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ORIGINAL ARTICLE

Energy metabolism in BPH/2J genetically hypertensive mice

Kristy L Jackson[1,2], Thu-Phuc Nguyen-Huu[1], Pamela J Davern[1,3] and Geoffrey A Head[1,2,3]

Recent evidence indicates that genetic hypertension in BPH/2J mice is sympathetically mediated, but these mice also have lower body weight (BW) and elevated locomotor activity compared with BPN/3J normotensive mice, suggestive of metabolic abnormalities. The aim of the present study was to determine whether hypertension in BPH/2J mice is associated with metabolic differences. Whole-body metabolic and cardiovascular parameters were measured over 24 h by indirect calorimetry and radiotelemetry respectively, in conscious young (10–13 weeks) and older (22–23 weeks) BPH/2J, normotensive BPN/3J and C57Bl6 mice. Blood pressure (BP) was greater in BPH/2J compared with both normotensive strains at both ages ($P<0.01$). Metabolic rate was greater in young BPH/2J compared with BPN/3J mice ($P<0.01$) but similar to C57Bl6 mice indicating that high metabolic rate is not necessarily related to the hypertension per say. The slope of the BP-metabolic rate relationship was comparable between BPH/2J and normotensive mice when adjusted for activity ($P>0.1$) suggesting differences in this relationship are not responsible for hypertension. EchoMRI revealed that percentage body composition was comparable in BPN/3J and BPH/2J mice ($P>0.1$) and both strains gained weight similarly with age ($P=0.3$). Taken together, the present findings indicate that hypertension in BPH/2J mice does not appear to be related to altered energy metabolism.
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Keywords: BPH/2J mice; energy; metabolism

INTRODUCTION

BPH/2J mice have been used for nearly 40 years as a genetic mouse model of hypertension together with their normotensive (BPN/3J) control mice counterparts. BPH/2J mice were selectively bred for high blood pressure (BP), whereas normotensive BPN/3J mice were concurrently bred by random selection from the same base population.[1] BPH/2J mice have been studied extensively in relation to physiological and genetic determinants of hypertension.[2–5] Recently the hypertension in BPH/2J mice has been recognized as neurogenic as ganglion blockade abolished the hypertension in BPH/2J mice, and spectral analysis of BP revealed a greater power in the mid-frequency band indicating overactivity of the sympathetic nervous system (SNS).[6] Hypertensive BPH/2J mice also appear to have indications of underlying abnormalities in energy metabolism. They are hyperactive during the dark (active) period compared with normotensive BPN/3J mice[5,6] and have markedly lower body weight (BW) from as young as 1 month of age[4,6,7], suggestive of a possible imbalance in energy input and output. BPH/2J mice are also reported to have abnormal thermoregulation based on greater thermosensitivity to acute heat exposure[8] and following chronic heat exposure markedly greater hypotension[9] suggesting a relationship between thermoregulation and BP. Taken together, these hypertensive mice have a number of signs of abnormal energy metabolism but energy homeostasis has not been explicitly assessed in BPH/2J mice.

The metabolic abnormalities that are commonly associated with hypertension include obesity, dyslipidaemia and diabetes, which have been speculated to be linked to hypertension via factors including insulin signaling and activation of the sympatho-adrenal system.[10] Elevations in metabolic rate are also associated with hypertension,[11,12] although even in studies of normotensive individuals there appears to be a positive correlation between the BP level and basal metabolic rate.[13,14] The factors contributing to this relationship are unclear but differences in SNS tone are recognized as one of the possible mechanisms mediating this association.[11,15,16] Yet metabolic rate is also influenced by factors including BW, body composition and physical activity.[14,17,18] Therefore, these variables should be factored into any assessment of metabolic rate.

The main aim of the present study was to determine whether hypertension in BPH/2J mice is associated with an altered energy metabolism. First, a comprehensive characterization of metabolic parameters using an indirect calorimetric system and EchoMRI was undertaken to determine whether there were differences in BPH/2J

[1]Neuropharmacology Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia and [2]Department of Pharmacology, Monash University, Clayton, Victoria, Australia
[3]These authors contributed equally to this work as senior authors.
Correspondence: Professor GA Head, Neuropharmacology Laboratory, Baker IDI Heart and Diabetes Research Institute, 75 Commercial Road, Melbourne, Victoria 8008, Australia.
E-mail: geoff.head@baker.edu.au
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Glossary

SNS	Sympathetic nervous system
BP	Blood pressure
HR	Heart rate
MAP	Mean arterial pressure
SAP	Systolic arterial pressure
DAP	Diastolic arterial pressure
BW	Body weight
VO2	Oxygen consumption
VCO2	Carbon dioxide production
RER	Respiratory exchange ratio

mice compared with two normotensive control strains. Furthermore circadian and developmental differences were determined by assessing changes over a 24-h period as well as by a comparison between two different ages. Normotensive BPN/3J mice were assessed because they were bred alongside BPH/2J mice from the same base population, but these mice have lower locomotor activity levels than BPH/2J mice.[1,6] To take into consideration the contribution of physical activity to metabolic rate, normotensive C57Bl6 were also assessed because they have a similar locomotor activity profile as BPH/2J mice.[6,18]

MATERIALS AND METHODS

Metabolic, EchoMRI and cardiovascular measurements were assessed in young (10–13 weeks old) and older (22–23 weeks old) hypertensive BPH/2J mice and normotensive BPN/3J and in C57Bl6 male mice ($n = 5$–6 per group). Experiments were conducted at room temperature ($24 \pm 0.5\,^{\circ}C$) and $37 \pm 1.3\%$ humidity. The mice were individually housed in a room with 12:12 h light–dark cycle with access *ad libitum* to water and mouse chow (Specialty Feeds, Glen Forrest, Western Australia, 19% protein, 5% fat, 5% fiber and 0.2% sodium). The experiments were approved by the Alfred Medical Research Education Precinct Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for Scientific Use of Animals.

Cardiovascular measurements

BP and activity radiotelemetry transmitters (model TA11PA-C10; Data Sciences International, St Paul, MN, USA) were implanted under isoflurane open circuit anesthesia (5% induction and 1.5–2% maintenance). Carprofen ($5\,mg\,kg^{-1}$) (Rimadyl, Pfizer Australia Pty Ltd, West Ryde, NSW, Australia) was administered subcutaneously just before surgery and 24-hours post-surgery for analgesia. The catheter of the telemetry device was inserted into the carotid artery and the transmitter probe was positioned subcutaneously along the right flank.[19] After a 10-day surgical recovery period, a 48-h measurement of BP and locomotor activity was recorded. Continuous recordings of systolic (SAP), diastolic (DAP) and calculated mean arterial pressure (MAP), heart rate and locomotor activity were measured in freely moving mice in their home cage. The recordings were sampled at 1000 Hz using an analog-to-digital data acquisition card (National Instruments 6024E) as described previously.[20]

Body composition measurements

Body composition was measured using an EchoMRI body composition analyser (EchoMRI-4in1, Columbus instruments, Columbus, OH, USA). Mice were restrained but not anesthetized during measurements, and lean mass, fat mass and total water mass were determined based on radio pulse emission properties to differentiate between tissue types.

Metabolic measurements

Mice were placed individually into open circuit indirect calorimeters (Comprehensive Lab Animal Monitoring System (CLAMS), Columbus Instruments, Columbus, OH, USA) and following at least 2-h of acclimatization[21], oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured at 15-min intervals over the 24-h period. Respiratory exchange ratio (RER) was defined as the ratio of VCO_2/VO_2. Metabolic rate was calculated using the following equation.[22]

$$\text{Metabolic rate (heat production)} = (3.815 + 1.232 \times \text{RER}) \times VO_2$$

Activity was measured by counting the interruption of infrared beams aligned on three axes and total activity was represented as the log (base10) of the sum of counts in both horizontal planes. Food intake was determined as the difference in food weight between the initial and final 24-h period as measured by a hopper weight scale built into the chamber.

Statistical analysis

Cardiovascular, metabolic and EchoMRI data were expressed as mean ± standard error of the mean (s.e.m.). The data were analyzed by multifactor, nested split-plot analysis of variance, which allowed for within animal and between animal contrasts.[23] A combined residual was used that pooled the between and within animal variance as described previously.[24] Multiple

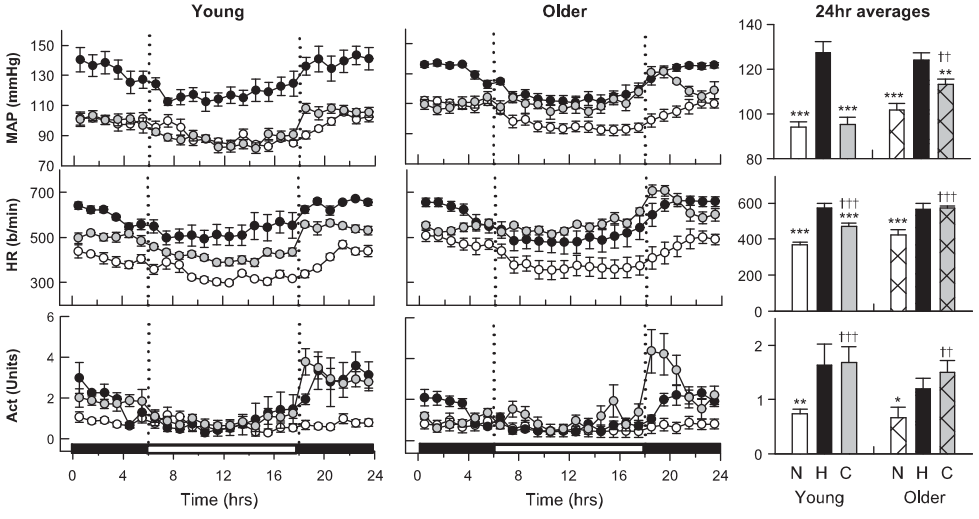

Figure 1 Hourly averaged data showing the circadian variation of MAP (mmHg), heart rate (HR) (beats per min) and activity (units) during the dark (active) (outer panels) and light (inactive) (middle panel) periods in BPN/3J (white circles), BPH/2J (black circles) and C57Bl6 mice (gray circles). Data are from young (left) and older mice (right). Bar graph shows BPN/3J (N, white bars), BPH/2J (H, black bars) and C57Bl6 (C, gray bars) when young (non-hatched) and old (hatched). Values are mean ± s.e.m. Comparison of BPH/2J with either normotensive BPN/3J or C57Bl6 mice represented by $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$. Comparison between BPN/3J *vs.* C57Bl6 mice represented by $^{\dagger\dagger}P<0.01$; $^{\dagger\dagger\dagger}P<0.001$.

regressions were analyzed using analysis of covariance. A probability of $P<0.05$ was considered significant.

RESULTS

Cardiovascular measurements

MAP was 35% higher in young BPH/2J mice ($n=5$) than in normotensive BPN/3J ($n=5$) or C57Bl6 mice ($n=6$, $P<0.001$ both, Figure 1), whereas similar levels were recorded in both normotensive strains ($P=0.7$). MAP in older BPH/2J ($n=6$) was 22% higher than that observed in BPN/3J ($n=6$, $P<0.001$) and 10% greater than C57Bl6 mice ($n=6$, $P=0.002$). Heart rate in young BPH/2J mice was higher than both normotensive strains ($P<0.001$ both, Figure 1). However, in older mice, heart rate was similar in BPH/2J and C57Bl6 mice ($P=0.8$), but BPN/3J mice had a markedly lower heart rate than both BPH/2J and C57Bl6 mice ($P<0.001$ both). Locomotor activity of young and older BPH/2J and C57Bl6 mice was greater than BPN/3J mice ($P<0.05$ all, Figure 1).

Metabolic rate

Raw metabolic rate (kcal h^{-1}) in young BPH/2J was greater than BPN/3J ($P=0.008$) but similar to C57Bl6 mice ($P=0.07$, Table 1). However, raw metabolic rate was comparable between all three strains in older mice ($P>0.2$). If normalized to BW, metabolic rate (kcal kg^{-1} per day) in young BPH/2J was 39% greater than BPN/3J ($P<0.001$) but similar to C57Bl6 mice ($P=0.2$, Figure 2). Metabolic rate in C57Bl6 was also 31% greater than BPN/3J mice ($P<0.001$). Older BPH/2J had 26% greater metabolic rate per kg (kcal kg^{-1} h^{-1}) than BPN/3J ($P<0.001$), which was similar to the 21% greater rate in C57Bl6 mice ($P=0.4$). There was a reduction in metabolic rate (per kg BW) in all strains with age ($P_{age}<0.01$ all).

Table 1 Average values of metabolic rate, VO_2, VCO_2, RER, activity, food and water intake over a 24-h period in BPN/3J, BPH/2J and C57Bl6 mice

	Young			*Old*		
	BPN/3J	*BPH/2J*	*C57Bl6*	*BPN/3J*	*BPH/2J*	*C57Bl6*
Metabolic rate (Raw) (kcal h^{-1})	0.56 ± 0.02**	0.65 ± 0.03	0.71 ± 0.03†††	0.61 ± 0.02	0.63 ± 0.02	0.65 ± 0.03
Metabolic rate (normalized to BW) (kcal kg^{-1} h^{-1})	22.1 ± 0.7***	30.6 ± 1.6	29.1 ± 1.1†††	18.6 ± 0.6***	23.5 ± 1.1	22.4 ± 0.8††
Metabolic rate (normalized to lean mass) (kcal kg^{-1} h^{-1})	25.6 ± 0.8***	33.3 ± 1.6	31.9 ± 1.4†††	24.4 ± 0.6***	29.3 ± 0.8	25.0 ± 1.0**
VO_2 (l kg^{-1} h^{-1})	4.5 ± 0.1***	6.2 ± 0.3	5.9 ± 0.2†††	3.8 ± 0.1***	4.9 ± 0.2	4.6 ± 0.2††
VCO_2 (l kg^{-1} h^{-1})	3.9 ± 0.1***	5.6 ± 0.3	5.2 ± 0.2†††	3.2 ± 0.1**	3.9 ± 0.2	4.0 ± 0.1††
RER (Units)	0.86 ± 0.01	0.89 ± 0.02	0.89 ± 0.01	0.85 ± 0.01***	0.79 ± 0.02	0.87 ± 0.01***
Total activity (log counts per hour)	7.7 ± 0.4***	11.1 ± 0.7	8.9 ± 0.6***,††	6.2 ± 0.3***	9.1 ± 0.8	8.4 ± 0.7†††
Food intake (grams)	3.7 ± 0.3*	4.8 ± 0.3	4.0 ± 0.0	3.1 ± 0.4	2.9 ± 0.6	5.0 ± 0.4***,††
Water intake (ml)	5.7 ± 0.3	6.3 ± 0.4	6.6 ± 0.4	3.2 ± 0.3	3.9 ± 0.5	3.3 ± 0.2

Abbreviations: RER, respiratory exchange ratio; VO_2, oxygen consumption; VCO_2, carbon dioxide production.
Comparison of BPH/2J with either normotensive BPN/3J or C57Bl6 mice represented by *$P<0.05$; **$P<0.01$; ***$P<0.001$.
Comparison between BPN/3J *vs.* C57Bl6 mice represented by ††$P<0.01$; †††$P<0.001$.

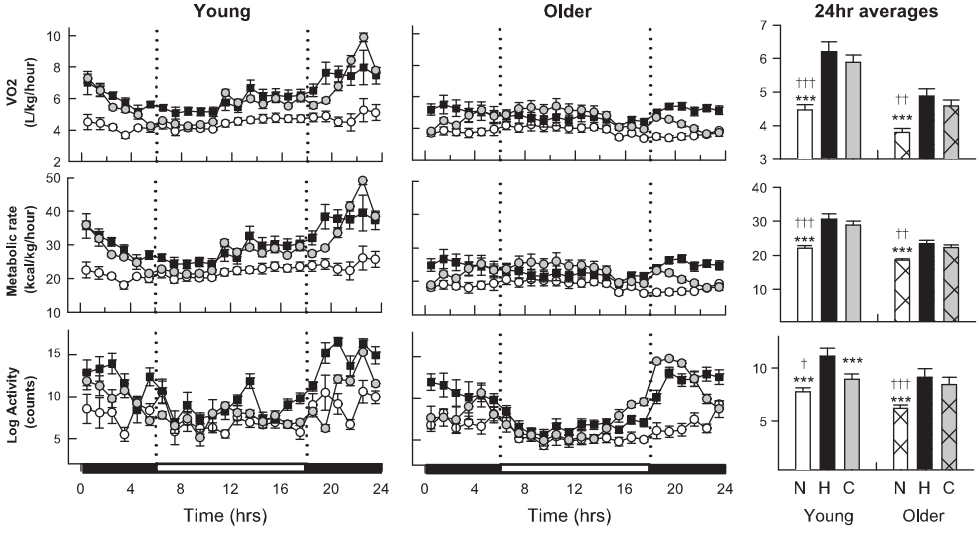

Figure 2 Hourly averaged data showing the circadian variation of VO_2 (l kg^{-1} h^{-1}), metabolic rate (kcal kg^{-1} h^{-1}) and log activity (counts) during the dark (active) (outer panels) and light (inactive) (middle panel) phases in BPN/3J (white circles), BPH/2J (black circles) and C57Bl6 mice (gray circles). Data are from young (left) and older mice (right). Bar graph shows BPN/3J (N, white bars), BPH/2J (H, black bars) and C57Bl6 mice (C, gray bars) when young (non-hatched) and older (hatched). Values are mean ± s.e.m. Comparison of BPH/2J with either normotensive BPN/3J or C57Bl6 mice represented by ***$P<0.001$. Comparison between BPN/3J *vs.* C57Bl6 mice represented by †$P<0.05$; ††$P<0.01$; †††$P<0.001$.

Ambulatory activity

Total (log) ambulatory activity in young BPH/2J was greater than both BPN/3J and C57Bl6 mice ($P<0.001$ both, Table 1, Figure 2). Even so, activity in C57Bl6 was also greater than BPN/3J mice ($P=0.007$). Older BPH/2J mice were more active than BPN/3J ($P<0.001$) but similar to C57Bl6 mice ($P=0.1$). Although activity declined with age in both BPN/3J and BPH/2J ($P_{age}<0.001$), there was no change observed in C57Bl6 mice ($P_{age}=0.2$).

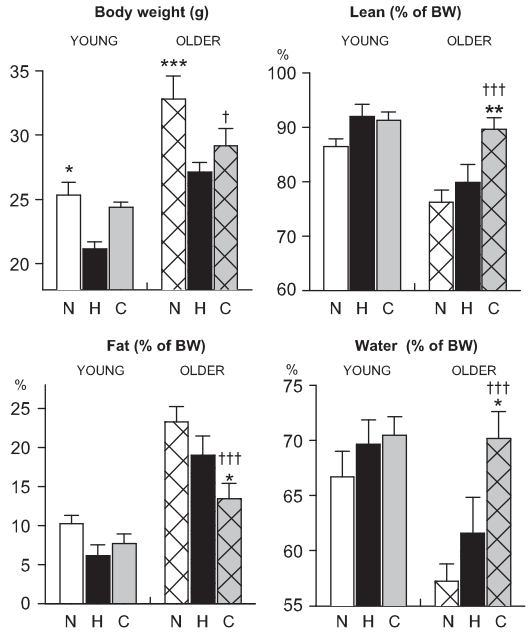

Figure 3 Bar graphs represents BW, percentage lean mass, percentage fat mass and percentage water content in BPN/3J (N, white, $n=6$), BPH/2J (H, black, $n=6$) and C57Bl6 mice (C, gray, $n=5$) in young (unhatched) and older mice (hatched). Values are mean ± s.e.m. Comparison of BPH/2J with either normotensive BPN/3J or C57Bl6 mice represented by $*P<0.05$; $**P<0.01$; $***P<0.001$. Comparison between BPN/3J *vs.* C57Bl6 mice represented by $\dagger P<0.05$; $\dagger\dagger\dagger P<0.001$.

Respiratory exchange ratio

RER was comparable between all strains in young mice ($P>0.07$, Table 1). However, RER in older BPH/2J (0.79) was lower than BPN/3J (0.85) and C57Bl6 mice (0.87, $P<0.001$ both). There was also an 11% reduction in RER with age in BPH/2J mice ($P_{age}<0.001$), which did not occur in the normotensive strains ($P_{age}>0.2$ both).

Food and water intake

Food intake over a 24-h period was 32% greater in young BPH/2J than BPN/3J ($P=0.03$, Table 1) but similar to C57Bl6 mice ($P=0.14$). Although only BPH/2J mice showed a reduction in food intake with age ($P_{age}=0.004$), by contrast, older C57Bl6 ingested more food than BPN/3J and BPH/2J mice ($P<0.01$ both). Water intake was similar between strains at both ages ($P>0.2$ all) and decreased with age ($P_{age}<0.001$, Table 1).

BODY WEIGHT

BW in young BPH/2J mice was 16% lower than BPN/3J ($P=0.010$) and 13% lower than C57Bl6 mice ($P=0.05$, Figure 3). All three strains gained more than 20% of their BW over the 13-week experimental period ($P<0.001$ all) but the absolute increase in BW with age was similar between BPH/2J mice and both normotensive strains ($P>0.3$, Figure 4). BW of older BPN/3J was greater than both BPH/2J and C57/Bl6 mice ($P<0.05$ both) but similar between BPH/2J and C57Bl6 mice ($P=0.2$).

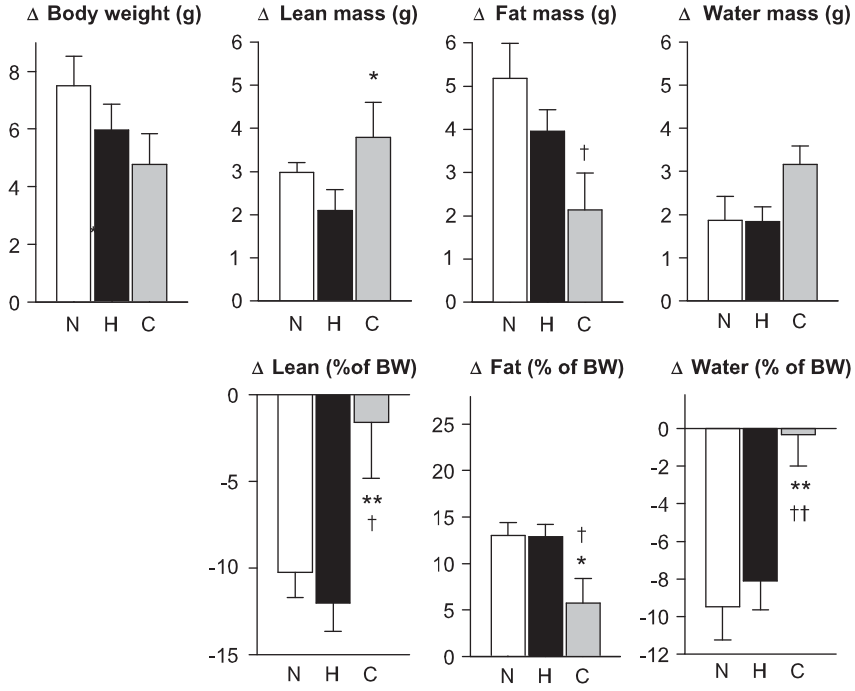

Figure 4 Bar graphs represents change in BW (g), lean mass (g), fat mass (g), water mass (g), and percentage lean mass (% of BW), percentage fat mass (% of BW) and percentage water mass (% of BW), in BPN/3J (N, white, $n=6$), BPH/2J (H, black, $n=6$) and C57Bl6 mice (C, gray, $n=5$) from young to older mice. Values are mean ± s.e.m. Comparison of BPH/2J with either normotensive BPN/3J or C57Bl6 mice represented by $*P<0.05$; $**P<0.01$. Comparison between BPN/3J *vs.* C57Bl6 mice represented by $\dagger P<0.05$; $\dagger\dagger P<0.01$.

Lean mass

Lean mass as a percentage of BW was similar in young BPH/2J (92%) compared with C57Bl6 (91%, $P=0.8$) and BPN/3J mice (86%, $P=0.09$, Figure 3). Percentage lean mass was maintained with age in C57Bl6 ($P_{age}=0.5$) but reduced by a comparable 12–13% in BPN/3J and BPH/2J mice ($P=0.6$, $P_{age}<0.001$ *vs.* C57Bl6, Figure 4). Similar percentage lean mass was found in older BPN/3J and BPH/2J mice ($P=0.2$) but older C57Bl6 mice had markedly higher lean content than BPN/3J and BPH/2J mice ($P<0.01$, Figure 3).

Fat mass

Percentage fat content was similar in all three strains when young ($P>0.1$, Figure 3) and this increased with age in all strains ($P_{age}<0.01$). Percentage fat mass in older C57Bl6 mice was lower than BPN/3J ($P=0.001$) and BPH/2J mice ($P=0.039$), whereas BPN/3J and BPH/2J mice were similar ($P=0.09$, Figure 3).

Correlations between BW with metabolic rate

There were negative correlations between BW and metabolic rate in all three strains ($P<0.05$ all, Figure 5a) and the slope and elevation of

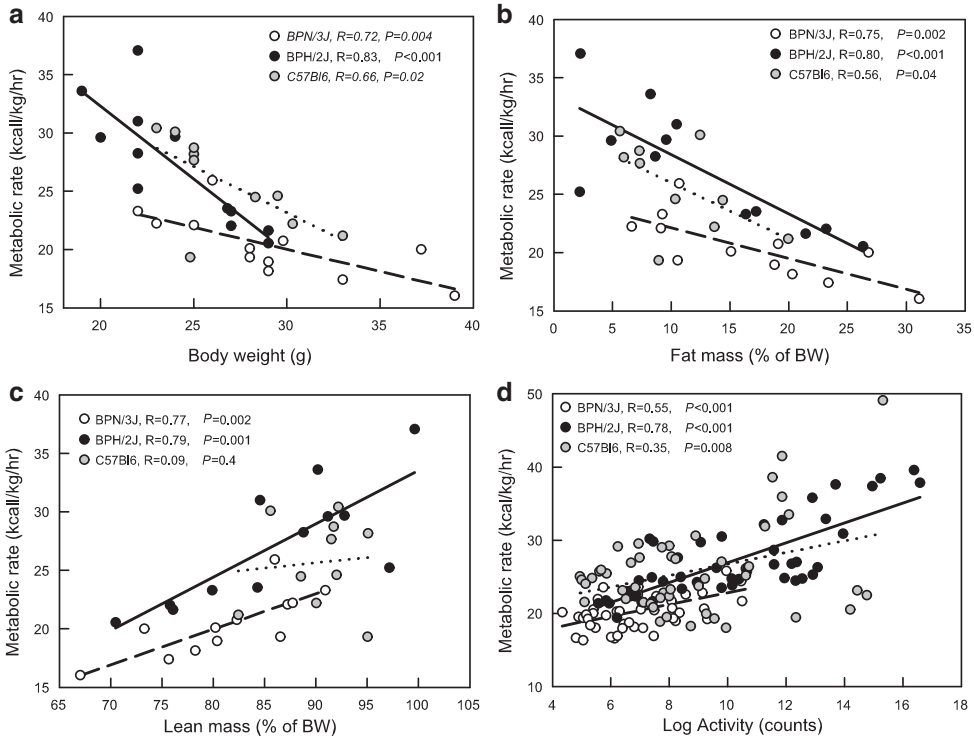

Figure 5 Correlation between metabolic rate (24-h average, kcal kg^{-1} of BW per hour) versus (**a**); BW (grams) (**b**); percentage fat mass and (**c**); percentage lean mass (**d**); average hourly values of metabolic rate versus log activity from BPN/3J (white circles), BPH/2J (black circles) and C57Bl6 mice (gray circles) of both ages. Dashed lines represent regression lines for BPN/3J mice, solid line represents regression lines for BPH/2J mice and dotted line represents regression line for C57Bl6 mice.

Table 2 Slope and elevation of regressions reported in Figures 5 and 6

		Slope			*Elevation*		
		BPN/3J	*BPH/2J*	*C57Bl6*	*BPN/3J*	*BPH/2J*	*C57Bl6*
Metabolic rate (kcal kg^{-1} h^{-1}) correlated with							
Body weight (BW, g)	Figure 5a	−0.4	−1.2	−0.8	31	57	47
Fat mass (% of BW)	Figure 5b	−0.3	−0.5	−0.5	25*	34	31
Lean mass (% of BW)	Figure 5c	0.3	0.5	0.1	−4	−12	17
Log activity (% of BW)	Figure 5d	0.8	1.4	0.8	15*	13	19†
Mean arterial pressure (mmHg) correlated with							
Raw metabolic rat (kcal h^{-1})	Figure 6a	27.6	99.5	1.9*			
Raw metabolic rate (kcal h^{-1})-activity adjusted	Figure 6b	27.0	27.8	−12.4	82***	108	113**
Metabolic rate (kcal kg^{-1} BW per hour)	Figure 6c	−1.3*	1.3	−0.5*			
Metabolic rate (kcal kg^{-1} BW per hour)-activity adjusted	Figure 6d	−2.6	−0.2	−0.9	151**	130	127**,†
Metabolic rate (kcal kg^{-1} lean per hour)	Figure 6e	−0.8*	1.7	−0.4*			
Metabolic rate (kcal kg^{-1} lean per hr)-activity adjusted	Figure 6f	−1.5	0.1	−0.8	135**	122	126**,†

Comparison of BPH/2J with either normotensive BPN/3J or C57Bl6 mice represented by *$P<0.05$; **$P<0.01$; ***$P<0.001$.
Comparison between BPN/3J *vs.* C57Bl6 mice represented by †$P<0.05$.

regression lines were similar between strains ($P>0.07$ all, Table 2). The average slope of the regression including all mice from the three strains was -0.82.

On the basis of this regression we can estimate the difference in metabolic rate (y) that could be attributed to the average difference in BW between BPN/3J and BPH/2J mice (-4.9 g); $y=-4.9\times-0.82=4.0$ kcal kg^{-1} h^{-1}. On the basis of these estimates, the difference in BW could explain 60% of the actual difference in metabolic rate between BPN/3J and BPH/2J mice.

Alternatively, we can estimate the difference in BW (x) that could be attributed to the average differences in metabolic rate between BPN/3J and BPH/2J mice (6.7 kcal kg^{-1} h^{-1});

$x=+6.7/-0.82=-8.2$ g. On the basis of these estimates, the difference in metabolic rate could produce a 1.6-fold greater BW difference than those actually measured.

Correlations between lean mass and activity with metabolic rate

Percentage fat mass and metabolic rate negatively correlated in all strains ($P<0.05$ all, Figure 5b), whereas percentage lean mass and metabolic rate were positively correlated in BPN/3J and BPH/2J mice ($P<0.01$ both, Figure 5c). Strong positive correlations were found between activity level and metabolic rate in all strains ($P<0.001$ all, Figure 5d) and, although regression lines of BPH/2J and C57Bl6 mice were different compared with BPN/3J mice ($P<0.05$ both), the slope was similar between all strains ($P>0.2$, Table 2).

Correlations between metabolic rate and BP

BPH/2J mice were the only strain to display a positive correlation between metabolic rate and MAP, which was apparent regardless of whether metabolic rate was raw or normalized to BW or lean mass ($P<0.001$, Figures 6a, c and e). However, this positive correlation did

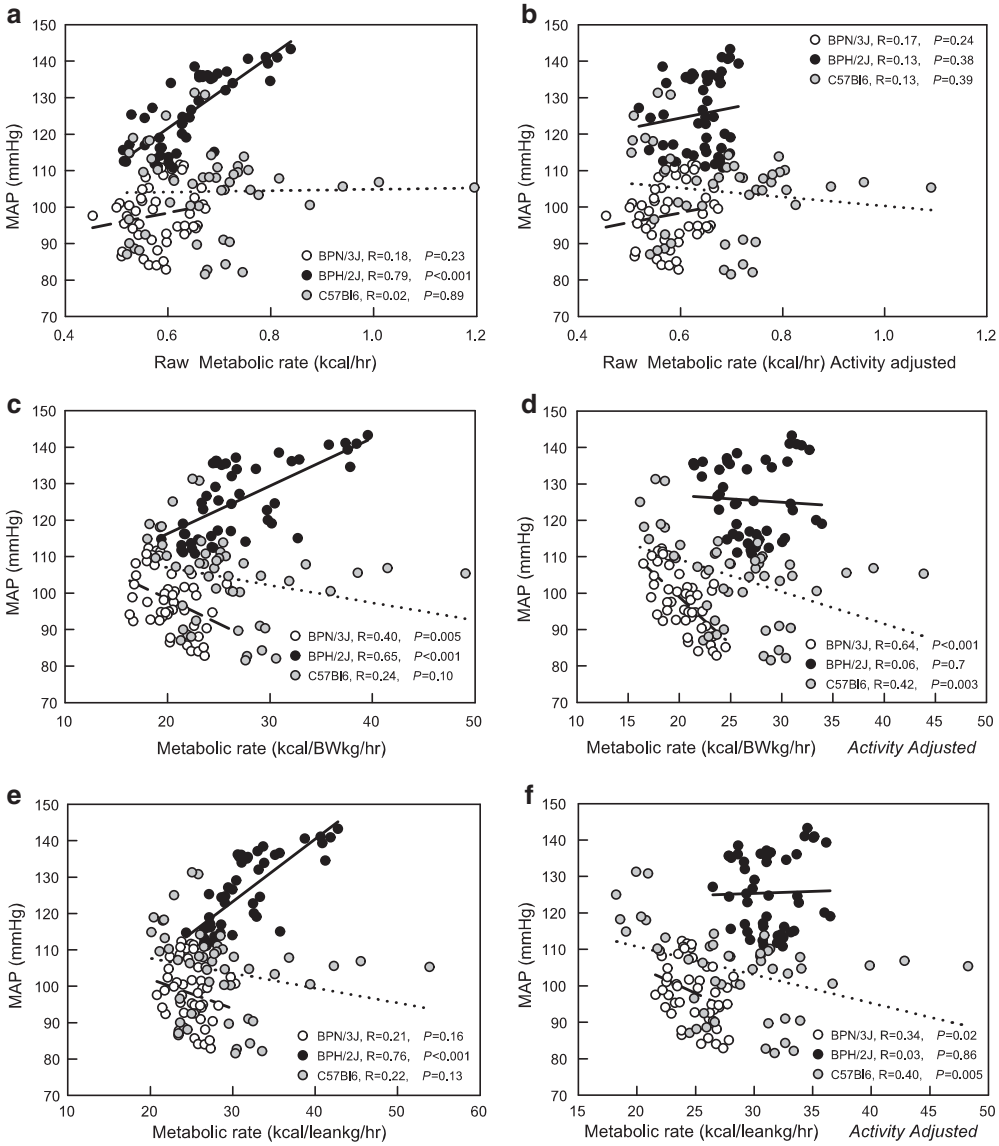

Figure 6 MAP (mmHg) correlated with (**a**) raw metabolic rate (kcal h^{-1}), (**b**) raw metabolic rate (adjusted for activity level) (**c**) metabolic rate normalized to BW (kcal kg^{-1} h^{-1}), (**d**) metabolic rate normalized to BW (kcal kg^{-1} h^{-1}) and adjusted for activity level, (**e**) metabolic rate normalized to lean mass (kcal per lean kg per hour), (**f**) metabolic rate normalized to lean mass (kcal per lean kg per hour) and adjusted for activity level. Average hourly values included BPN/3J (white circles), BPH/2J (black circles) and C57Bl6 mice (gray circles) of mice at both ages. Dashed lines represent regression lines for BPN/3J mice, solid line represents regression lines for BPH/2J mice and dotted line represents regression line for C57Bl6 mice.

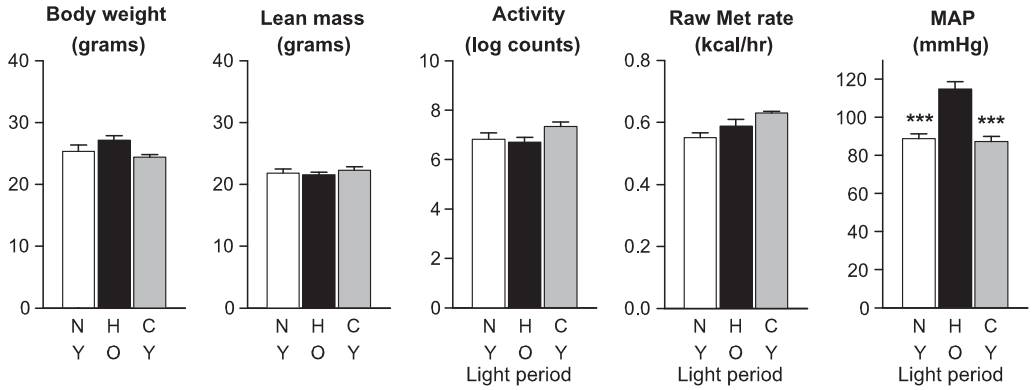

Figure 7 Bar graphs represents BW (g) and lean mass (g) in young BPN/3J (N, Y, white, $n=6$), older BPH/2J (H, O, black, $n=6$) and young C57Bl6 mice (C, Y, gray, $n=5$). Ambulatory activity (log counts), raw metabolic rate (kcal kg^{-1}) and mean arterial pressure were measured during the light (inactive) period in young BPN/3J, older BPH/2J and young C57Bl6 mice. Values are mean ± s.e.m. Comparison of normotensive BPN/3J and C57Bl6 mice with BPH/2J represented by ***$P<0.001$. Comparisons between BPN/3J *vs.* C57Bl6 mice were not significant.

not persist when metabolic rate was adjusted for activity level ($P>0.38$, Figures 6b, d and f). The slope of the regression line for metabolic rate (activity adjusted) versus MAP in BPH/2J mice was comparable with BPN/3J and C57Bl6 mice ($P>0.07$) but it remained elevated compared with both strains regardless of whether metabolic rate was raw or normalized to BW or lean mass ($P<0.01$).

BP in mice 'controlled' for raw metabolic rate, BW, lean mass and activity

During the light (inactive) period, young BPN/3J and C57Bl6 mice and older BPH/2J mice were found to have comparable BW ($P=0.23$), lean mass ($P=0.76$), ambulatory activity level ($P=0.57$) and raw metabolic rate ($P=0.24$). Thus, although the influence of these variables on raw metabolic rate between all three strains was eliminated, BPH/2J mice remained hypertensive compared with both BPN/3J ($P<0.001$) and C57Bl6 mice ($P<0.001$, Figure 7).

DISCUSSION

The main aim of the present study was to determine whether hypertension in BPH/2J mice is associated with an altered energy metabolism. We found that in younger mice, metabolic rate was greater in BPH/2J compared with normotensive BPN/3J mice. However, metabolic rate was the same as in normotensive C57Bl6 mice indicating that a high metabolic rate is not axiomatically associated with hypertension. Nevertheless, our finding that metabolic rate was similar in both normotensive C57Bl6 and hypertensive BPH/2J mice does not necessarily preclude the possibility that BP in BPH/2J mice may be more sensitive to changes in energy metabolism, which could also contribute to hypertension. There is evidence that BP in spontaneously hypertensive rats may be more sensitive to changes in energy metabolism.[25,26] Thus, in the present study circadian patterns of metabolic rate and BP were assessed, and the BP-metabolic rate relationship was compared in the three mouse strains. Indeed, BPH/2J mice were the only strain to show a positive correlation between hourly values for metabolic rate and BP, initially indicating that there may be a hypertension-specific relationship. However, the positive correlation between activity and metabolic rate observed in all strains also demonstrated that circadian patterns of metabolic rate are strongly influenced by ambulatory activity. Consequently, once metabolic rate was adjusted for activity level, the positive correlation between metabolic rate and BP in BPH/2J mice did not persist, suggesting the relationship was driven by differences in activity rather than metabolic rate. These data suggest that differences in the BP-metabolic rate relationship are not a major factor influencing hypertension, and therefore the hypertension in BPH/2J mice is not related to an altered metabolic state.

Currently there is extensive consideration given to the correct method of representation of metabolic rate in mice.[27] Our analysis identified that BW, body composition and activity strongly influence metabolic rate and as such presentation of raw metabolic rate alone can be misleading. For instance, there was no difference observed between strains in raw (non-normalized) metabolic rate in older mice, yet metabolic rate normalized to BW or lean mass, is disproportionately high in BPH/2J mice. Therefore, it is somewhat difficult to assess the potential relationship between metabolic rate and hypertension, independent of these other influences. One statistical approach is to analyze the influence of variables such as BW or body composition on metabolic rate using analysis of covariance.[27] Another experimental approach is to remove the contribution from all of these variables (BW, lean mass and activity) from our assessment by 'matching' the animals to eliminate the differences between strains and to then examine the effect of metabolic rate on hypertension in BPH/2J mice. This obviates the need to intervene to manipulate BW, lean mass or activity by a treatment, which in itself may well confound the relationships. Indeed, when BW, lean mass and activity were comparable between strains, raw metabolic rate was also similar between strains (Figure 7). Importantly, when raw metabolic rate is 'controlled' between strains, BPH/2J mice remain hypertensive, supporting the notion that this form of hypertension is independent of metabolic rate.

Interestingly, the present findings show that when metabolic rate is adjusted for activity there is either no relationship or even a negative relationship between BP and metabolic rate in mice. These findings tend to refute the idea that metabolic rate is positively related to BP level. However, the positive relationship between metabolic rate and BP is primarily based on correlative analysis of human data[13,14] and, although correlations can suggest a relationship, they do not necessarily suggest a causal role. To assess a causal relationship between metabolic rate and BP, an intervention to manipulate metabolic rate could be an interesting approach but the findings may still be difficult to interpret. Influencing metabolic rate by interventions such as fat feeding or cold exposure followed by assessment of the effect on BP could be useful to examine the general metabolic rate-BP relationship in mice but such manipulations are likely to influence BP

by inducing sympathetic activation.[28,29] Therefore, such interventions are unlikely to clarify whether the higher metabolism is causing the existing hypertension in BPH/2J mice. Additionally, as these metabolic interventions are likely to influence BP via activation of the SNS, any future studies to assess the general metabolic rate-BP relationship would ideally assess the role of the SNS. However, this aspect would also be challenging to interpret. For instance treatment with antihypertensive beta blockers has been shown to reduce the elevated metabolic rate in obese hypertensive patients.[11] However, such drugs that target the actions of the SNS can independently affect both metabolism and BP.[30] Therefore, findings would not necessarily prove a causal relationship between metabolic rate and BP. Both correlative and interventional analysis appear to have advantages and limitation; thus, a combination of approaches will likely be necessary to yield a more comprehensive understanding of this potential relationship between metabolic rate and BP.

The lower BW of BPH/2J mice reported throughout the literature[31] was one of the most notable indications that energy balance may be different in BPH/2J compared with BPN/3J mice. Lower BW in BPH/2J mice was recorded in mice as young as 1 month of age.[7] We speculated that this lower BW may be reflective of an energy imbalance caused by either higher energy expenditure or lower energy intake. Indeed, based on the BW-metabolic rate relationship alone, we estimate that the differences in metabolic rate between BPN/3J and BPH/2J mice could explain the entire difference in BW between strains. Although the size of an animal can also be a major determinant of metabolic rate,[32] on the basis of the same BW-metabolic rate regression, the differences in BW between BPN/3J mice and BPH/2J mice could also explain ~60% of the metabolic rate differences. Energy homeostasis is extremely complex and involves a multitude of contributing factors[33] and, although we have not performed a precise assessment of energy balance in the present study, we have measures of metabolic rate, food intake and body composition, which when considered together may provide a rough indication of the state of energy balance in BPH/2J mice. Our data show that metabolic rate is indeed greater in young BPH/2J than in young BPN/3J mice, but this is accompanied by greater consumption of food in BPH/2J mice than BPN/3J mice. This might reflect the high energy usage being balanced by high energy intake, although without more information, one cannot assume that digestive efficiency is comparable between strains.[34] Nonetheless, if there were a minor imbalance in energy input or expenditure in BPH/2J mice, one might expect this to be reflected more clearly in changes in BW as the mice grow over time.[35] However, both strains gained weight at a comparable rate over the study period suggesting BPH/2J mice are not likely to be energy deficient. Furthermore, if there was an energy imbalance it might also be expected that this would result in differences in body composition between strains.[36] However, we observed no difference in body composition in BPH/2J and BPN/3J mice in either young or old mice. Taken together these findings suggest that energy balance does not appear to be overtly abnormal in BPH/2J mice and is therefore not a likely contributing factor in hypertension in BPH/2J mice. One of the few metabolic differences that was exclusive to BPH/2J mice in this study was the reduction in RER that occurred with age, which indicates a shift in substrate utilization toward greater fat oxidation.[37] However, given that BPN/3J and BPH/2J mice gain a similar percentage of fat content with age, this suggests that BPH/2J mice are not utilizing excessive amounts of stored fat. We have not assessed the mechanism that might mediate this change in substrate utilization, but it was not present in young BPH/2J mice, which already have established hypertension. Although this suggests that it is unlikely to be involved in the development of hypertension, it could potentially be a consequence.

The present study provides a comprehensive characterization of metabolic parameters in hypertensive BPH/2J mice. Furthermore, an integrative physiological approach was used to determine whether hypertension in BPH/2J mice was associated with differences in metabolism in this strain. Overall, our findings suggest that, although BPH/2J mice may have metabolic differences compared with normotensive BPN/3J mice, these differences do not appear to be associated with the hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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