2 ECKO mice were obtained by crossing PHD2-floxed mice with VE-Cadherin (Cdh5)-Cre transgenic mice. The effect of PHD2 ECKO on obesity and exercise capacity in PHD2 ECKO mice and control PHD2$^{f/f}$ mice were determined in young mice (6 to 7 months) and aged mice (16–18 months). We found that aged PHD2 ECKO mice, but not young mice, exhibited a lean phenotype, characterized by lower fat mass, and its ratio to lean weight, body weight, or tibial length, while their food uptake was not reduced compared with controls. Moreover, as compared with aged control mice, aged PHD2 ECKO mice exhibited increased oxygen consumption at rest and during exercise, and the maximum rate of oxygen consumption (VO$_2$ max) during exercise. Furthermore, as compared with corresponding control mice, both young and aged PHD2 ECKO mice demonstrated improved glucose tolerance and lower insulin resistance. Together, these data demonstrate that inhibition of vascular endothelial PHD2 signaling significantly attenuates aging-related obesity, exercise intolerance, and glucose intolerance.

Lihong Pan and Xiaochen He are contributed equally to this work.

L. Pan · X. He · R. Xu · U. Bhattarai · Z. Niu · J. do Carmo · J. Clemmer · Y. Chen (✉)
Department of Physiology and Biophysics, University of Mississippi Medical Center, School of Medicine, 2500 North State Street, Jackson, MS 39216, USA
e-mail: ychen2@umc.edu

Y. Sun
Department of Nutrition, Texas A&M University, College Station, TX, USA

H. Zeng · J.-X. Chen
Department of Pharmacology and Toxicology, University of Mississippi Medical Center, School of Medicine, Jackson, MS 39216, USA

Introduction

Obesity and reduced exercise capacity or mobility disorder are major medical problems that greatly decrease the life quality of older populations. Obesity, reduced exercise capacity, and metabolic dysfunction also contribute to the development of various diseases during the aging process [1, 2].

Prolyl hydroxylase domain-containing protein 2 (PHD2), encoded by the EGLN1 gene, is a key enzyme responsible for mediating hypoxia-inducible factors (HIFs) that respond to decreases in oxygen bioavailability in the cellular environment. HIFs are heterodimeric proteins consisting of an O2-sensitive HIF-1α, HIF-2α, or HIF-3α subunit and a constitutively expressed HIF-1β subunit. PHD2 knockdown or inhibition is known to induce HIF activity in most cell types [3, 4]. Hypoxia, a condition characterized by reduced oxygen levels, has been shown to decrease body weight [5–7]. Although the mechanism behind hypoxia-induced suppression of fatty acid synthesis and reduction in fat mass is not fully understood [8, 9], the role of the oxygen-sensing pathway in metabolism has garnered considerable attention. This pathway involves both HIFs and PHDs [10]. PHDs catalyze the oxygen-dependent hydroxylation of specific proline residues in HIF-1α, HIF-2α, or HIF-3α, leading to its degradation. Hypoxia diminishes PHD activity, resulting in increased HIF expression and the activation of target genes involved in metabolism [11]. HIF-α has been implicated in the attenuation of obesity, fatty liver disease, and type 2 diabetes [12, 13], as well as in regulating energy balance and metabolism in the hypothalamus [14–17]. Furthermore, loss of skeletal muscle HIF-1α has resulted in metabolic shift away from glycolysis and toward oxidation, and increased sensitivity to exercise-induced muscle injury in mice [18]. Moreover, studies have demonstrated that PHD2 plays a critical role in diet-induced obesity and glucose intolerance [19]. While HIF may hold therapeutic potential, direct manipulation of HIF in vivo is challenging. In contrast, PHDs represent an ideal target for modulating HIF levels, and several PHD inhibitors have been developed.

In this study, we investigated the effects of endothelial-specific PHD2 deficiency on obesity and exercise tolerance in young adult and aged mice. We hypothesized that the deletion of endothelial PHD2 will alleviate obesity and enhance exercise capacity in aged mice.

Materials and methods

Animals

The experimental procedures conducted in this study adhered to the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The research protocol was approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center. Vascular endothelial cell-specific PHD2 knockout mice (**PHD2 ECKO**) mice were generated using PHD2 flox/flox (PHD2$^{f/f}$) mice [20] and VE-Cadherin (Cdh5)-Cre transgenic mice (Jackson Lab, # Strain #:**006137**) [21] as previously described [22]. PHD2$^{f/f}$ mice were used as the control mice for PHD2 ECKO mice. Young male PHD2 ECKO mice (n = 9), young male PHD2$^{f/f}$ mice (n = 9), aged male PHD2 ECKO mice (n = 19), and aged male PHD2$^{f/f}$ mice (n = 13) were used for the study. Mice were fed ad libitum with normal chow throughout their lives, and experimental tests were conducted in mice at young mice (6 to 7 months), and aged mice (16 to 18 months). Samples were collected after intraperitoneal injection of 100 mg/kg Ketamine and 10 mg/kg Xylazine.

Glucose tolerance test and insulin tolerance test

Mice were fasted for 6-h prior to the glucose tolerance test as previously described [23]. After baseline glucose measurement, the mice were intraperitoneally injected with a bolus of glucose (1 mg/g of lean body weight). Blood glucose levels were measured 0, 15, 30, 60, 90 and 120 min after the injection. For the insulin tolerance test, the mice received an intraperitoneal injection of a bolus of insulin (0.75 IU/kg of lean body weight). Blood samples were collected from the tail vein and measured 0, 15, 30, 45, 60 and 90 min after the injection. blood glucose concentrations were measured using the Glutest Every kit (Sanwa Kagaku Kenkyusho, Japan).

Tissue weight and body composition analysis

The fat and lean masses content from PHD2 ECKO and control mice were assessed by using a magnetic resonance imaging system (EchoMRI-900TM, Echo Medical System, Houston, TX). Subsequently, the mice were euthanized and measurements of body weight, tibia length, white adipose tissue weight, and liver weight were taken.

Energy expenditure measurements in aged mice

Aged PHD2 knockout (KO) mice and PHD2f/f control mice were individually housed in metabolic cages (Promethion Metabolic and Behavioral System, Sable Systems International, Las Vegas, NV) to assess oxygen consumption, food intake, and energy expenditure (EE). These mice were allowed to acclimate to the new environment for 3 days before recording data for 3 consecutive days.

Food intake measurement in young mice

Daily food intake and body weight were also measured in young PHD2 ECKO and age-matched control mice over 3 days period.

Graded maximal exercise capacity test

Mice were acclimated on the exercise treadmill 1 time /day for 3 consecutive days then followed by a graded maximal exercise capacity test as previously described by Petrosino et al [24]. Briefly, the graded maximal exercise capacity test protocol was performed by placing the mice on the treadmill (Columbus Instruments, OH) at 5° inclination. The treadmill speed was then gradually increased as follows (speed, duration, inclination): 9 m/minute, 2 min, 5°; 12 m/minute, 2 min, 10°; 15 m/minute, 2 min, 15°; 18 m/minute, 1 min, 15°; 21 m/minute, 1 min, 15°; 23 m/minute, 1 min, 15°; and an increase in speed 1 m/minute for every minute thereafter. Exhaustion is defined as the point at which mice maintained continuous contact with the shock grid for 10 s without the intent of returning to the belt to continue to run. VO_2, VCO_2, ΔVO_2, respiratory exchange ratio (RER), and heat production were recorded every 30 s by the Oxymax software (Columbus Instruments, OH) and normalized to body weight. VO_2max was determined by the peak oxygen consumption reached during this test when RER was ≥ 1.0. The ΔVO_2 was determined by VO2max subtract by VO2 at rest.

Histological analysis

The adipose tissues and liver were fixed in a 10% neutral buffered formaldehyde solution overnight and subsequently embedded in paraffin. Paraffin sections were then subjected to hematoxylin and eosin (H&E) staining. Ten images of H&E staining sections were captured from each sample.

Statistical analysis

The results are presented as means ± SE. To evaluate significant differences between the two groups, an unpaired Student's t-test was employed. For comparisons between two groups over time, two-way ANOVA with repeated measures was conducted, followed by Bonferroni post hoc multiple comparisons. Statistical significance was determined at a level of $P < 0.05$. Additionally, three-way ANOVA tests were utilized to assess the effects of different variables (such as age and strain) and their interactions on body fat/lean mass, body weight, food intake, energy expenditure, and motor activity.

Results

PHD2 Deficiency in endothelial cells abolished age-related obesity in Mice

PHD2 ECKO mice showed a remarkable prevention of aging-related obesity, with no significant differences in body weight, fat mass, or fat mass ratios compared to control group in young mice. In contrast, aged control mice exhibited significant increases in body weight, fat mass, and the ratio of fat mass to bodyweight or lean mass (Fig. 1A-D). While PHD2 ECKO mice exhibited increased lean mass and lean mass ratios to tibial length in youth, there were no discernible differences in terms of lean mass ratios to body weight and fat mass ratios to lean mass (Fig. 1E-H). We also observed significantly increased lean mass ratios to body weight and fat mass ratios to lean mass (Fig. 1F, H). Furthermore, aged PHD2 ECKO mice had substantially reduced fat mass, smaller adipocytes, and lower white adipose tissue, emphasizing the role of PHD2 in age-related obesity prevention (Fig. 1I-L). Additionally, liver fat content and weight were lower in aged PHD2 ECKO mice (Fig. 1M-O). Overall, PHD2 deficiency demonstrated a pivotal role in mitigating age-related obesity. We also reconfirmed that PHD2 expression was reduced in vascular endothelial cells in PHD2 ECKO mice, while HIF1α protein expression was significantly increased in heart and lung tissues in PHD2 ECKO mice (Supplemental Fig. 1).
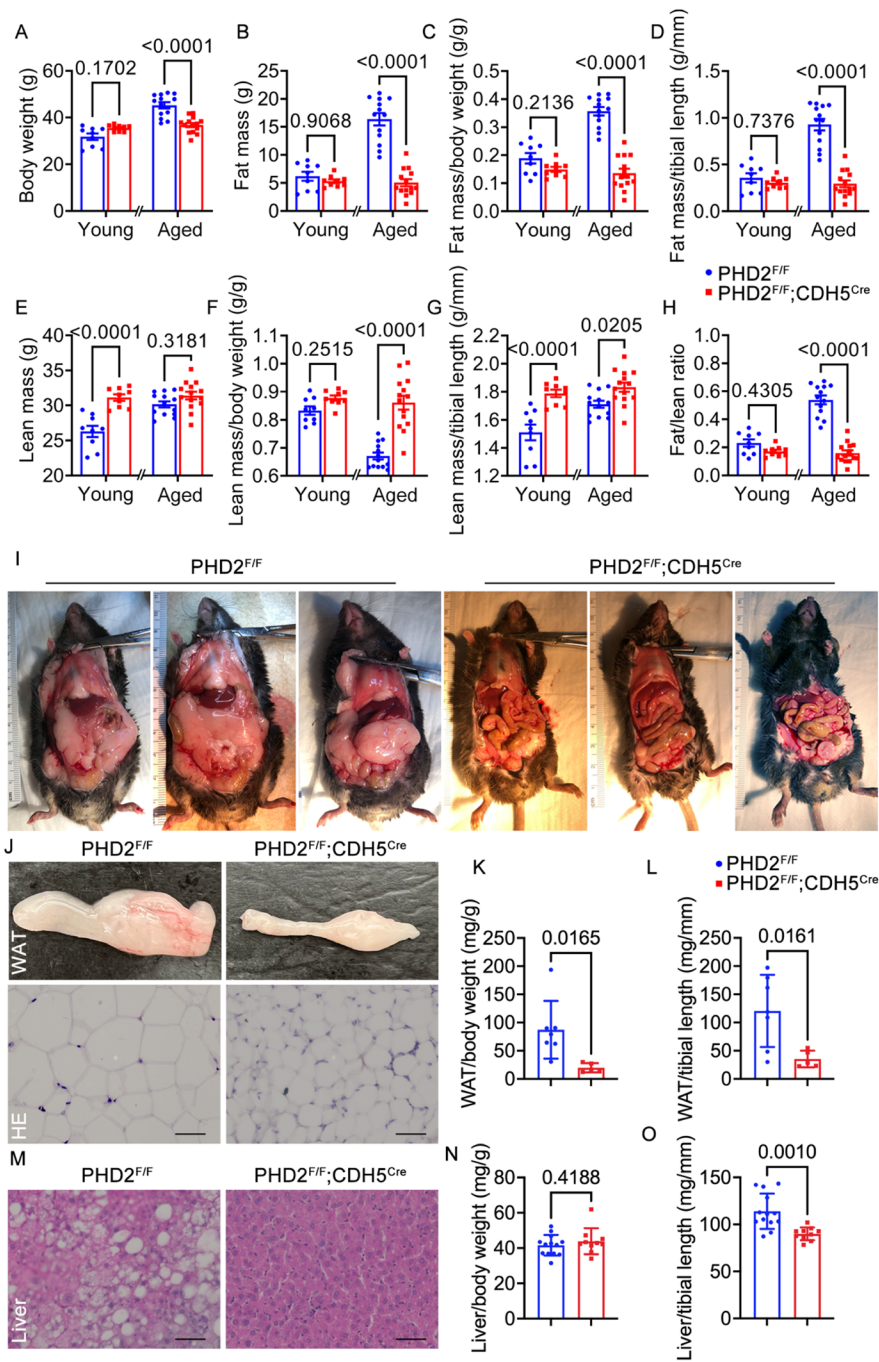
PHD2 Deficiency increased the overall energy expenditure without affecting the food uptake in aged mice

To gain further insights into the mechanism of age-related obesity in PHD2 ECKO mice, we conducted metabolic rate measurements using indirect calorimetry in metabolic chambers. Interestingly, aged PHD2 ECKO mice exhibited higher oxygen consumption than the PHD2$^{f/f}$ control mice (Fig. 2A, B). We also observed an increase in carbon dioxide production in aged PHD2 ECKO mice as compared with the aged PHD2$^{f/f}$ control

◄**Fig. 1** PHD2 ECKO mice are protected from aging-dependent obesity. **A**, PHD2 ECKO mice, and control mice were allowed to age in the laboratory animal center, and their body weight was monitored, two-way ANOVA, *$p < 0.05$. **B-D,** Fat mass, fat mass normalized to body weight, fat mass normalized to the tibial length of both young and aged PHD2 ECKO mice and control PHD2$^{f/f}$ mice, unpaired 2-tailed t-test. **E–G,** Lean mass, lean mass normalized to body weight, lean mass normalized to the tibial length of PHD2 ECKO mice and control PHD2$^{f/f}$ mice, unpaired 2-tailed t-test. **H,** fat/ lean ratio of PHD2 ECKO mice and control PHD2$^{f/f}$ mice. **I,** representative image of aged PHD2 ECKO mice and aged control PHD2$^{f/f}$ mice. **J,** representative image and hematoxylin and eosin staining of white adipose tissue (WAT) from aged PHD2 ECKO mice and aged control PHD2$^{f/f}$ mice. Scale bar, 50 μm. **K,** quantification of WAT weight normalized to body weight. **L,** quantification of WAT weight normalized to tibial length, unpaired 2-tailed t-test. **M,** representative hematoxylin and eosin staining of liver tissue from aged PHD2 ECKO mice and aged control PHD2$^{f/f}$ mice. Scale bar, 50 μm. **N,** quantification of WAT weight normalized to body weight. **O,** quantification of liver weight normalized to tibial length, unpaired 2-tailed t-test. N = 9, 9, 13, 14

mice (Figs. 2C, 2D). However, the respiratory quotient (RQ) was not significantly different between the aged PHD2 ECKO and PHD2$^{f/f}$ control mice (Fig. 2E).

PHD2 ECKO mice consistently exhibited significantly higher daytime energy expenditure (EE), regardless of whether normalized or not by body weight (Fig. 2F, G). There were no notable differences in water intake between the two groups (Fig. 2H and I). Furthermore, we did not observe differences in food intake at different time points (Fig. 2J, K). Interestingly, total food uptake per mouse was unchanged, but the food uptake after normalized to body weight was significantly increased in PHD2 ECKO mice (Fig. 2K). However, the food intake of young PHD2 ECKO mice was unchanged as compared with young PHD2$^{f/f}$ control mice (Fig. 2L). These findings indicate that the reduced fat mass in aged PHD2 ECKO mice is mainly an outcome of increased energy expenditure but not an outcome of reduced food consumption.

PHD2 deficiency preserved metabolic and exercise capacity in aged mice

Since we observed an increased in daily oxygen consumption in aged PHD2 ECKO mice, we further assessed VO_2 consumption, VCO_2 production, and endurance exercise capacity, utilizing a graded maximal exercise capacity test protocol in both aged and young PHD2 ECKO mice. During the test, as the speed increased, some mice reached their endurance limit and were removed from the analysis. In our study, once 25% of the mice dropped out, their VO_2 and VCO_2 recordings were excluded from the final presentation. Consistent with the findings from metabolic chambers, we found increased VO_2 consumption in the PHD2 ECKO group, both at baseline and maximum levels (Fig. 3A-C). No significant differences were observed in the $\Delta VO2$ (Supplemental Fig. 2A). Similarly, we observed elevated VCO_2 production in the PHD2 ECKO group under both baseline and maximum production conditions (Fig. 3D-F). Regarding nutrient utilization, as indicated by the respiratory exchange ratio (RER), we observed a higher baseline RER in the PHD2 ECKO group but no significant difference in the maximum condition (Fig. 3G-I). Furthermore, we detected increased heat production in both baseline and maximum conditions within the PHD2 ECKO group (Fig. 3J-L). Notably, the PHD2 ECKO group demonstrated a significantly longer running time during the exercise protocol, indicating enhanced endurance (Fig. 3N). Moreover, the maximum running speed in PHD2 ECKO mice was also significantly higher (Fig. 3M). However, the life span of aged PHD2 ECKO mice was reduced as compared with aged control mice, as 5 out of 19 aged PHD2 ECKO died during the study.

Together, these findings showed that PHD2 ECKO significantly abolished the aging-induced reduction of exercise capacity and the reduction of aerobic capacity, but these changes failed to improve the life span of PHD2 ECKO mice.

Minimal influence of PHD2 ECKO on metabolic responses to treadmill exercise in young mice

Baseline VO_2 and VO_2 max consumption were measured in PHD2 ECKO mice and control mice, revealing no significant differences between the two groups (Fig. 4A-C, Supplemental Fig. 2A). Similarly, we assessed VCO_2 production in both groups and obtained comparable results (Fig. 4D-F). Furthermore, nutrient utilization was analyzed using indirect calorimetry, where measurements of VCO_2 production and VO_2 consumption were employed to calculate the respiratory exchange ratio (RER). Consistent with previous findings, no notable differences were observed between young PHD2 ECKO and control groups (Fig. 4G-I).
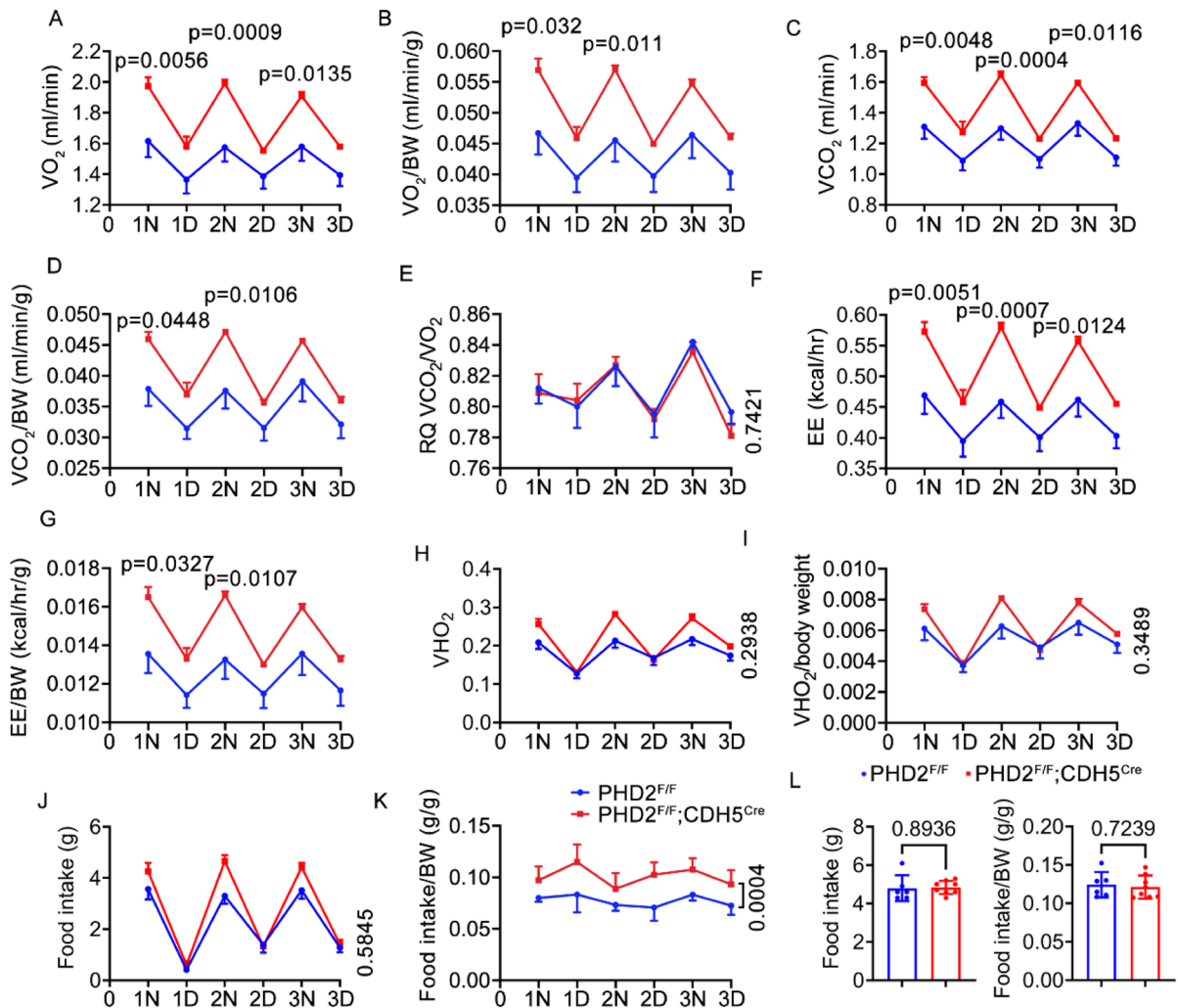
Fig. 2 Energy Expenditure in Aged Mice. **A**, **B**, the daytime and nighttime oxygen consumption in PHD2 ECKO and control mice, both normalized to body weight (BW) and without normalization. **C, D,** daytime and nighttime carbon dioxide production in PHD2 ECKO and control mice, both normalized to body weight (BW) and without normalization. **E,** daytime and nighttime respiratory quotient (RQ) on PHD2 ECKO and control mice. **F, G,** daytime and nighttime energy expenditure (EE) in PHD2 ECKO and control mice, both normalized to body weight (BW) and without normalization. **H, I,** daytime and nighttime water intake in PHD2 ECKO and control mice, both normalized to body weight (BW) and without normalization. **J, K,** daytime and nighttime food intake in PHD2 ECKO and control mice, both normalized to body weight (BW) and without normalization. **L,** food intake with or without normalized to BW in young PHD2 ECKO and young PHD2$^{f/f}$ control mice, unpaired 2-tailed *t-test*, n = 6,7

Intriguingly, we investigated heat production in the two groups and observed a significantly higher heat production in PHD2 ECKO mice, both at baseline and during maximum heat production (Fig. 4J-L). Additionally, the PHD2 ECKO group exhibited a significantly longer running time (Fig. 4M). However, the duration of RER above 1 remained similar between the two groups (Fig. 4N). These findings collectively demonstrate that deficiency in young mice has minimal impact on metabolic responses, including VCO_2 production, VO_2 consumption, and nutrient utilization as indicated by RER. Notably, PHD2 ECKO mice exhibit increased heat production and enhanced exercise endurance, suggesting potential effects on thermogenesis and physical performance.
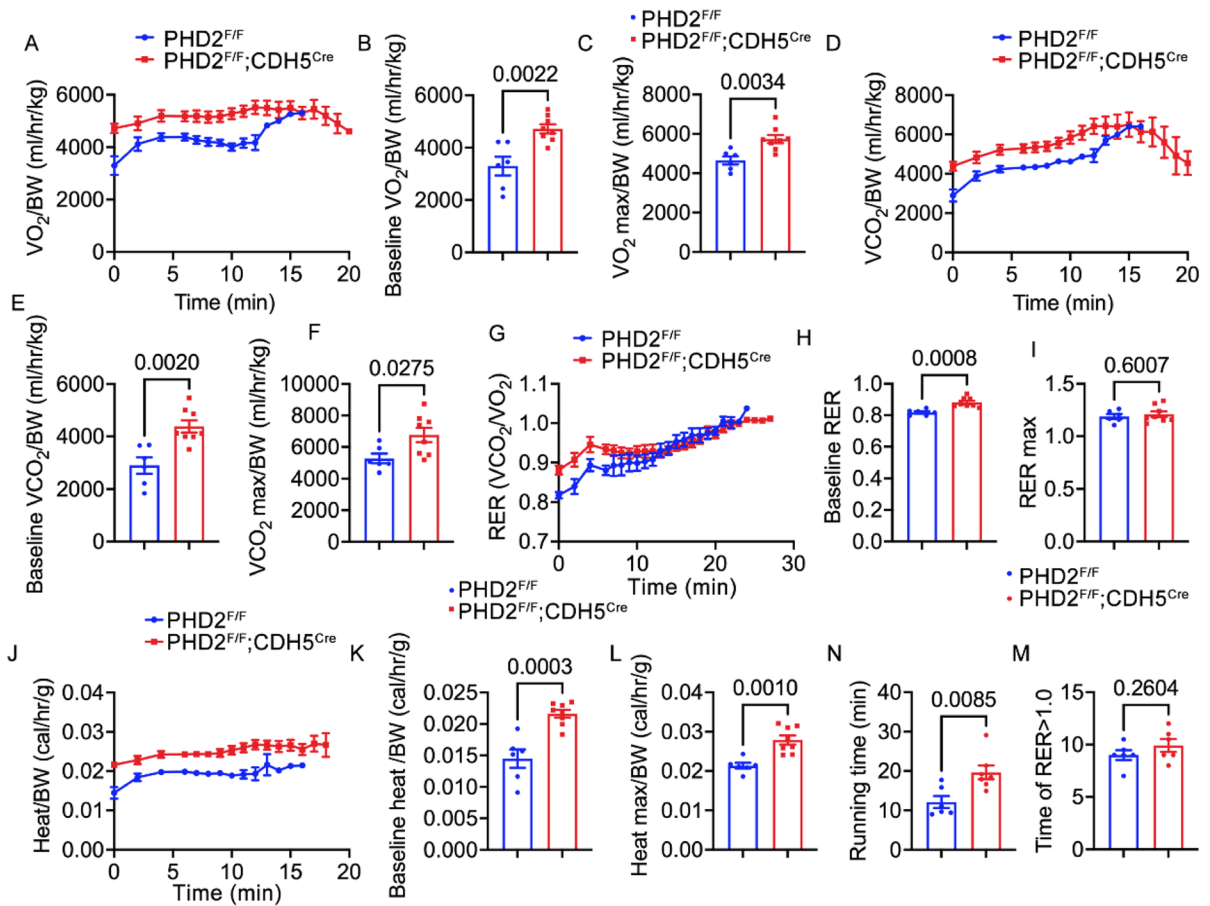
Fig. 3 The respiratory exchange ratio (RER) during treadmill exercise in aged mice. **A-M,** PHD2 ECKO mice and control groups were run on the treadmill and their metabolism and running duration were monitored. **A,** O_2 consumption. **B,** quantification of baseline O_2 consumption. **C,** quantification of maximum O_2 consumption. **D,** CO_2 production. **E,** quantification of baseline CO_2 production. **F,** quantification of maximum CO_2 production. **G,** RER. **H,** quantification of baseline RER. **I,** quantification of maximum RER. **J,** heat production. **K,** quantification of baseline heat production. **L,** quantification of maximum heat production. **N,** quantification of running time for each group. **M,** quantification of the maximum running speed. Unpaired 2-tailed t-test, n = 6, 8

PHD2 deficiency improved glucose tolerance and insulin sensitivity in both young and aged mice

Given the lean phenotype observed in PHD2 ECKO mice and the well-known association between obesity and insulin resistance, we further investigated the impact of PHD2 ECKO on glucose metabolism. In young and aged mice, PHD2 ECKO mice displayed lower glucose intolerance compared with control mice during the intraperitoneal glucose tolerance test (IPGTT) (Fig. 5A-D). Additionally, in the insulin tolerance test (ITT) (Fig. 5E-H), PHD2 ECKO mice demonstrated increased insulin

sensitivity compared with controls. These findings indicate that a deficiency of PHD2 in endothelial cells has a positive effect on insulin sensitivity in both young and aged mice.

Discussion

The present study has several major new findings. First, we found that vascular endothelial-specific mPHD2 gene KO (PHD2 ECKO) significantly attenuates age-related obesity without affecting lean mass gain. Second, aged PHD2 ECKO mice displayed a
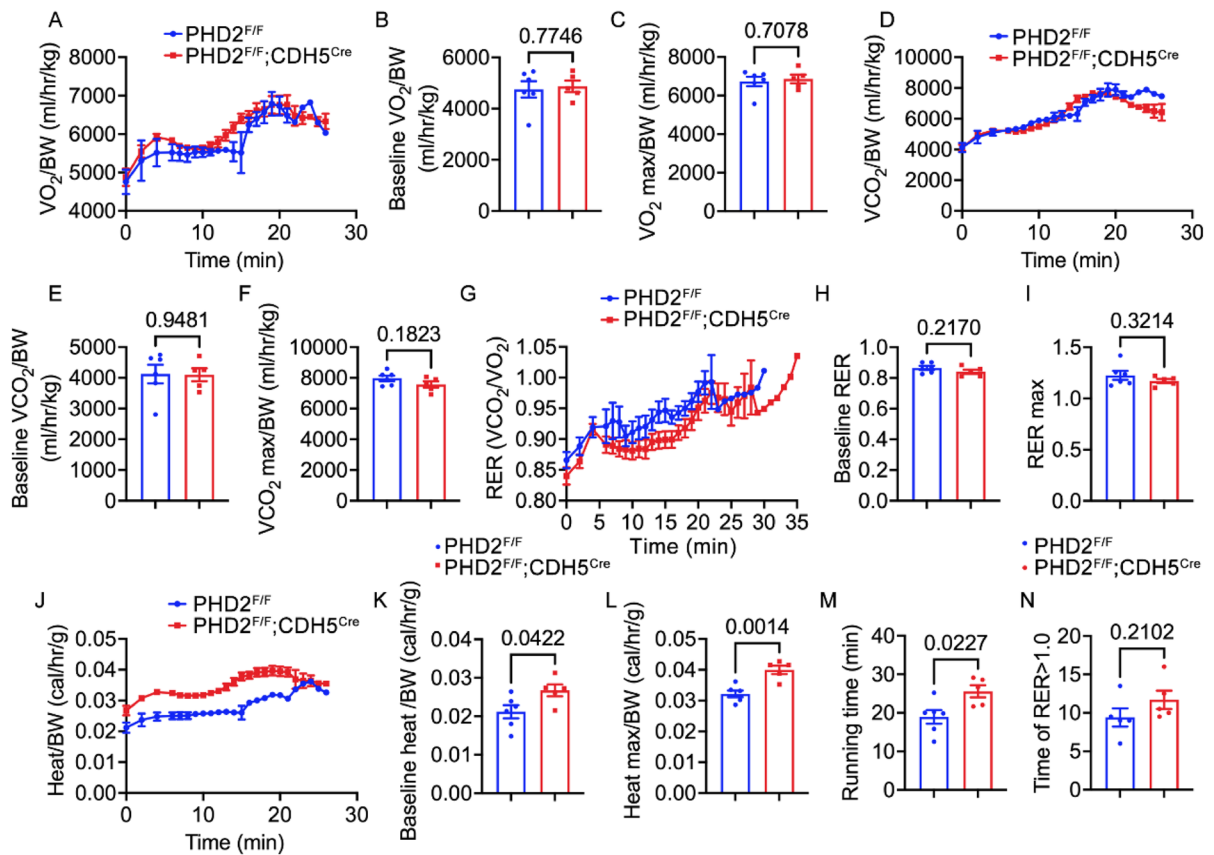
Fig. 4 The respiratory exchange ratio (RER) during treadmill exercise in young mice. **A-M,** PHD2 ECKO mice and control groups were run on the treadmill and their metabolism and running duration were monitored. **A,** O_2 consumption. **B,** quantification of baseline O_2 consumption. **C,** quantification of maximum O_2 consumption. **D,** CO_2 production. **E,** quantification of baseline CO_2 production. **F,** quantification of maxi-mum CO_2 production. **G,** RER. **H,** quantification of baseline RER. **I,** quantification of maximum RER. **J,** heat production. **K,** quantification of baseline heat production. **L,** quantification of maximum heat production. **M,** quantification of the running time for each group. **N,** quantification of the running duration of RER over 1. Unpaired 2-tailed t-test, n = 6, 5

significantly increased exercise capacity as compared to aged PHD2$^{F/F}$ mice. However, the exercise capacity was similar between young PHD2 ECKO mice and young PHD2$^{F/F}$ mice. Third, we found that the daily energy expenditure was significantly increased with no significant changes in food intake in PHD2 ECKO mice. Furthermore, we found that glucose tolerance capacity and insulin sensitivity were significantly improved in both young and aged PHD2 ECKO mice.

One of the most interesting findings is that aged PHD2 ECKO mice exhibited significantly reduced obesity, characterized by reduced fat mass and its ratio to lean mass, tibial length, or body weight. The reduction of fat mass was accompanied by a decrease in adipocyte size in WAT. Aged PHD2 ECKO mice also exhibited higher oxygen consumption without reduction of food update as compared with control mice, suggesting the increased energy expenditure contributes to their leaner phenotyping. While the finding of reduced age-related obesity in vascular endothelial PHD2 deficient mice has not been previously reported, the effect of global PHD2 deficiency in attenuating obesity has been previously observed. Thus, using a HIF-p4h-2 hypomorph mouse line, previous studies also demonstrated that an increase of HIF stability by systemic inhibition of PHD2 significantly attenuated obesity and metabolic dysfunction [25]. A recent study from the same group further showed that HIF-p4h-2 hypomorph mice were resistant to aging-induced obesity and glucose intolerance
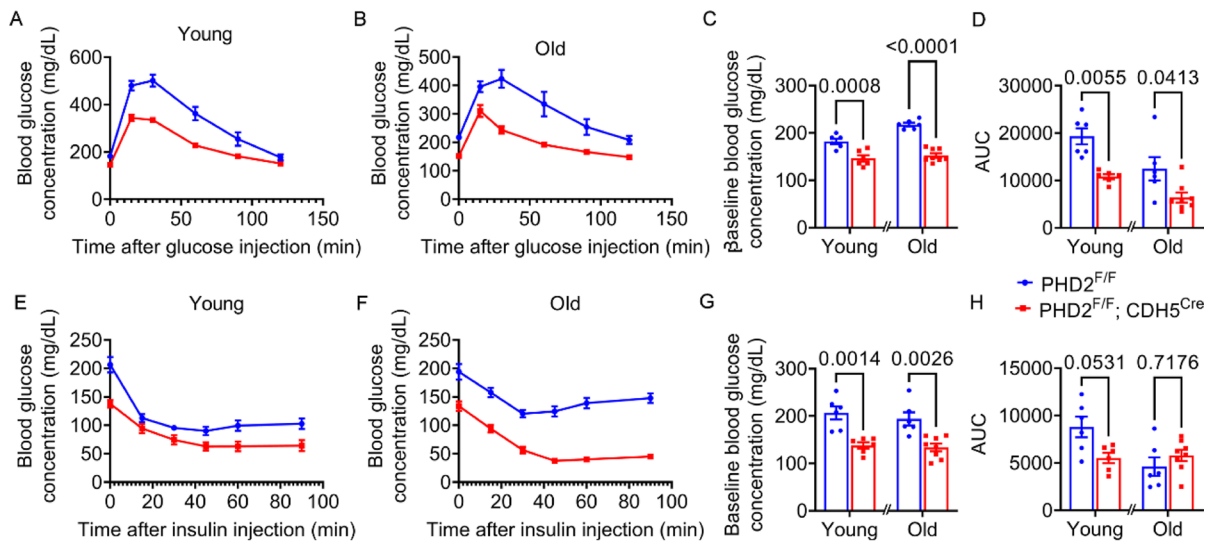
Fig. 5 PHD2 ECKO mice show improved glycemic control. **A, B,** Intraperitoneal glucose tolerance test performed on PHD2 ECKO and control young and aged mice. Glucose was measured from blood at given time points after injection of glucose. **C,** quantification of baseline blood glucose, two-way ANOVA, data are represented as mean ± SEM. **D,** area under the curve (AUC) of blood glucose profiles during IPGTT. unpaired 2-tailed t-test. **E, F,** Insulin tolerance test performed on PHD2 ECKO and corresponding control mice. Glucose was measured from blood at given time points after injection of insulin. **G,** quantification of baseline blood glucose, unpaired 2-tailed t-test, data are represented as mean ± SEM. **H,** Area under the curve (AUC) of blood glucose profiles during ITT. unpaired 2-tailed t-test, data are represented as mean ± SEM, n = 6,8

[26]. In addition, a previous study also demonstrated that PHD2 deficiency in adipocytes significantly attenuated diet-induced obesity and glucose intolerance in mice [19, 27]. One of the potential mechanisms for the reduced obesity in the aged PHD2 ECKO mice might be due to the elevated energy expenditure by increased heat production in these mice. The increased heat generation in PHD2 ECKO mice is likely an adaptive response because extra heat generation might be needed to maintain the core body temperature for mice with lower subcutaneous adipose tissue to form an insulating barrier around the body.

Another interesting finding is that PHD2 ECKO resulted in a significant increase in exercise capacity in aged mice. PHD2 ECKO mice also had significantly higher oxygen consumption and VCO2 production under control conditions and during treadmill exercise in these mice. The increased oxygen consumption and VCO2 production were observed at both nighttime and daytime, indicating the systemic metabolic rate and energy expenditure are upregulated in these PHD2 ECKO mice. The potential mechanisms underlying the enhanced exercise

capacity and energy expenditure in these mice likely involve, but are not limited to, the activation of HIF1 and HIF2 signaling pathways. First, PHD2 deficiency can enhance angiogenesis, vessel remodeling/generation, and collateral vessel formation by increasing HIF1 stability in skeletal and cardiac muscles partially through modulating HIF1, VEGF, and NO signaling pathways [28, 29]. Indeed, studies showed that inducible PHD2 deficiency led to hyperactive angiogenesis in the heart and other tissues [30]. In addition, PHD2 deficiency increases erythropoietin (EPO) production [20, 30, 31], while EPO can improve exercise performance through its interaction with EPO receptors broadly distributed in both hematopoietic and non-hematopoietic tissues [32]. For example, EPO stimulates red blood cell production to improve oxygen transportation during exercise. EPO also stimulates skeletal muscle mitochondrial biogenesis gene expression (such as PGC-1α) and skeletal muscle fiber programming to type I muscle fibers [33]. In addition, EPO attenuates diet-induced obesity, improves glucose tolerance, reduces insulin resistance, and attenuates fat mass accumulation [32, 34]. Lastly, the reduced fat mass and body weight in aged

PHD2 ECKO mice can certainly decrease work load and directly enhance exercise capacity.

While systemic and endothelial-specific PHD2 inhibition attenuates obesity and metabolic dysfunction in mice, we also noticed that several aged PHD2 ECKO mice died during the study, indicating a detrimental effect of endothelial-specific PHD2 inhibition on the life span. In addition, several previous studies have demonstrated that both systemic and endothelial-specific PHD2 inhibition resulted in severe diseases such as idiopathic pulmonary hypertension and congestive heart failure in mice [35, 36]. For example, Tie2[Cre]-mediated PHD2 deletion caused a spontaneous severe PAH, as evidenced by extensive pulmonary vascular remodeling, vascular occlusion, plexiform-like lesions, and right ventricular (RV) hypertrophy and failure [36]. The above complex pathological and molecular changes were mainly attributed to the activation of HIF-2α after PHD2 deletion [36]. In addition, CDH5[Cre]-mediated PHD2 deletion also caused a mild age-dependent PAH in mice [37]. A recent study further demonstrated that Tie2[Cre]-mediated PHD2 deletion caused LV hypertrophy and dysfunction through HIF2a dependent pathway [35]. Furthermore, global PHD2 deficiency leads to erythrocytosis and thrombosis formation by enhancing EPO production [20]. Moreover, conditional inactivation of PHD2 (using chicken beta-actin-Cre-ER mice together with tamoxifen injection) resulted in an increase in red blood cell production, venous congestion, and dilated cardiomyopathy in mice [38]. The increased mortality in aged male PHD2 ECKO mice also indicate a detrimental effect of PHD2 inhibition on life span in mice. Thus, treating obesity and metabolic dysfunction by either systemic or endothelial-specific PHD2 inhibition may cause unwanted side effects such as cardiac dysfunction and increased mortality.

Overall, the findings of this study highlight the importance of endothelial PHD2 in regulating metabolic status and maintaining metabolic homeostasis, particularly during aging. While the absence of PHD2 in endothelial cells appears to confer apparent metabolic benefits against obesity, systemic or endothelial-specific PHD2 deficiency also causes detrimental effects in the cardiopulmonary system.

Thus, caution should be taken to explore inhibition of PHD2 as a potential therapeutic target for addressing age-related metabolic abnormalities and obesity-related disorders. In the context that sex is an important biological factor that affects obesity, glucose sensitivity, and exercise capacity, there is a possibility that female and male mice may have different changes of obesity and exercise capacity after endothelial-specific PHD2 deficiency. Thus, studying only male mice is a major limitation of our study. Further research is clearly needed to fully understand any potential sex differences, the underlying mechanisms, and the potential clinical implications of targeting PHD2 in the context of metabolic health.

Data availability Data will available when it is requested.

Declarations

Ethics The animal study was approved by Animal Care and Use Committee at the University of Mississippi Medical Center.

Conflict of interest The authors declare no competing interests.

References

1. Kirkland JL. Translating advances from the basic biology of aging into clinical application. Exp Gerontol. 2013;48:1–5. https://doi.org/10.1016/j.exger.2012.11.014.

2. Kuk JL, Saunders TJ, Davidson LE, Ross R. Age-related changes in total and regional fat distribution. Ageing Res Rev. 2009;8:339–48. https://doi.org/10.1016/j.arr.2009.06.001.

3. Berra E, Benizri E, Ginouves A, Volmat V, Roux D, Pouyssegur J. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. EMBO J. 2003;22:4082–90. https://doi.org/10.1093/emboj/cdg392.

4. Appelhoff RJ, Tian YM, Raval RR, Turley H, Harris AL, Pugh CW, Ratcliffe PJ, Gleadle JM. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. J Biol Chem. 2004;279:38458–65. https://doi.org/10.1074/jbc.M406026200.

5. Shukla V, Singh SN, Vats P, Singh VK, Singh SB, Banerjee PK. Ghrelin and leptin levels of sojourners and acclimatized lowlanders at high altitude. Nutr Neurosci. 2005;8:161–5. https://doi.org/10.1080/10284150500132823.

6. Allahdadi KJ, Walker BR, Kanagy NL. Augmented endothelin vasoconstriction in intermittent hypoxia-induced hypertension. Hypertension. 2005;45:705–9. https://doi.org/10.1161/01.HYP.0000153794.52852.04.

7. Simler N, Grosfeld A, Peinnequin A, Guerre-Millo M, Bigard AX. Leptin receptor-deficient obese Zucker rats reduce their food intake in response to hypobaric hypoxia. Am J Physiol Endocrinol Metab. 2006;290:E591-597. https://doi.org/10.1152/ajpendo.00289.2005.

8. Quintero P, Milagro FI, Campion J, Martinez JA. Impact of oxygen availability on body weight management. Med Hypotheses. 2010;74:901–7. https://doi.org/10.1016/j.mehy.2009.10.022.

9. Yun Z, Maecker HL, Johnson RS, Giaccia AJ. Inhibition of PPAR gamma 2 gene expression by the HIF-1-regulated gene DEC1/Stra13: a mechanism for regulation of adipogenesis by hypoxia. Dev Cell. 2002;2:331–41. https://doi.org/10.1016/s1534-5807(02)00131-4.

10. Kaelin WG Jr, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell. 2008;30:393–402. https://doi.org/10.1016/j.molcel.2008.04.009.

11. Majmundar AJ, Wong WJ, Simon MC. Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell. 2010;40:294–309. https://doi.org/10.1016/j.molcel.2010.09.022.

12. Virtue S, Vidal-Puig A. Nothing Iffy about HIF in the Hypothalamus. PLoS Biol. 2011;9:e1001116. https://doi.org/10.1371/journal.pbio.1001116.

13. Girgis CM, Cheng K, Scott CH, Gunton JE. Novel links between HIFs, type 2 diabetes, and metabolic syndrome. Trends Endocrinol Metab. 2012;23:372–80. https://doi.org/10.1016/j.tem.2012.05.003.

14. Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev. 2002;16:1466–71. https://doi.org/10.1101/gad.991402.

15. Bento CF, Pereira P. Regulation of hypoxia-inducible factor 1 and the loss of the cellular response to hypoxia in diabetes. Diabetologia. 2011;54:1946–56. https://doi.org/10.1007/s00125-011-2191-8.

16. Zhang H, Zhang G, Gonzalez FJ, Park SM, Cai D. Hypoxia-inducible factor directs POMC gene to mediate hypothalamic glucose sensing and energy balance regulation. PLoS Biol. 2011;9:e1001112. https://doi.org/10.1371/journal.pbio.1001112.

17. Ban JJ, Ruthenborg RJ, Cho KW, Kim JW. Regulation of obesity and insulin resistance by hypoxia-inducible factors. Hypoxia (Auckl). 2014;2:171–83. https://doi.org/10.2147/HP.S68771.

18. Mason SD, Howlett RA, Kim MJ, Olfert IM, Hogan MC, McNulty W, Hickey RP, Wagner PD, Kahn CR, Giordano FJ, et al. Loss of skeletal muscle HIF-1alpha results in altered exercise endurance. PLoS Biol. 2004;2:e288. https://doi.org/10.1371/journal.pbio.0020288.

19. Matsuura H, Ichiki T, Inoue E, Nomura M, Miyazaki R, Hashimoto T, Ikeda J, Takayanagi R, Fong GH, Sunagawa K. Prolyl hydroxylase domain protein 2 plays a critical role in diet-induced obesity and glucose intolerance. Circulation. 2013;127:2078–87. https://doi.org/10.1161/CIRCULATIONAHA.113.001742.

20. Takeda K, Ho VC, Takeda H, Duan LJ, Nagy A, Fong GH. Placental but not heart defects are associated with elevated hypoxia-inducible factor alpha levels in mice lacking prolyl hydroxylase domain protein 2. Mol Cell Biol. 2006;26:8336–46. https://doi.org/10.1128/MCB.00425-06.

21. Alva JA, Zovein AC, Monvoisin A, Murphy T, Salazar A, Harvey NL, Carmeliet P, Iruela-Arispe ML. VE-Cadherin-Cre-recombinase transgenic mouse: a tool for lineage analysis and gene deletion in endothelial cells. Dev Dyn. 2006;235:759–67. https://doi.org/10.1002/dvdy.20643.

22. Wang S, Zeng H, Chen ST, Zhou L, Xie XJ, He X, Tao YK, Tuo QH, Deng C, Liao DF, et al. Ablation of endothelial prolyl hydroxylase domain protein-2 promotes renal vascular remodelling and fibrosis in mice. J Cell Mol Med. 2017;21:1967–78. https://doi.org/10.1111/jcmm.13117.

23. Ayala JE, Samuel VT, Morton GJ, Obici S, Croniger CM, Shulman GI, Wasserman DH, Mcguinness OP, Consortium NIHMMPC. Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. Dis Model Mech. 2010;3:525–34. https://doi.org/10.1242/dmm.006239.

24. Petrosino JM, Heiss VJ, Maurya SK, Kalyanasundaram A, Periasamy M, LaFountain RA, Wilson JM, Simonetti OP, Ziouzenkova O. Graded Maximal Exercise Testing to Assess Mouse Cardio-Metabolic Phenotypes. PLoS ONE. 2016;11:e0148010. https://doi.org/10.1371/journal.pone.0148010.

25. Rahtu-Korpela L, Karsikas S, Horkko S, Blanco Sequeiros R, Lammentausta E, Makela KA, Herzig KH, Walkinshaw G, Kivirikko KI, Myllyharju J, et al. HIF prolyl 4-hydroxylase-2 inhibition improves glucose and lipid metabolism and protects against obesity and metabolic dysfunction. Diabetes. 2014;63:3324–33. https://doi.org/10.2337/db14-0472.

26. Laitakari A, Huttunen R, Kuvaja P, Hannuksela P, Szabo Z, Heikkila M, Kerkela R, Myllyharju J, Dimova EY,

Serpi R, et al. Systemic long-term inactivation of hypoxia-inducible factor prolyl 4-hydroxylase 2 ameliorates aging-induced changes in mice without affecting their life span. FASEB J. 2020;34:5590–609. https://doi.org/10.1096/fj.201902331R.

27. Feng T, Zhao X, Gu P, Yang W, Wang C, Guo Q, Long Q, Liu Q, Cheng Y, Li J, et al. Adipocyte-derived lactate is a signalling metabolite that potentiates adipose macrophage inflammation via targeting PHD2. Nat Commun. 2022;13:5208. https://doi.org/10.1038/s41467-022-32871-3.

28. Zimna A, Kurpisz M. Hypoxia-Inducible Factor-1 in Physiological and Pathophysiological Angiogenesis: Applications and Therapies. Biomed Res Int. 2015;2015:549412. https://doi.org/10.1155/2015/549412.

29. Luo Z, Tian M, Yang G, Tan Q, Chen Y, Li G, Zhang Q, Li Y, Wan P, Wu J. Hypoxia signaling in human health and diseases: implications and prospects for therapeutics. Signal Transduct Target Ther. 2022;7:218. https://doi.org/10.1038/s41392-022-01080-1.

30. Takeda K, Cowan A, Fong GH. Essential role for prolyl hydroxylase domain protein 2 in oxygen homeostasis of the adult vascular system. Circulation. 2007;116:774–81. https://doi.org/10.1161/CIRCULATIONAHA.107.701516.

31. Gardie B, Percy MJ, Hoogewijs D, Chowdhury R, Bento C, Arsenault PR, Richard S, Almeida H, Ewing J, Lambert F, et al. The role of PHD2 mutations in the pathogenesis of erythrocytosis. Hypoxia (Auckl). 2014;2:71–90. https://doi.org/10.2147/HP.S54455.

32. Suresh S, Rajvanshi PK, Noguchi CT. The Many Facets of Erythropoietin Physiologic and Metabolic Response. Front Physiol. 2019;10:1534. https://doi.org/10.3389/fphys.2019.01534.

33. Wang L, Jia Y, Rogers H, Suzuki N, Gassmann M, Wang Q, McPherron AC, Kopp JB, Yamamoto M, Noguchi CT. Erythropoietin contributes to slow oxidative muscle fiber specification via PGC-1alpha and AMPK activation. Int J Biochem Cell Biol. 2013;45:1155–64. https://doi.org/10.1016/j.biocel.2013.03.007.

34. Wang L, Di L, Noguchi CT. Erythropoietin, a novel versatile player regulating energy metabolism beyond the erythroid system. Int J Biol Sci. 2014;10:921–39. https://doi.org/10.7150/ijbs.9518.

35. Dai Z, Cheng J, Liu B, Yi D, Feng A, Wang T, An L, Gao C, Wang Y, Zhu MM, et al. Loss of Endothelial Hypoxia Inducible Factor-Prolyl Hydroxylase 2 Induces Cardiac Hypertrophy and Fibrosis. J Am Heart Assoc. 2021;10:e022077. https://doi.org/10.1161/JAHA.121.022077.

36. Dai Z, Li M, Wharton J, Zhu MM, Zhao YY. Prolyl-4 Hydroxylase 2 (PHD2) Deficiency in Endothelial Cells and Hematopoietic Cells Induces Obliterative Vascular Remodeling and Severe Pulmonary Arterial Hypertension in Mice and Humans Through Hypoxia-Inducible Factor-2alpha. Circulation. 2016;133:2447–58. https://doi.org/10.1161/CIRCULATIONAHA.116.021494.

37. Wang S, Zeng H, Xie XJ, Tao YK, He X, Roman RJ, Aschner JL, Chen JX. Loss of prolyl hydroxylase domain protein 2 in vascular endothelium increases pericyte coverage and promotes pulmonary arterial remodeling. Oncotarget. 2016;7:58848–61. https://doi.org/10.18632/oncotarget.11585.

38. Minamishima YA, Moslehi J, Bardeesy N, Cullen D, Bronson RT, Kaelin WG Jr. Somatic inactivation of the PHD2 prolyl hydroxylase causes polycythemia and congestive heart failure. Blood. 2008;111:3236–44. https://doi.org/10.1182/blood-2007-10-117812.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.