

# PARP1 deficiency exacerbates diet-induced obesity in mice

Kishor Devalaraja-Narashimha<sup>1</sup> and Babu J Padanilam<sup>1,2</sup>

<sup>1</sup>Department of Cellular and Integrative Physiology and <sup>2</sup>Section of Nephrology, Department of Internal Medicine, University of Nebraska Medical Center, 985850 Nebraska Medical Center, Omaha, Nebraska 68198-5850, USA

(Correspondence should be addressed to B J Padanilam at Department of Cellular and Integrative Physiology, 985850 Nebraska Medical Center; Email: bpadanilam@unmc.edu)

## Abstract

Poly (ADP-ribose) polymerase-1 (PARP1) regulates gene expression as a transcriptional cofactor and protein functions via poly (ADP-ribosylation). This study was aimed to determine the effect of *Parp1* gene deficiency on diet-induced obesity and energy metabolism. *Parp1*-knockout (*Parp*-KO) and wild-type (WT) mice on the same genetic background were fed either normal chow or high-fat (HF) diet. Food intake and weight gain were monitored weekly. Plasma levels of glucose, leptin, and insulin were monitored monthly. At 19 weeks, locomotor activity, body composition, respiratory quotient and heat production, glucose and insulin tolerance, and fat reabsorption were analyzed. *Parp*-KO mice are highly

susceptible to diet-induced obesity, accumulation of fat tissue, and they develop hyperleptinemia and insulin resistance and glucose intolerance compared with their WT counterparts. The increased weight gain is due to decreased metabolic rate, heat production, and total energy expenditure (EE). Paradoxically, food intake is less, and the motor activity and oxidation of fat are higher in *Parp*-KO mice. Absorption of fatty acids is not altered between the groups after HF diet. These results suggest that malfunction of PARP1 signaling exacerbates diet-induced obesity, hyperleptinemia, and insulin resistance, and that it decreases EE in 129 mice.

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## Introduction

Obesity has become a worldwide epidemic over the years (James *et al.* 2001), and it is tightly associated with type 2 diabetes (Smyth & Heron 2006). It is a multifactorial disorder, and inter- and intrapopulation genetic variations exist in susceptibility to diet-induced obesity in humans (Comuzzie & Allison 1998) as well as among various inbred strains of mice (Brockmann & Bevova 2002). Both mono- and polygenic variations contribute to the development of obesity phenotype in mice. Spontaneous mutations or variations in the expression levels of selective genes can induce obesity when a high-fat (HF) diet is fed, but little or no effect is observed when a low-fat diet is fed (Martinez-Botas *et al.* 2000). Some inbred strains of mice vary in their susceptibility to diet-induced obesity indicating that obesity is a complex trait and modifications of multiple genes may be necessary for its etiology (Surwit *et al.* 1995, Brockmann & Bevova 2002). We and others have recently reported that C57BL/6 mice are readily susceptible to diet-induced obesity and associated type 2 diabetes (Surwit *et al.* 1988, Wei *et al.* 2004), while the 129 mouse strain is shown to be resistant to diet-induced obesity and insulin resistance (Almind & Kahn 2004, Ishimori *et al.* 2004). Genetic analysis by various groups using cross between different mouse strains has identified more than 100 chromosomal loci, some loci representing high number of genes with differential expression, that may contribute to obesity (Brockmann & Bevova 2002,

Almind & Kahn 2004, Ishimori *et al.* 2004). The possible contribution of this high number of genes and multiple changes at individual loci suggest that transcriptional regulation (Spiegelman & Flier 2001), post-translational modification, methylation, chromatin structure, or histone binding may be altered in different strains to affect gene expression or protein functions (Almind & Kahn 2004).

Poly (ADP-ribose) polymerase-1 (PARP1), a nuclear DNA repairing enzyme, regulates gene expression as a transcriptional cofactor and protein functions via poly (ADP-ribosylation). Loss of PARP1 is shown to affect gene expression profile in a genome-wide manner (Ogino *et al.* 2007). PARP1 has nucleosome binding properties and a transcription-related regulation of chromatin structure (Tulin & Spradling 2003, Kim *et al.* 2004, Krishnakumar *et al.* 2008). In actively transcribed genes, it is shown that PARP1 acts to exclude histone H1 from their promoters, and thus a reciprocal binding between PARP1 and histone H1 may determine gene expression outcome (Krishnakumar *et al.* 2008). Poly (ADP-ribosylation) can have different effects on various proteins including activation, downregulation, changes in protein confirmation, and promotion of protein-protein interactions (Zaniolo *et al.* 2007, Hassa & Hottiger 2008, Devalaraja-Narashimha & Padanilam 2009). Thus, PARP1 exerts its effects via poly(ADP-ribosylation), transcriptional regulation of gene expression, and transcription-related regulation of chromatin structure.

Despite the role of PARP1 in functions that are important in normal cellular homeostasis and in a variety of disease models (reviewed in Chiarugi (2002)), a role for PARP1 in obesity has not been explored. de Murcia *et al.* (1997) reported that the weight of adult *Parp*-KO mice on C57BL/6 background was significantly lower than their wild-type (WT) littermates in their knockout model. In contrast, *Parp*-KO on 129 background developed by Wang *et al.* (1995) it was stated in 'note added in proof' that 'a large fraction of *Parp*-KO female mice older than 15 months become obese, suggesting an important role for ADPRT/poly(ADP-ribosylation) in general metabolism'. This study is recently supported by Piskunova *et al.* (2008) demonstrating that *Parp*-KO female mice on 129 background older than 21 months gained body weight more than their WT littermates when fed a normal diet. Although these reports suggest a role for PARP in general metabolism, its role in high-calorie diet-induced obesity has not been explored.

Given the complexity of energy intake and expenditure program that includes hormones, transcription factors, and multiple genes, we hypothesized that coordination of the functions and interactions of multiple factors may require regulation at the transcriptional and/or at the post-translational level by a master regulatory gene such as *Parp1*. In this study, we sought to determine the effect of high-calorie intake in PARP1-deficient mice on 129 background, and we investigated whether the body weight, food intake, energy expenditure (EE), body fat stores as well as glucose tolerance, and insulin and leptin sensitivity are altered in 129 mice in the absence of PARP1.

## Materials and Methods

### *Animal care and feeding studies*

*Parp*-KO mice and WT (129/SvImJ) counterparts for *Parp*-KO mice were purchased from Jackson Labs (Bar Harbor, ME, USA). Male and female mice of age 35–40 days were maintained on a 12 h light:12 h darkness cycle with free access to either a control diet (10% fat by calories, 3.85 kcal/g) or a HF diet (45% fat by calories, 4.76 kcal/g, Research Diets, Inc, New Brunswick, NJ, USA). Food intake and body weight were measured weekly. All animal studies and procedures were approved by the institutional animal care and usage committee.

### *Histological and morphological evaluation*

WT and *Parp*-KO male mice were killed, and a comprehensive set of organs and tissues from each animal was fixed in 10% buffered formalin or Bouin's, embedded in paraffin, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin (H&E). Nonoverlapping fields were randomly selected and were photographed under 40 $\times$  magnification. The number of cells per camera field in the tissue is quantified using Image Pro Software (Media Cybernetics, Inc., Bethesda, MD, USA).

The average cell size was quantified by dividing the total area of the camera field by the total number of cells in the field.

### *Locomotor activity*

Locomotor activity was measured in an infrared beam operated open field ActiMot/MoTil system (25 $\times$ 25 $\times$ 25 cm, ActiMot System, Midland, MI, USA; Michalakis *et al.* 2006). Horizontal motor activity was evaluated by monitoring the beam breaks in the X and Y coordinates of the animal and thus its location for 24 h.

### *Body composition analysis by nuclear magnetic resonance method*

At the end of the long-term feeding study (19 weeks on the HF diet), body composition of *Parp*-KO and WT male mice was analyzed with a commercial nuclear magnetic resonance (NMR) machine (EchoMRI-100; Echo Medical Systems, Houston, TX, USA; Strader *et al.* 2004). Body fat and lean tissue were measured during the test.

### *Energy expenditure*

On the same group of animals indicated above, oxygen consumption and carbon dioxide production is measured using an indirect calorimeter (AccuScan Instruments Inc, Columbus, OH, USA). Constant airflow (0.75 l/min) was drawn through the chamber and was monitored by a mass-sensitive flowmeter. Variables provided by this measurement include  $\text{VO}_2$ ,  $\text{VCO}_2$ , respiratory quotient (RQ), and HEAT (Strader *et al.* 2004). To calculate oxygen consumption ( $\text{VO}_2$ ), carbon dioxide production ( $\text{VCO}_2$ ), and RQ (ratio of  $\text{VCO}_2$  to  $\text{VO}_2$ ), gas concentrations were monitored at the inlet and outlet of the sealed chambers.

### *Temperature measurements*

Body temperatures were measured using a rectal digital thermometer probe (model 4600; Yellow Springs Instruments, Yellow Springs, OH, USA).

### *Measurement of blood glucose, and serum insulin and leptin*

Blood was collected from fasted (12 h) mice at various time points, and different assays were performed as indicated. Blood glucose was assayed with a glucometer (Hemocue glucose 201 analyzer). Serum insulin and leptin were determined by ELISA, using mouse insulin and leptin respectively as standards using a commercially available kit (Crystal Chem Inc., Chicago, IL, USA).

### *Intraperitoneal glucose tolerance test*

At the end of the long-term feeding study (19 weeks on the HF diet), 5-h fasted mice were injected i.p. with D-glucose (30% solution; 2 mg/g of body weight), and blood glucose

values were determined at 0, 15, 30, 60, and 120 min post injection. Baseline and test samples were all obtained from the tail vein (Strader *et al.* 2004).

*Insulin tolerance tests*

Insulin tolerance tests (ITT) were performed on the same group of animals 10 days after the intraperitoneal glucose tolerance test (IPGTT). Mice were fasted for 5 h, and blood was collected for baseline glucose values. Animals were injected i.p. with 0.5 mU/g insulin (100 mU/ml solution), and blood glucose values were determined at 0, 15, 30, and 60 min post injection.

*Statistical analysis*

Data from long-term body weight studies were analyzed by two-way ANOVA with repeated measures. For comparisons of body composition measures, food intake (kcal), mean kcal/h, fasting plasma glucose, and leptin and insulin, a Student's *t*-test was utilized. *P* values <0.05 were considered statistically significant. All values are presented as means ± S.E.M. unless otherwise specified.

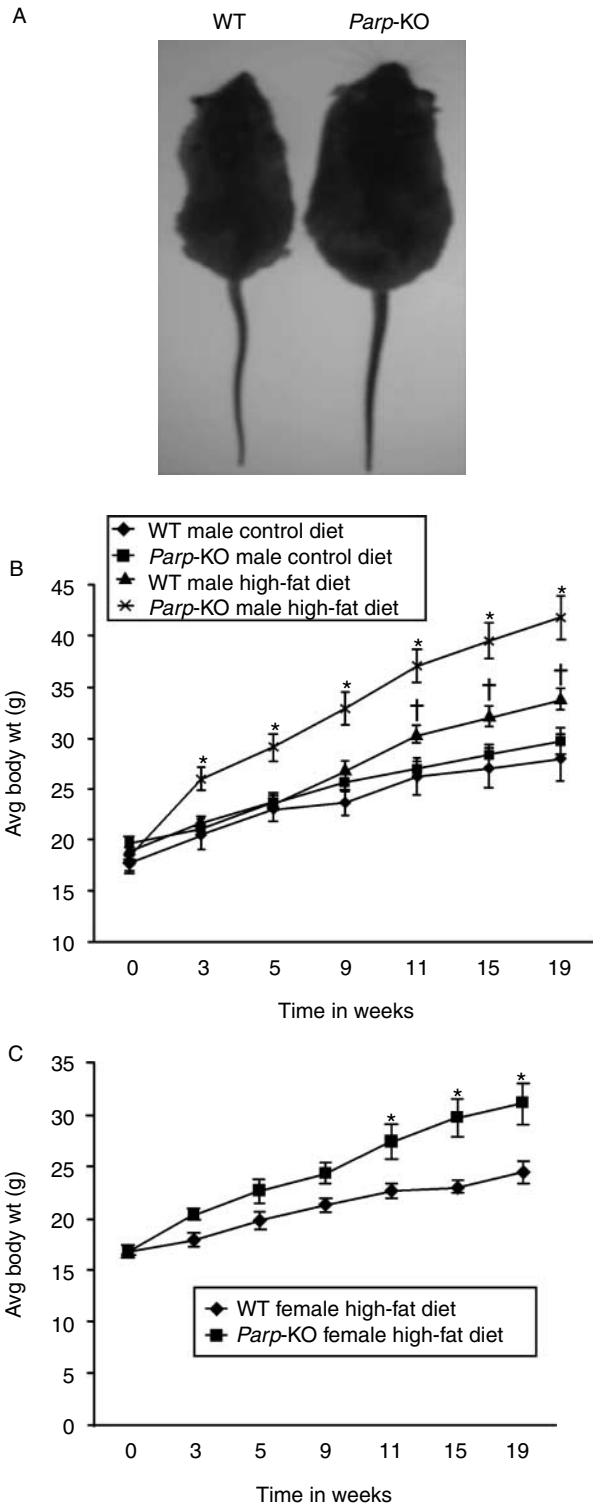
The area under the curve (AUC) is calculated using the trapezium rule as presented by Matthews *et al.* (1990). The baseline area is defined as the value at time zero multiplied by the total time. The percent deflection from baseline is a simple percentage calculation of the difference of the final value minus the baseline value divided by the baseline value (final–baseline)/baseline. For AUC and percent deflection summary measures, we used a two-sample Mann–Whitney *U* test (also called a Wilcoxon–Mann–Whitney test or a Two-Sample Wilcoxon test) to judge whether the medians for the two groups of mice were equal. The analyses were conducted using the NPAR1WAY procedure in SAS. The internal procedure algorithm was used to calculate exact *P* values for each test.

**Results**

*Development of exacerbated obesity in Parp-KO mice following HF diet feeding*

Age and weight of mice from different groups were similar at the beginning of the study. A representative photograph of male WT and *Parp*-KO mice on HF diet for 15 weeks is shown in Fig. 1A. Male *Parp*-KO mice on HF diet appeared larger in

**Figure 1** Development of exacerbated obesity in *Parp*-KO mice. (A) Representative photograph of WT and *Parp*-KO male mice that were on high-fat diet for a period of 15 weeks. (B) Average body weights of WT and *Parp*-KO male mice: \**P*<0.005 compared with WT high-fat diet-fed mice or WT and *Parp*-KO control diet-fed mice; †*P*<0.05 compared with WT control diet-fed mice. *n*=4–5 for control diet-fed mice. *n*=7–9 for high-fat diet-fed mice. (C) Average body weight of female mice: \**P*<0.05 (*n*=4–5) compared with WT high-fat diet-fed mice.

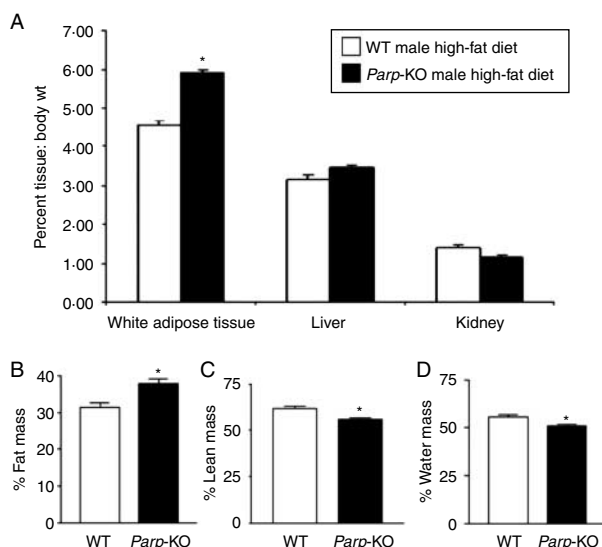


size compared with their WT counterparts. The difference in body weight became significant as early as 3 weeks post HF feeding in male *Parp*-KO mice compared with WT on HF diet or on control diet or *Parp*-KO mice on control diet (Fig. 1B). The body weight of HF diet-fed male WT mice was significantly increased from WT control diet only at and after 11 weeks (Fig. 1B). The body weight in female *Parp*-KO mice on HF diet significantly increased compared with their WT counterparts at and after 11 weeks (Fig. 1C).

#### Augmentation of body weight in HF-fed male *Parp*-KO mice was due to enhanced adipose stores

Next, we examined if the increase in body weight gain was due to increase in the mass of specific tissues. A comparison of the weight of the white adipose tissue (WAT) from omental depot demonstrated that it accounted for 5.91% of total body weight in male *Parp*-KO mice compared with 4.56% in WT mice at 15 weeks post HF feeding (Fig. 2A). No significant difference in the size or weight of liver, kidneys (Fig. 2A) brown adipose tissue, pancreas, or spleen (data not shown) was observed between male *Parp*-KO and WT mice after correction for body weight.

Analysis of the body composition of the male *Parp*-KO and WT mice, using quantitative NMR method, at the 19 week post-HF feeding revealed that *Parp*-KO mice have significantly higher fat mass (Fig. 2B), decreased lean mass (Fig. 2C), and water content (Fig. 2D) compared with WT mice.



**Figure 2** (A) WT and *Parp*-KO male mice were killed at 15 weeks post HF feeding, and tissue weights were compared. Average tissue weight is expressed as a percentage of body weight: \* $P < 0.05$  compared with WT mice on high-fat diet ( $n = 3$ ). (B) The percent of fat mass (\* $P < 0.01$ ), (C) lean mass (\* $P < 0.004$ ), and (D) water content (\* $P < 0.005$ ) in WT and *Parp*-KO male mice fed HF diet for 19 weeks were analyzed by the NMR method ( $n = 4$  per group).

#### Histological disparities in male *Parp*-KO mice post HF feeding

For histological evaluation, WAT from abdominal fat pad, liver, and brown fat (BF) were sectioned and stained using H&E followed by microscopic evaluation. Analysis of adipocyte cell size after H&E staining of WAT demonstrated a significant increase in size of the cells in male *Parp*-KO mice compared with WT mice at 19 weeks post HF feeding (Fig. 3A). Quantitation of the total number of adipocyte cells per unit area demonstrated that there was a 34% decrease implicating an increase in the size of the cells (Fig. 3B). These data suggest that the difference in body size and weight can at least partially be due to increase in WAT size and weight. Analysis of BF tissue demonstrated increased lipid content in the tissue in male *Parp*-KO mice compared with WT mice at 19 weeks post HF feeding (Fig. 3A). Quantitation of the area occupied by lipid droplets per unit area in BF tissue demonstrated an 84% increase (Fig. 3C). The increased lipid content in BF may be indicative of less number of adipocyte cells that can function to produce heat by uncoupled respiration. The lipid content in liver tissues per unit area, however, showed no significant alteration (Fig. 3A and D).

#### *Parp*-KO mice consume decreased energy

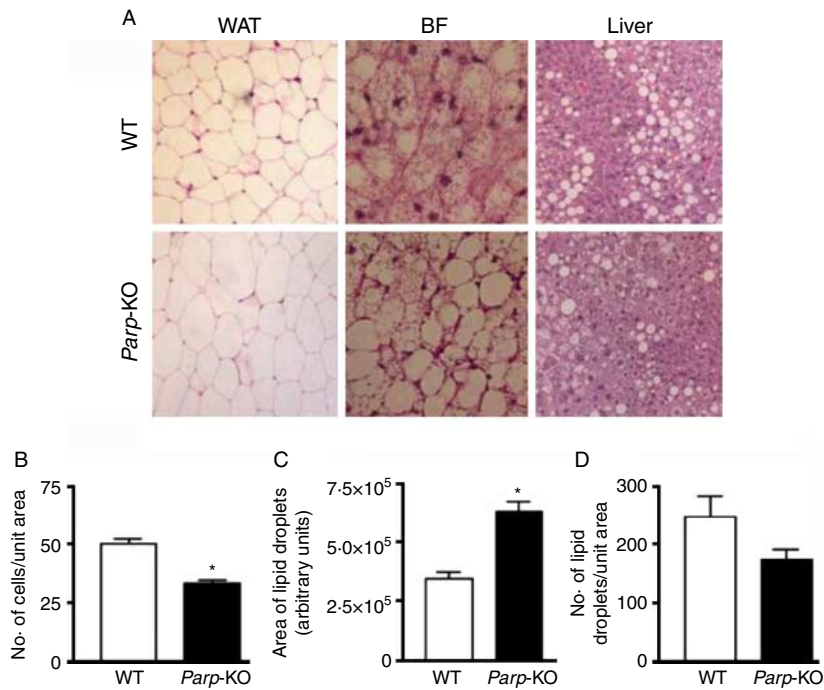
Measurement of food intake in *Parp*-KO and WT male mice demonstrated that the average feed consumption in *Parp*-KO mice is higher through the 19-week HF feeding period compared with WT mice on HF diet (Fig. 4A). Next, we examined if increased HF feed consumption in *Parp*-KO mice is a cause for their augmented body weight compared with their WT counterparts. The cumulative energy intake over a period of 19 weeks adjusted for body mass, however, demonstrated a significant decrease in male *Parp*-KO mice (12.9%) compared with their WT counterparts (Fig. 4B) ruling out hyperphagia as a cause of increased weight gain in *Parp*-KO mice.

Since the main effect of PARP1 deletion on body weight occurs during the first 3–5 weeks of the HF diet in the male mice, we analyzed the data on food intake during this period. The kcal consumption/100 g body mass was significantly lower at 3–5 week-time period in *Parp*-KO males compared with WT, and it was consistent with the data over 19 weeks. However, no significant difference was observed in females at 5 weeks. These data are in agreement with our observation that, in females, significant difference in the body weight appears at and after 11 weeks only.

#### Food absorption is not altered in *Parp*-KO mice

In order to determine if the differences in fat absorption efficiency between WT and *Parp*-KO mice were due to a difference in fat absorption, fecal lipid output was measured directly after they were fed the HF diet for 18 weeks.





**Figure 3** (A) Paraffin embedded sections of WAT, BF, and liver from WT and *Parp*-KO mice that were fed HF diet for 19 weeks were stained with hematoxylin and eosin and were photographed under 40× magnification. (B) The number of cells per camera field in WAT is quantitated using Image Pro software. \* $P < 0.005$ ;  $n = 20$ . (C) The area occupied by lipid droplets in BF per camera field. \* $P < 0.00001$ ;  $n = 8$ . (D) The number of lipid droplets in liver per camera field were also quantitated NS –  $P < 0.06$ ;  $n = 21$ . Full colour version of this figure available via <http://dx.doi.org/10.1677/JOE-09-0402>

The percent of fatty acids content was not significantly altered between HF-fed WT mice compared with *Parp*-KO mice at 19 weeks of age. The percent of palmitate (16:0) in the fecal sample was  $32.86 \pm 2.358$  ( $n = 6$ ) and  $40.63 \pm 3.470$  ( $n = 4$ ) in WT and *Parp*-KO mice respectively. Similarly, the percent of stearate (18:0) in the fecal sample was  $59.59 \pm 4.488$  ( $n = 6$ ) and  $49.34 \pm 4.081$  ( $n = 4$ ) in WT and *Parp*-KO mice respectively. These data suggest that the increased obesity in *Parp*-KO mice is not due to an alteration in the fecal lipid output when maintained on a HF diet.

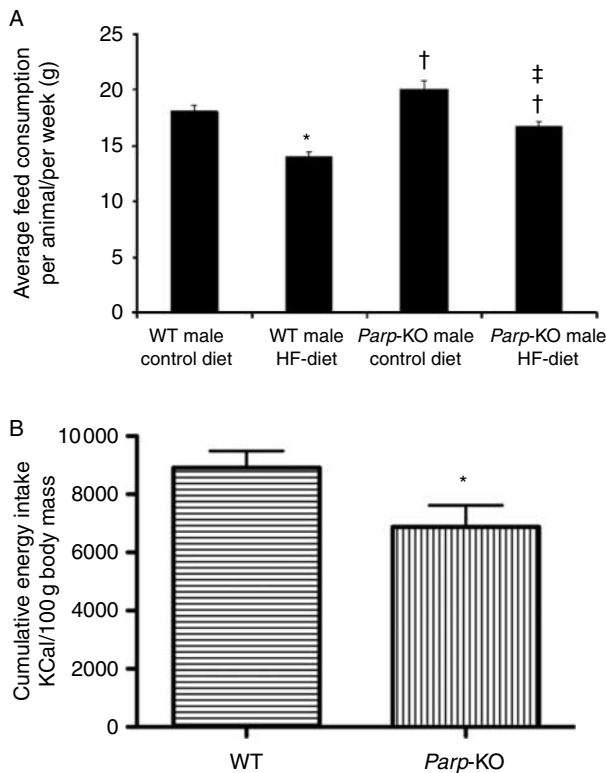
*Increased body weight in Parp-KO mice is not due to decreased fat burning or physical activity*

Locomotor activity measurements indicated that the dark cycle activity was significantly increased in *Parp*-KO mice by 59.6% compared with WT mice (Fig. 5A). The RQ of 0.76 in *Parp*-KO mice indicates that these mice used fatty acids as the main energy source, while a RQ of 0.82 in WT mice indicated the use of both carbohydrates and fat as their energy source suggesting *Parp*-KO mice burning more fat than WT mice (Fig. 5B and C). These data suggest that the increase in body weight in male *Parp*-KO mice is not due to a decrease in the physical activity or fat burning.

*Hyperleptinemia and decreased EE in male Parp-KO mice*

The circulating levels of leptin were assessed at regular intervals (weeks 1, 5, 9, and 15). The plasma levels of leptin were significantly increased as early as 1 week ( $17.89 \pm 2.89$  ng/ml compared with  $3.05 \pm 0.54$  at week 0) post HF feeding in *Parp*-KO mice, and it continued to rise throughout the 15-week period that we monitored (Fig. 6A), whereas it took 5 weeks for WT mice on HF diet to show significant increase in plasma leptin levels ( $9.22 \pm 1.16$  ng/ml compared with  $3.25 \pm 0.15$  at week 0). Interestingly, even though the plasma leptin levels at week 0 were similar, they were significantly higher right from the first week through 15 weeks in *Parp*-KO mice on HF diet compared with their WT counterparts. These results demonstrate that although the levels of leptin were increased, the food and calorie intake were also increased in *Parp*-KO mice suggesting leptin resistance. The plasma levels of leptin and insulin in mice of either groups on control diet were not significantly altered throughout the 19-week period (data not shown).

Indirect calorimetry experiments were carried out to investigate whether the increased weight gain in *Parp*-KO mice is due to decreased EE. *Parp*-KO mice consistently demonstrated a decrease in oxygen consumption compared



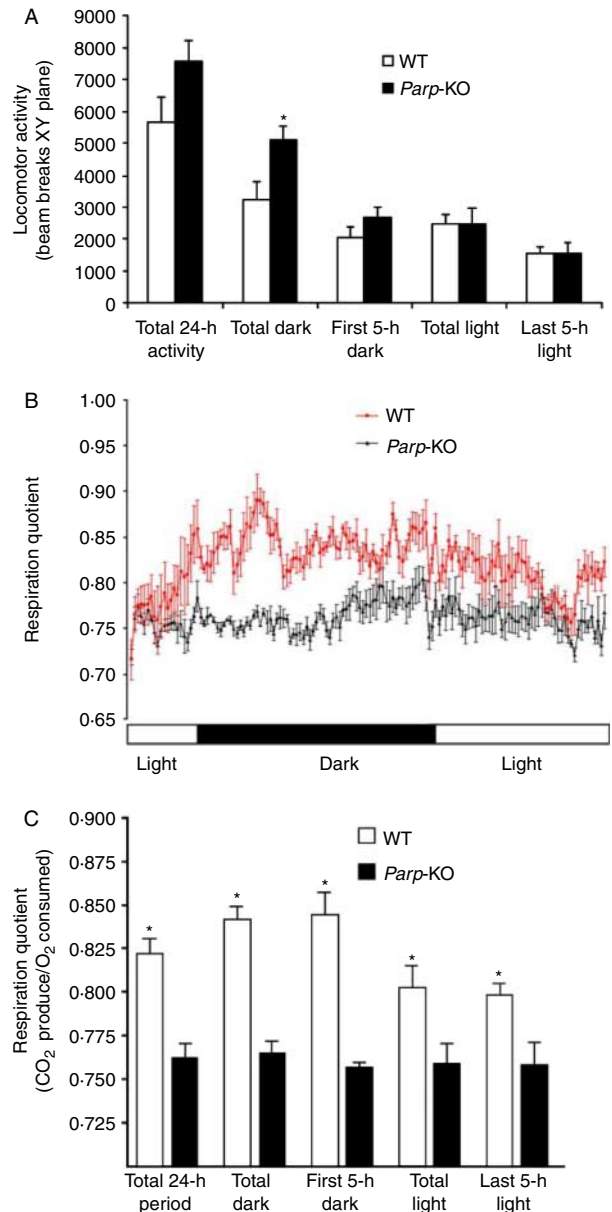
**Figure 4** (A) Average feed consumption of male mice over 19-week period: \* $P < 0.005$  compared with WT mice on control diet. <sup>†</sup> $P < 0.005$  compared with *Parp*-KO mice on control diet. <sup>‡</sup> $P < 0.005$  compared with WT mice on high-fat diet ( $n = 4-5$  for control group and  $n = 7-9$  for high-fat group). (B) Cumulative energy intake adjusted for body mass in male mice on HF diet for 19 weeks: \* $P < 0.05$  compared with WT mice on high-fat diet ( $n = 7$ ).

with WT mice ( $67.45 \pm 0.33$  in WT mice versus  $60.49 \pm 0.35$  ml/kg of body weight/min of  $VO_2$  in *Parp*-KO mice) suggesting decreased metabolic rate (Fig. 6B). Heat (cal/h per kg) was measured for 24 h (Fig. 6C), and the mean EE was calculated separately during both the dark and light cycles. *Parp*-KO mice demonstrated decreased EE during both the cycles. The mean EE of *Parp*-KO mice and WT mice during the dark cycle was 18 376 and 20 577 cal/kg per hour respectively ( $P < 0.05$ ,  $n = 4$ ), and during light cycle, it was 16 759 and 18 917 cal/kg per hour respectively ( $P < 0.05$ ,  $n = 4$ ). Consistent with decrease in EE, *Parp*-KO mice had lower core body temperature ( $36.58 \pm 0.54$ ) compared with WT mice ( $37.25 \pm 0.44$ ;  $n = 4$ ;  $P = 0.181$ ).

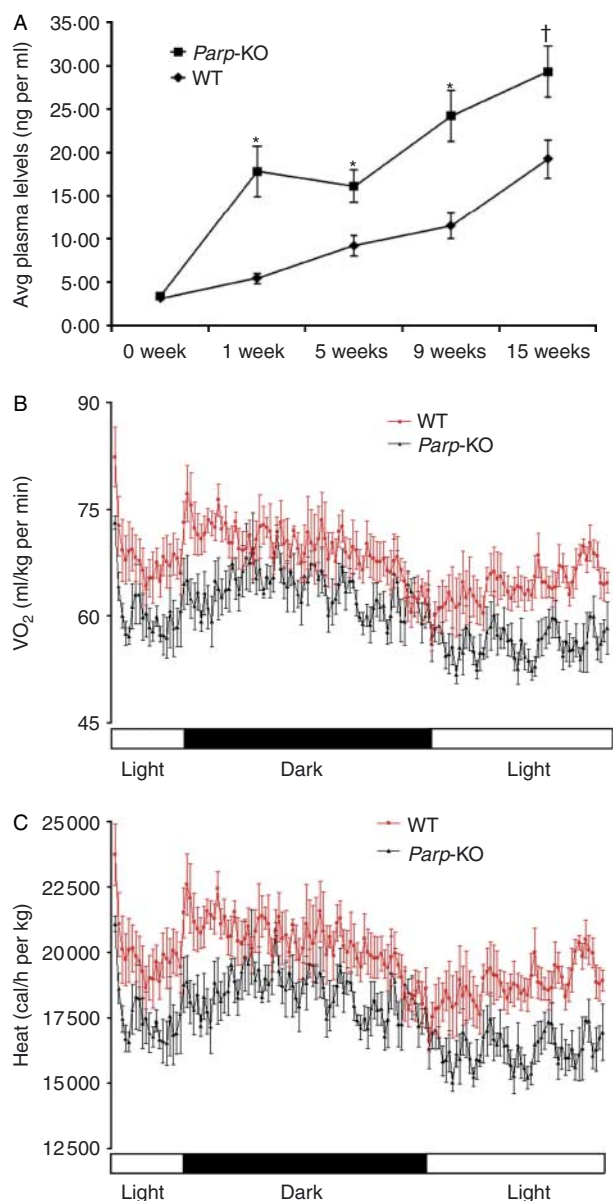
#### Insulin resistance and impaired glucose tolerance in *Parp*-KO mice

The circulating levels of insulin were assessed at regular intervals (weeks 1, 5, 9, and 15 post HF feeding). At 5–15 weeks post HF feeding, insulin levels persisted at higher levels in *Parp*-KO mice compared with their WT counterparts

suggesting the development of hyperinsulinemia in the *Parp*-KO mice (Fig. 7A). A significant increase in plasma insulin levels was observed in HF-fed *Parp*-KO mice at 9 weeks compared with mice at 0 week, while it was not significantly altered in HF-fed WT mice until 15 weeks



**Figure 5** (A) Locomotor activity of mice fed a HF diet. Locomotor activity was measured for *Parp*-KO and WT male mice that were on HF diet for a period of 19 weeks. Data are expressed as mean values  $\pm$  S.E.M. \* $P < 0.0007$ ,  $n = 4$ . (B) Comparison of the respiration quotient for a period of 24 h in WT and *Parp*-KO mice that were fed HF diet for 19 weeks. (C) The mean RQ for the dark and light cycles were quantitated separately for each group of mice. The data are expressed as mean  $\pm$  S.E.M. ( $n = 4$  per group;  $P < 0.005$ ). Full colour version of this figure available via <http://dx.doi.org/10.1677/JOE-09-0402>.

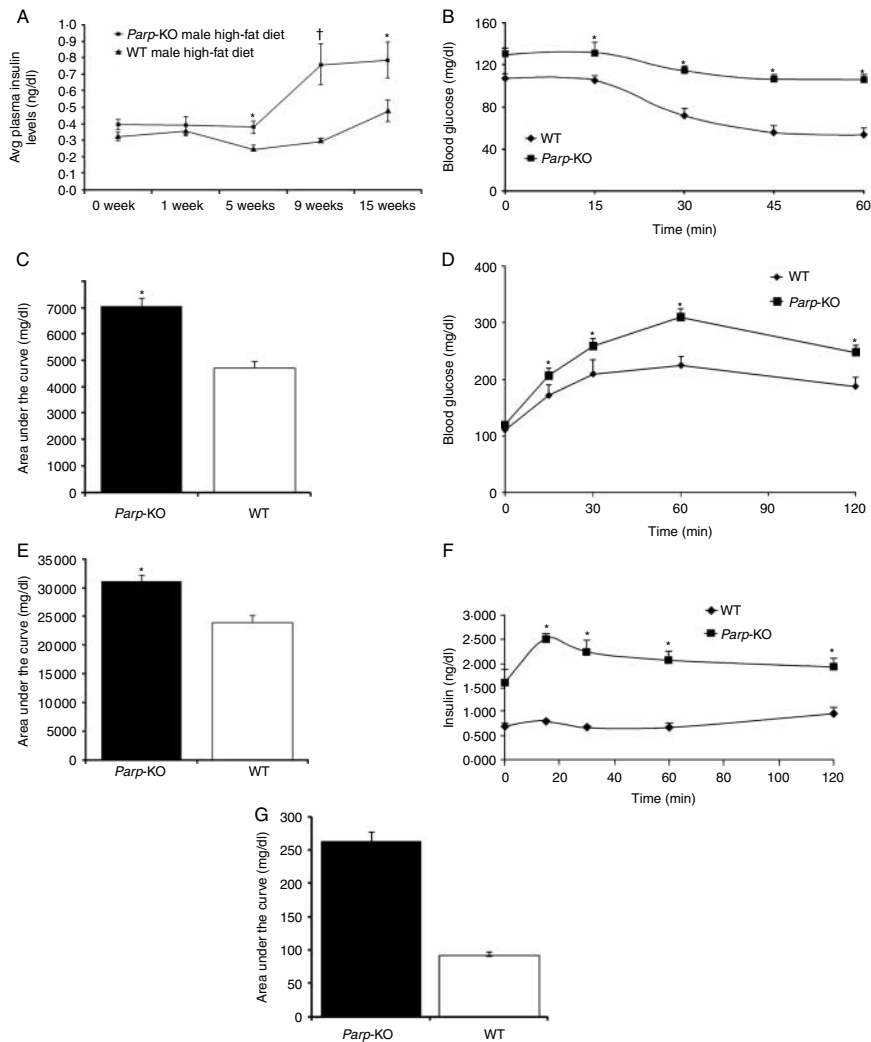


**Figure 6** (A) Average plasma leptin levels in male WT and *Parp*-KO mice; \* $P < 0.005$  compared with WT mice on high-fat diet. † $P < 0.05$  compared with WT mice on high-fat diet ( $n = 7-8$ ). Basal metabolic rate and energy expenditure are decreased in *Parp*-KO mice. (B) Comparison of the oxygen consumption for a period of 24 h for WT and *Parp*-KO mice that were fed HF diet for 19 weeks. (C) Comparison of energy expenditure by indirect calorimetry of WT and *Parp1*<sup>-/-</sup> mice that were fed HF diet for 19 weeks. Full colour version of this figure available via <http://dx.doi.org/10.1677/JOE-09-0402>.

suggesting that hyperinsulinemia sets up at earlier time in *Parp*-KO mice compared with their WT counterparts. The plasma levels of insulin in mice of either groups on control diet were not significantly altered throughout the 19-week period (data not shown).

The ability of insulin to acutely moderate the levels of glucose or its clearance was assessed by performing ITT. At 19 weeks post HF feeding, the ability of insulin to acutely stimulate glucose disposal in *Parp*-KO mice was significantly blunted throughout the 60-min period monitored suggesting decreased insulin sensitivity (Fig. 7B). The blood level of glucose was  $105.75 \pm 5.2$  mg/dl in *Parp*-KO mice compared with  $53.5 \pm 6.4$  in WT mice;  $P < 0.05$  ( $n = 4$  per group) at the 60-min time point post insulin administration. The AUC for the glucose-time profile (Fig. 7C) was  $7052.5 \pm 297.4$  mg/dl in *Parp*-KO mice compared with  $4712.5 \pm 235.9$  in WT mice;  $P < 0.001$ ; ( $n = 4$  per group).

In order to determine if the increased obesity and adiposity in *Parp*-KO mice are accompanied by abnormalities in glucose homeostasis, we performed IPGTT. WT mice and *Parp*-KO mice that were fed HF diet for 19 weeks were fasted for 5 h followed by i.p. injection with 20% D-glucose. Tail blood was collected for measurement of glucose and insulin. Consistent with their increased obesity and adipose stores, *Parp*-KO mice had decreased ability for glucose clearance as the serum glucose levels were significantly higher over a 120-min period. Serum glucose levels were elevated at 15 min and reached even higher levels at later time points. The average values of glucose in mg/dl in WT were 110.1, 173.5, 210.1, 225.8, and 187.4, and of those in *Parp*-KO mice were 117.4, 205.0, 267.6, 309.6, and 247.8 at 0, 15, 30, 60, and 120 min post glucose administration (Fig. 7D). The AUC for glucose values 120 min post glucose administration during IPGTT (Fig. 7E) was  $31117.2 \pm 1074.9$  in *Parp*-KO mice compared with  $23941.3 \pm 1270.1$  in WT mice;  $P < 0.005$ ;  $n = 4$  per group. In a separate IPGTT, insulin measurements were performed over the 120-min post-glucose administration period. Interestingly, the plasma insulin levels were significantly higher in *Parp*-KO mice (1.588, 2.489, 2.230, 2.062, and 1.912 ng/dl) compared with WT mice (0.707, 0.791, 0.661, 0.681, and 0.968 ng/dl) at 0, 15, 30, 60, and 120 min post glucose administration (Fig. 7F). The AUC measurements for insulin values 120 min post glucose administration during IPGTT (Fig. 7G) was  $262.5 \pm 14.9$  in *Parp*-KO mice compared with  $91.75 \pm 6.2$  in WT mice;  $P < 0.0001$ ;  $n = 4$  per group. Despite the increase in insulin levels observed in *Parp*-KO mice, the glucose levels remained at higher levels in *Parp*-KO mice compared with WT mice during this period. These data suggest that at 19 weeks post HF feeding, *Parp*-KO mice have developed insulin resistance and impaired glucose tolerance. However, the average fasting blood glucose levels post HF diet at 1 week (92.44 mg/dl) and 15 weeks (109.89 mg/dl) in WT mice did not differ significantly from that in *Parp*-KO mice at corresponding time points (90.14 mg/dl at 1 week and 114.29 mg/dl at 15 weeks). The percent deflection from baseline was not varied between the two groups in the summary measures for the three graphs 7B–D.



**Figure 7** (A) Average plasma insulin levels in male WT and *Parp*-KO mice: \* $P < 0.05$  compared with WT mice on high-fat diet. † $P < 0.005$  compared with WT mice on high-fat diet ( $n = 5-8$ ). (B) Comparison of insulin tolerance by ITT between WT and *Parp*-KO male mice at 19 weeks post HF feeding: blood glucose levels, \* $P < 0.05$ ,  $n = 4$ . (C) Total area under the curve (AUC) for the glucose-time profile during ITT. (D) Comparison of glucose tolerance by IPGTT between WT and *Parp*-KO male mice at 19 weeks post HF feeding: blood glucose levels, \* $P < 0.05$ ,  $n = 4$ . (E) Total AUC for the glucose-time profile during IPGTT. (F) Comparison of glucose tolerance between WT and *Parp*-KO male mice: plasma insulin levels, \* $P < 0.05$ ,  $n = 4$ . (G) Total AUC for insulin values during IPGTT. Values are expressed as means  $\pm$  S.E.M.

## Discussion

In this study, we demonstrated that when mice with *Parp1* deficiency on a 129 background are challenged with a HF diet, the mice exhibited increased susceptibility to weight gain, energy imbalance, hyperleptinemia and insulin resistance, and glucose intolerance when compared with their WT counterparts. The male PARP1-deficient mice on HF diet gained higher body weight as early as 3 weeks compared with WT mice on HF diet or control diet. Whereas, male WT mice on

HF diet attained significantly higher body weight only at and after 11 weeks compared with WT mice on control diet. These results suggest that PARP1-mediated signaling pathways, such as poly (ADP-ribosyl)ation of PARP or other substrates and/or its function as a transcriptional cofactor, is a requirement in controlling the energy metabolism, accumulation of fat depots and augmented weight gain during HF feeding in 129 mice. It would be interesting to determine the effect of *Parp1* gene deletion on the development of obesity in obesity-prone genetic background such as the B6 background.



To understand the mechanism by which PARP1 deficiency leads to exacerbation of obesity, the body weight and fat mass, food intake, metabolic level, EE, hormonal milieu, and physical activity level were measured in *Parp*-KO mice. Our data indicate that several possible mechanisms could contribute to the increased obesity demonstrated in mice due to PARP1 deficiency. Cumulative energy intake data demonstrate that HF-fed *Parp*-KO male mice consumed lower energy on HF diet. These data suggest that the weight gain in *Parp*-KO mice is not due to increased consumption of energy. Our data also demonstrate that there are no significant changes in absorption of the fat content as the fecal content of fat was not altered between the groups. Taken together, these findings suggest that an altered energy utilization may account for the exacerbated obesity phenotype in *Parp*-KO mice. The histological appearance of brown adipose tissue in HF-fed *Parp*-KO mice demonstrated increased lipid droplets with much larger size than those from WT mice. These data suggest that the number of functional brown adipose cells is reduced in *Parp*-KO mice, and that their capacity to uncouple respiration to promote EE and thus decrease in fat content may be compromised.

Paradoxically, despite the weight gain observed in HF-fed *Parp*-KO mice, they have demonstrated a 59.6% increase in locomotor activity during the darkness cycle compared with their WT counterparts. Similarly, PARP1 deficiency decreased the oxygen consumption ( $\text{VO}_2$ ) as well as the  $\text{CO}_2$  produced ( $\text{VCO}_2$ ) throughout the 24-h time period that we measured. The alterations in  $\text{VO}_2$  and in  $\text{VCO}_2$  resulted in a decreased RQ of 0.76 compared with RQ of 0.82 in WT mice. The decrease in RQ suggests that PARP1 deficiency increases fat oxidation and thus the utilization of fat as an energy source. Thus, the stimulatory effect of PARP1 on body weight and fat mass may not be influenced by the RQ and the counterintuitive increase in fat oxidation or physical activity. It should be noted that when  $\text{VO}_2$  is corrected for lean mass, no changes in  $\text{VO}_2$  was observed between WT and *Parp*-KO mice suggesting that alterations in basal metabolic rate may not be a key determinant in the exacerbation of weight gain in *Parp*-KO mice.

In this study, we show that HF-fed *Parp*-KO mice compared with their WT counterparts had decreased EE. EE derives from thermogenesis resulting from cellular metabolic processes, including basal metabolism, adaptive thermogenesis, and physical activity. Basal metabolism represents the heat production of the body in a thermoneutral environment, under resting conditions (Rolfe & Brown 1997). Our core body temperature measurements indicate that the basal metabolism is lowered in HF-fed *Parp*-KO mice, consistent with a decrease in total EE and decreased oxygen consumption. On the contrary, physical activity in HF-fed *Parp*-KO mice was not altered, and in fact, there was a trend towards increased activity. Adaptive thermogenesis is the process by which energy is dissipated in the form of heat in response to environmental changes such as exposure to cold and alterations in diet (Spiegelman & Flier 2001). Under homeostatic

conditions, adaptive thermogenesis is a key mechanism by which body maintains core temperature and regulates EE, and its dysregulation promotes obesity (Fan *et al.* 2005).

The leptin-regulated central melanocortin system is the best characterized and most clinically relevant pathway that regulates energy intake and thermogenesis (Spiegelman & Flier 2001). The circulating leptin level was increased in HF-fed *Parp*-KO mice compared with their WT counterparts as early as 1 week post HF feeding, a time at which the body weights were not significantly altered between WT and *Parp*-KO mice. Given the association between obesity and increased production of leptin in most human subjects (Hamilton *et al.* 1995) and in most animal models of obesity, the increased leptin levels would be expected to decrease food intake and weight gain in *Parp*-KO mice. On the contrary, despite the increased levels of leptin, the animals are hyperphagic, suggesting that the animals may have developed leptin resistance. The cellular basis for the leptin resistance observed in *Parp*-KO mice has not been addressed in this study.

An important question that is raised from the results of the study pertains to the association between PARP1, obesity and insulin resistance in the setting of high-calorie intake. Insulin resistance following HF diet may involve multiple target organs including adipose tissue, liver, skeletal muscle and interactions among these three organs linking to neurogenic mechanisms (Uno *et al.* 2006), and/or circulating factors (Yang *et al.* 2005). While increased obesity contributes to insulin resistance, it is possible that decreased insulin sensitivity in the skeletal muscle of HF-fed *Parp*-KO mice may lead to decreased metabolic rate and heat generation and the consequent accumulation of fat tissue.

To conclude, our findings define PARP1 expression and activation as a critical determinant in energy metabolism in 129 mice in the setting of HF feeding. Our data indicate that PARP1 deficiency reverses resistance to diet-induced obesity by decreasing EE suggesting that the PARP1 signaling pathways are upstream of effector mechanisms that regulate EE. Absence of PARP1 leads to the exacerbation of obesity, hyperleptinemia, and insulin resistance. Based on the current knowledge on PARP functions, PARP could regulate the expression of multiple genes by acting as a transcriptional cofactor, and it can modulate the functions of several proteins by poly (ADP-ribosylation). Identification of protein targets that are post-translationally regulated by PARP and genes whose expression is altered as a function of PARP1 expression might lead to elucidation of the molecular mechanisms by which PARP1 signaling modulate appetite and EE. Identification of such molecular mechanisms may provide new potential therapeutic opportunities for obesity and its associated pathologies.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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