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Skipping breakfast regimen induces an increase in body weight and a decrease in muscle weight with a shifted circadian rhythm in peripheral tissues of mice

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Abstract

Meal timing is a key factor in synchronising the circadian clock in peripheral tissues. Circadian disorders are associated with the metabolic syndrome. Previously, we demonstrated that a skipping breakfast regimen (SBR) with a high-fat diet increased body weight gain in rats. In this study, we investigated whether SBR with a normal diet led to abnormal lipid metabolism and muscle metabolism in mice. Male C57BL/6 mice were fed during zeitgeber time (ZT) 12–24 in the control group and ZT 16–24 in the SBR group for 2 weeks. SBR mice showed increased body weight gain and perirenal adipose tissue weight. The plantar muscle weight was decreased in the SBR group compared with that in the control group. Furthermore, SBR delayed the circadian oscillations in clock gene expression in peripheral tissues, such as the liver, adipose tissue and muscle, as well as the oscillations in the expression of lipid metabolism-related genes in the liver and adipose tissue. These results suggest that skipping breakfast over a long period of time is associated with a risk of obesity, the metabolic syndrome and muscle loss, such as sarcopenia.

Key words: **Skipping breakfast: Circadian rhythm: Metabolic syndrome: Sarcopenia: Chrono-nutrition**

The metabolic syndrome is a complex condition related to various co-morbidities such as diabetes, hypertension, hyperlipidaemia, obesity and CVD[1–3]. In recent years, the morbidity associated with the metabolic syndrome has increased. Lifestyle-related diseases caused by the metabolic syndrome may influence the quality of life. Eating habits are associated with the metabolic syndrome[4,5]. In addition to the metabolic syndrome, sarcopenia is also associated with dietary habits[6].

In mammals, the central circadian clock is controlled by light in the suprachiasmatic nucleus; it regulates 24-h cycles in biological rhythms and regulates peripheral organs[7]. The biological clock in the liver is known to be highly influenced by diet[8]. Diet is a major stimulus for the synchronisation in peripheral organs. The major clock genes *Clock* (circadian locomotor output cycles kaput), *Bmal1* (brain and muscle Arnt-like protein), *Per* (period) and *Cry* (cryptochrome) are all transcription factors. These genes form a negative feedback loop that show 24-h rhythm[7]. Clock genes are expressed in almost every organ of the human body, and each organ shows its own rhythm. The regulation of clock genes in each organ is involved in the regulation of organ metabolism. For example, *Rev-erba* (nuclear receptor subfamily 1, group D, member 1) which regulated by *Bmal1*, is involved in lipid metabolism in peripheral tissues[9].

Numerous reports have demonstrated that disordered eating habits result in obesity and several metabolic diseases[10–13]. Recent studies have shown that irregular eating habits induce dyslipidaemia by disrupting circadian oscillations[14,15]. The timing of meals, especially breakfast, plays an important role[16]. Skipping breakfast has been reported to increase the risk of the metabolic syndrome and obesity[17] and to increase the risk of lifestyle-related diseases[18,19]; daily breakfast eaters showed a reduced risk of hypertension compared with infrequent breakfast eaters[20]. Modern dietary patterns, such as the Western diet and irregular eating behaviour, are important contributors to the epidemic of lifestyle-related diseases. The Western diet, known to be a high-fat diet, has been reported to disrupt feeding behaviours and circadian rhythms in mice[21]. In our previous study, disrupted circadian oscillations and increased body weight were observed in rats fed a high-fat diet combined with time-shifted feeding[22]. To clarify the role of skipping breakfast on lipid metabolism, a normal diet was adopted in the present study. The feeding efficiency ratio of mice is known to be higher than that of rats[23,24]. Mice are also known to exhibit higher energy metabolic rates than rats[25] and to respond more to dietary conditions. The aim of this study was to clarify the impact of the skipping breakfast regimen (SBR) with a normal diet.

Abbreviations: SBR, skipping breakfast regimen; ZT, zeitgeber time.

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Methods

Animals

The animal experiments were approved by the Nagoya University Animal Care Committee (No. 2018011901). The experiments were performed in accordance with the guidelines for the Care and Use of Laboratory Animals at Nagoya University. Thirty-six male C57BL/6J mice were purchased from the CLEA Japan Inc. The mice were maintained in cages (23 (SD 1)°C) and kept under a 12-h light/dark cycle at each zeitgeber time (light: ZT 0–12, dark: ZT 12–24). During the experiment, the mice were allowed *ad libitum* access to water. Temperature data loggers (KN Laboratories Inc.) were implanted into the abdominal cavities of 7-week-old male mice. After 3 d of recovery from surgery, mice were randomly divided into control (*n* 9) or SBR (*n* 8) groups according to body weight and food intake. Another nineteen mice were divided into control (*n* 9) or SBR (*n* 10) groups at the same time. Mice were fed AIN-93G for all experimental periods. The control mice were permitted access to food during ZT 12–24. The SBR mice were permitted access to food during ZT 16–24. Body weight and food intake were measured daily. All mice were kept under breeding condition until just before sacrifice. Decapitation was used for sacrifice method. Decapitation was carried out without anaesthesia due to excluding an effect of anaesthetics. Blood was harvested from cut part. During day 14 to day 15, mice were killed every 4 h at ZT 2, 6, 10, 14, 18 and 22 (*n* 3 per time point). At each time point of sacrifice, blood, liver, adipose tissues and muscle tissues were harvested and frozen at –80°C for further analysis. Two weeks of experimental period altered the peripheral circadian rhythm by dietary intervention[8] and changes in energy expenditure were clearly observed until 4 h after active phase begun[26]. Based on the results of present studies, we set 4 h of SBR with susceptible to reflect the impact of SBR.

Measurement of body temperature

Seven-week-old mice were implanted with the data logger into the abdominal cavity, as described above. Body temperatures were recorded at 10-min intervals during the experiments. Data loggers were collected at the time of sacrifice, and the Rh Manager programme (KN Laboratories Inc.) was used to analyse temperature data.

Biochemical analysis

Serum TAG and cholesterol levels were measured as described previously[27]. Briefly, TAG and cholesterol levels were measured using the Triglyceride E-test kit and Cholesterol E-test kit (FUJIFILM Wako Pure Chemical Corp.). Serum insulin and corticosterone levels were measured using ELISA kits (Mouse Insulin ELISA kit; Morinaga Institute of Biological Science Inc.; Corticosterone ELISA kit; Assaypro).

RNA preparation and real-time quantitative PCR

Total RNA from the liver, epididymal adipose tissue and plantar muscles of mice were prepared using the method described by Chomczynski and Sacchi[28] or TRIzol Reagent (Invitrogen). Complementary DNA was synthesised using kits (Takara Bio Inc. and Toyobo). Gene expression was determined using the real-time quantitative PCR. PCR was performed using the Step One Plus Real-Time PCR system (Thermo Fisher Scientific) using the SYBR Green method (Thermo Fisher Scientific and Toyobo). *Apoe* in the liver and *18S* rRNA in the other tissues were used as the internal controls. Hepatic *Apoe* was unaffected by dietary sources in previous study and was used for normalisation[29]. A brief description of the gene groups analysed by qPCR is as follows. Clock genes: *Bmal1, Clock, Per1, Per2, Cry1, Cry2, Dec1* (differentiated embryo chondrocytes 2), *Dec2*, *Rev-erba, Rora* (nuclear receptor retinoic acid-related orphan receptor-*α*), *E4bp4 (*E4 promoter-binding protein 4), *Dbp* (D site of albumin promoter binding protein), *Hlf* (hepatic leukaemia factor) and *Tef* (thyrotroph embryonic factor). Lipid metabolism-related genes: *Acly* (ATP citrate lyase), *Fas* (fatty acid synthase), *Acc* (acetyl-CoA carboxylase), *Srebp1c* (sterol regulatory element-binding protein-1c), *Ppara* (peroxisome proliferator-activated receptor *α*), *Pparg* (peroxisome proliferator-activated receptor *γ*), *Mtp* (microsomal TAG transfer protein), *Elovl6* (elongation of very long chain fatty acids protein 6), *Atgl* (TAG lipase), *Fgf21* (fibroblast growth factor 21) and *Adn* (adiponectin). Muscle differentiation-related genes: *Myod* (myogenic determination protein 1), *Myog* (myogenin). Muscle atrophy related genes: *Atrogin-1* (muscle atrophy F-box 1) and *Murf-1* (muscle-specific RING finger protein 1). Apoptosis gene: *Bcl2* (B-cell/CLL lymphoma 2). Primer sequences are listed in online Supplementary Table S1.

Periodic analysis

Diurnal variations in body temperature, serum parameters and gene expression were analysed using the JTK_CYCLE software[30]. Significant differences in periodicity were determined at $P < 0{\cdot}05$. To ensure the reliability of the results of the non-parametric JTK_CYCLE analysis, a parametric cosinor analysis was also performed for comparison[31]. The comparison of acrophase by two methods is shown in online Supplementary Table S7. Those were similar in the most of cases. Therefore, results were mentioned with mainly JTK_CYCLE.

Statistical analysis

The results are presented as mean with their standard error of the mean. Body weight, organ weight and food intake were assessed using Student's *t* test. Statistical significance was set at $P < 0{\cdot}05$. Statistical analyses were performed using the SPSS software (IBM).

Results

Skipping breakfast regimen increased body weight gain

Compared with the control mice, the body weight gain of SBR mice significantly increased from day 5 onward (Fig. 1(a)). No differences were found in the total food intake between the groups (online Supplementary Fig. S1). As shown in Table 1, the weight of perirenal adipose tissue was higher in SBR mice than in control mice. There were no statistical differences in liver

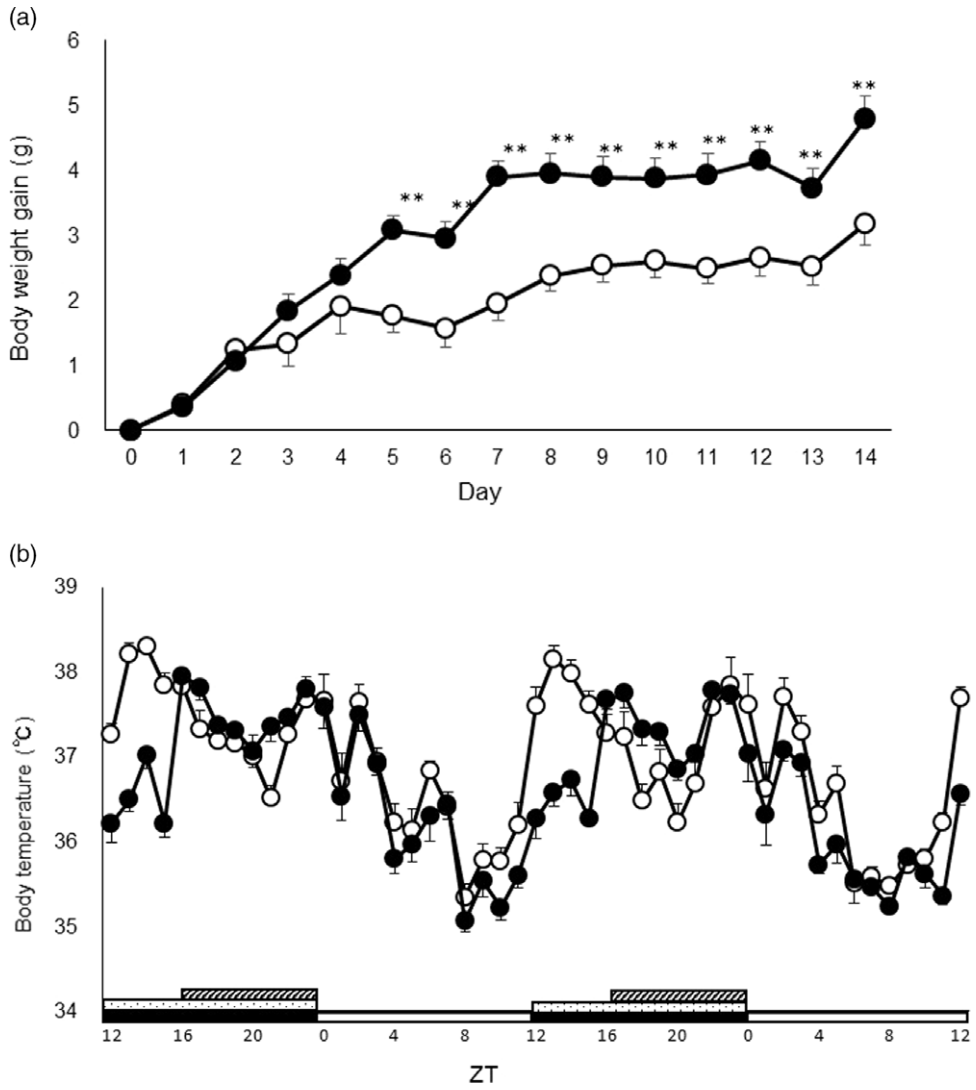

Fig. 1. SBR increased body weight gain and delayed rising body temperature in mice. (a) Body weight gain was measured for 14 d. The values are means with their standard error of the mean. $^{**}P < 0{\cdot}01$ (Student's *t* test); *n* 18. The open circles represent control mice, and the closed circles represent SBR mice. (b) The core body temperature was analysed using the data logger. The body temperature data were analysed using the hourly average for 2 d from day 14 to day 15. The open circles represent control mice, and the closed circles represent SBR mice. The light periods are shown with open bars (ZT 0–12), and the dark periods are shown with closed bars (ZT 12–24). The dot pattern bar represents the control feeding time (ZT 12–24), and the striped bar represents the SBR feeding time (ZT 16–24). The values are means with their standard error of the mean; *n* 8–9. SBR, skipping breakfast regimen; ZT, zeitgeber time.

weight between the control and SBR mice. These results indicate that SBR increased body weight and adipose tissue weight. In contrast, plantar muscle weight significantly decreased and tibial anterior muscle weight tended to be lower in the SBR group than in the control group (Table 1).

Skipping breakfast delayed the rise in body temperature

The circadian rhythm of body temperature is mainly controlled by the suprachiasmatic nucleus [32]. To determine the effects of SBR on body temperature variation, we analysed the oscillations in body temperature over 24 h in the data logger-implanted mice. The rhythmicity of body temperature was observed in both control and SBR groups (control: $P < 0{\cdot}001$, SBR: $P < 0{\cdot}001$). The amplitude of the rhythm was enhanced in the SBR group compared with the control group (control: 0·496, SBR: 0·919). The body temperature of the control mice rose at the beginning of the dark period (ZT 12) and decreased at the beginning of the light period (ZT 24); the body temperature of SBR mice slightly increased at ZT 12 and increased immediately at the start of the diet period (ZT 16). The body temperature in both groups decreased gradually during the light period (Fig. 1(b)).

Skipping breakfast regimen delayed peaks in serum TAG, NEFA and insulin levels

The concentrations of serum glucose, cholesterol, bile acids and adiponectin did not change in SBR mice compared with those in control mice (Fig. 2(a), (b), (e) and (g), and online Supplementary Table S2). The peak time of the TAG level in SBR mice was delayed by 8 h from ZT 18 in the control group to ZT 2 until the following day (Fig. 2(c) and online

Table 1. Changes in tissue weights in the SBR and control groups (Mean values with their standard errors of the mean)

	Tissue weight (g/100 g body weight)			
	Control		SBR	
	Mean	SEM	Mean	SEM
Liver	4·825	0·190	4·411	0·208
Epididimal white adipose tissue	1·599	0·105	1·756	0·082
Perirenal adipose tissue	0·282	0·021	0·404	0·025**
Plantar muscle	0·405	0·007	0·381	0·006*
Gastrocnemius muscle	0·728	0·012	0·700	0·012
Soleus muscle	0·068	0·001	0·069	0·002
Tibial anterior muscle	0·370	0·006	0·351	0·008
Extensor digitorum longus muscle	0·072	0·002	0·068	0·003

n 18. Control: normal diet *ad libitum* during the active phase (ZT 12–24); SBR: normal diet time-restricted feeding (ZT 16–24).
* $P < 0·05$.
** $P < 0·01$ (Student's *t* test).

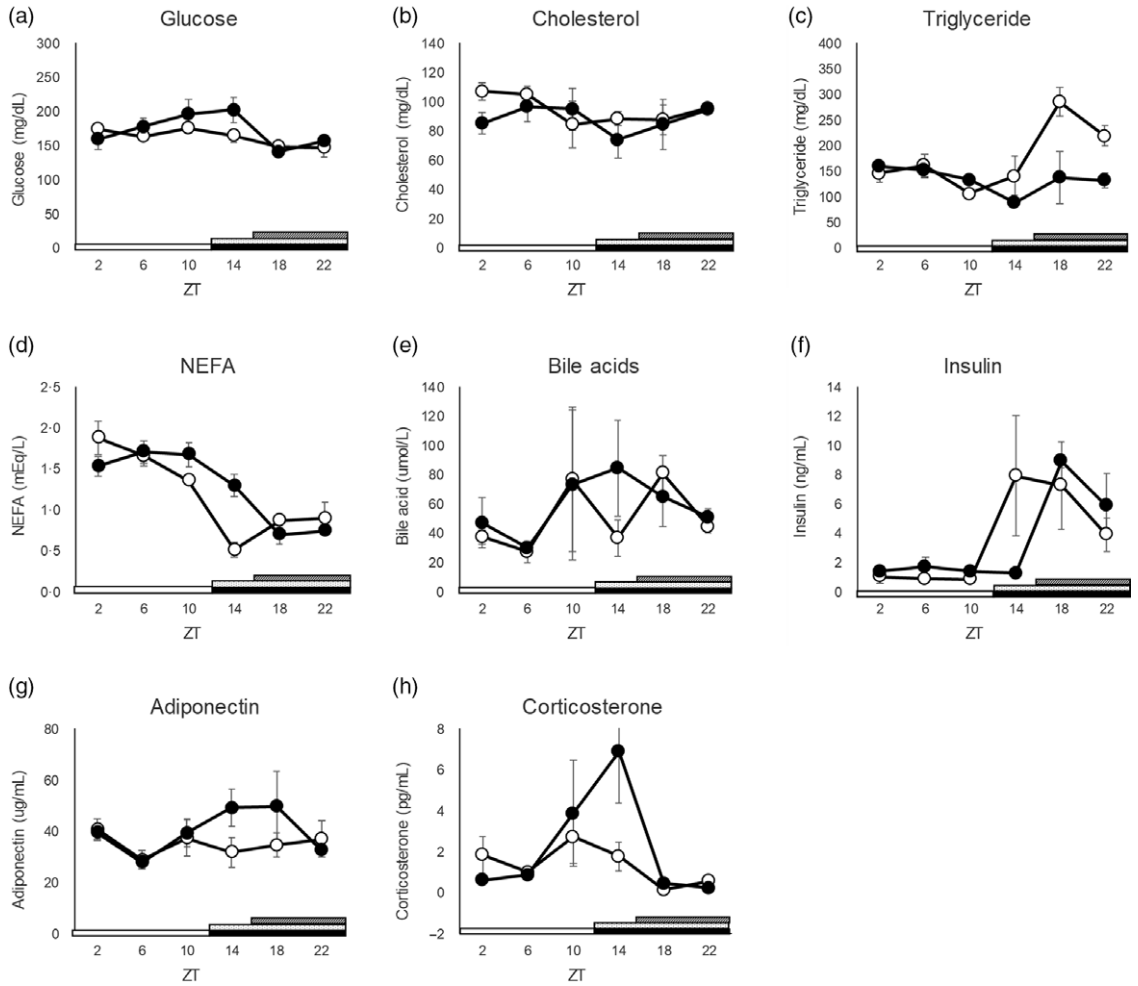

Fig. 2. SBR delayed the peaks of serum TAG, NEFA and insulin levels in mice. The serum (a) glucose, (b) cholesterol, (c) TAG, (d) NEFA, (e) bile acid, (f) insulin, (g) adiponectin and (h) corticosterone levels were analysed. The open circles represent control mice, and the closed circles represent SBR mice. The light periods are shown with open bars (ZT 0–12), and the dark periods are shown with closed bars (ZT 12–24). The dot pattern bar represents the control feeding time (ZT 12–24), and the striped bar represents the SBR feeding time (ZT 16–24). The rhythmicity was analysed by JTK_CYCLE. The results of the rhythmicity are shown in online Supplementary Table S2. SBR, skipping breakfast regimen; ZT, zeitgeber time.

Supplementary Table S2). The peak time of NEFA, insulin and corticosterone levels was delayed by 4 h, from ZT 14 in the control mice to ZT 18 in the SBR mice (Fig. 2(d), (f) and (h) and online Supplementary Table S2). These results indicate that the circadian rhythms of TAG, NEFA, insulin and corticosterone levels were delayed by SBR.

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Skipping breakfast regimen shifted the expression of clock genes and lipid metabolism-related genes in the liver

The peak time of *Clock* and *Dec2* did not differ between the groups (Fig. 3(b) and (h) and online Supplementary Table S3). *Cry2* and *Rora* showed no rhythmic mRNA expression in either group, as determined by JTK_CYCLE analysis (Fig. 3(f) and (j) and online Supplementary Table S3). Cosinor analysis showed that the peak time of *Cry2* was shifted in the SBR group, but JTK_CYCLE analysis did not show rhythmicity and any shift in peak time by SBR (online Supplementary Table S3 and S7). The peaks of *Bmal1*, *Per1*, *Per2*, *Cry1*, *Dec1*, *Rev-erba*, *E4bp4*, *Dbp*, *Hlf* and *Tef* expression were delayed by 4 h by SBR (Fig. 3(b), (c)–(e), (g), (i), 3(k)–(n) and online Supplementary Table S3). These results indicated that a 4-h shift in feeding induced a 4-h delay in the circadian rhythm of clock genes in the liver.

Since rhythmicity in the expression of various clock genes was delayed by SBR, we investigated the circadian oscillations of lipid metabolism-related genes in the liver, as several such genes are regulated by clock genes[9,33]. The rhythmic expression of *Acc* and *Srebp1c* was suppressed in SBR mice (Fig. 4(b) and (d) and online Supplementary Table S4). These data showed that SBR not only delayed the expression of lipid metabolism genes but also affected the amplitude of lipid metabolic gene expression in the liver. *Pparg* and *Mtp* showed no rhythmic expression in either group (Fig. 4(f) and (g) and online Supplementary Table S4).

Skipping breakfast regimen shifted the expression of clock genes in the adipose tissue and muscle

As mentioned above, we observed that adipose tissue weight increased, and muscle weight decreased in SBR mice. To elucidate the mechanism underlying the induction of increased adipose weight and decreased muscle weight by SBR, we investigated the expression of clock genes, lipid metabolism-related genes and muscle metabolism-related genes. As we stored only epididymal adipose tissues and plantar muscles, we analysed epididymal adipose tissues and plantar muscles by qPCR. The peaks of *Bmal1* and *Rev-erba* expression were delayed by SBR in the epididymal adipose tissues (Fig. 5(c) and (g) and online Supplementary Table S5). The expression of *Per1, Per2, Dec1, Cry1, Cry2, Elovl6*, *Atgl*, *Fgf21* and *Adn* did not differ between the groups (Fig. 5(a), (b), (d)–(f), (h)–(l) and online Supplementary Table S5).

The oscillations in *Per1*, *Per2*, *Bmal1, Rev-erba* and *Myod* expression were delayed by SBR in the plantar muscle (Fig. 6(a)–(c), (g), (h) and online Supplementary Table S6). *Atrogin-1* and *Murf-1* expression tended to increase at every time point (Fig. 6(j) and (k)). In cosinor analysis, peak time of *Murf-1* was not shifted drastically by SBR, but JTK_CYCLE analysis showed no rhythmicity and larger shift of peak time (online Supplementary Tables S6 and S7). The expression of *Cry1, Cry2, Dec1, Myog* and *Bcl2* was not different between the groups (Fig. 6(d)–(f), (i), (l) and online Supplementary Table S6).

Discussion

Skipping breakfast has been proposed to increase the risk of lifestyle-related diseases in humans[18,19]. In our previous study, time-shifted feeding of rats resulted in increased body weight gain and induced circadian disorders in the liver[22]. Our previous study suggested that the combination of a high-fat diet and irregular feeding behaviour would increase body weight[22]. However, the factors that contribute to this increase in body weight remain unknown. To clarify which factor directly impacts the increase in body weight, a normal diet was used in this study. As mentioned above, compared with rats, mice have a higher feeding efficiency ratio and higher energy metabolic rate[23–25]. It has been suggested that mice respond more to dietary conditions and are suitable for dietary intervention studies. The group we used as control is time restricted feeding condition. Time restricted feeding during inactive phase is reported not to influence lipid metabolism in mice[34]. In the present study, we limited mice to access food at light phase (ZT 0–12) to bring out the impacts of SBR more drastically. Furthermore, the feeding conditions in each mouse needed to be same just before the beginning of the active phase (ZT 12–16) to evaluate the effects of SBR.

In the present study, we investigated whether SBR induced negative effects on lipid and muscle metabolism in mice fed a normal diet. Our results indicated that 2 weeks of SBR increased body weight gain in mice (Fig. 1(a)). Despite SBR mice being fed for 4 h less than control mice, there was no significant difference in food intake between the groups throughout the experimental period (online Supplementary Fig. S1). SBR mice showed significantly increased perirenal adipose tissue weight and decreased plantar muscle weight compared with control mice (Table 1), whereas the liver weight did not change. SBR delayed circadian oscillations in the expression of clock genes and lipid metabolism-related genes, but liver weight did not change, suggesting that SBR did not affect lipid accumulation in the liver.

Skeletal muscle fibres are roughly divided into two types: fast-twitch muscle fibres and slow-twitch muscle fibres. The plantar, gastrocnemius, tibial anterior and extensor digitorum longus muscles are characterised by fast-twitch muscle fibres, and the soleus muscle is characterised by slow-twitch muscle fibres[35]. Fast-twitch muscle fibres are preferentially degraded during fasting[36]. In the present study, the weight of the four muscles with fast-twitch fibres, except the plantar muscle, tended to decrease with SBR (Table 1). These changes were not observed in the soleus muscle, which is characterised by slow-twitch fibres. In this study, the weight of muscles with fast-twitch fibres significantly decreased or tended to be decreased by SBR. We speculate that 4-h fasting owing to SBR in the first active phase induced the degradation of muscles with fast-twitch fibres. In the present study, only the perirenal adipose tissue weight was increased by SBR. We hypothesised that epididymal adipose tissue is the major adipose tissue affected by SBR and collected adipose tissue only from the epididymis region for gene expression analysis. However, only perirenal adipose tissue weight was changed by SBR. A similar finding was reported in our previous study[22]. Previous study suggested that lipid metabolism is regulated differently in different adipose regions[37]. Based on the differences

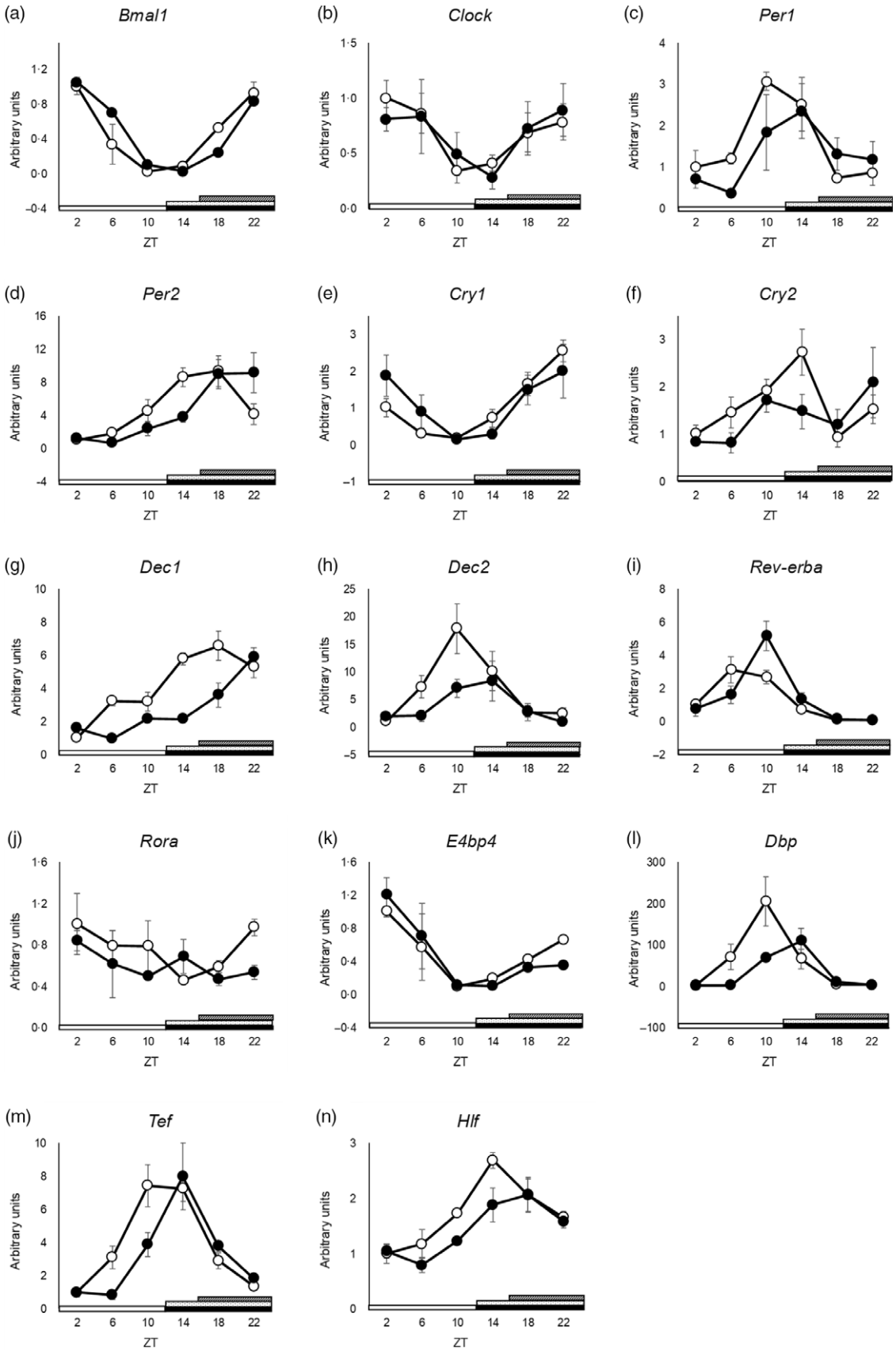

Fig. 3. SBR delayed circadian oscillation of hepatic clock gene expression in mice. (a) *Bmal1*, (b) *Clock*, (c) *Per1*, (d) *Per2*, (e) *Cry1*, (f) *Cry2*, (g) *Dec1*, (h) *Dec2*, (i) *Rev-erba*, (j) *Rora*, (k) *E4bp4*, (l) *Dbp*, (m) *Tef* and (n) *Hlf* mRNA expression in the liver was analysed using RT-qPCR. *ApoE* rRNA was used as a reference for the RT-qPCR. The open circles represent the control group, and the closed circles represent the SBR group. The light periods are shown with open bars (ZT 0–12), and the dark periods are shown with closed bars (ZT 12–24). The dot pattern bar represents the control feeding time (ZT 12–24), and striped bar represents the SBR feeding time (ZT 16–24). Each value in the ZT points is means with their standard error of the mean; *n* 3. The rhythmicity was analysed by JTK_CYCLE. The results of the rhythmicity are shown in online Supplementary Table S3. SBR, skipping breakfast regimen; ZT, zeitgeber time.

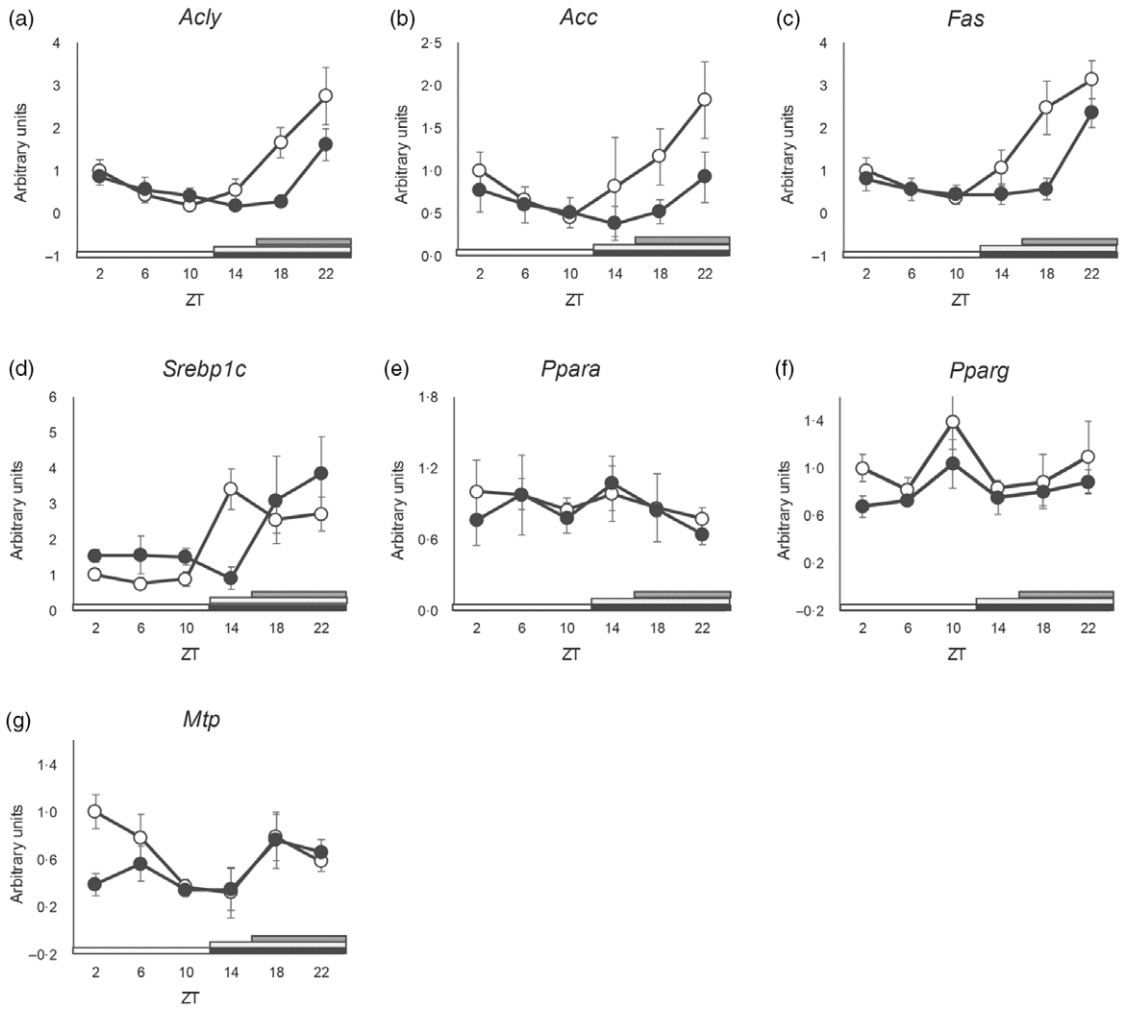

Fig. 4. Oscillation patterns of hepatic lipid metabolism-related gene expression in mice. (a) *Acly*, (b) *Acc*, (c) *Fas*, (d) *Srebp1c*, (e) *Ppara*, (f) *Pparg* and (g) *Mtp* mRNA expression in the liver was analysed using RT-qPCR. *18S* rRNA was used for normalisation in the RT-qPCR. The open circles represent the control group, and the closed circles represent the SBR group. The light periods are shown with open bars (ZT 0–12), and the dark periods are shown with closed bars (ZT 12–24). The dot pattern bar represents the control feeding time (ZT 12–24), and the striped bar represents the SBR feeding time (ZT 16–24). Each value in the ZT points is means with their standard error of the mean; *n* 3. The rhythmicity was analysed by JTK_CYCLE. The results of the rhythmicity are shown in online Supplementary Table S4. SBR, skipping breakfast regimen; ZT, zeitgeber time.

in lipid metabolic regulation, perirenal fat may have been significantly affected by SBR in the present study.

In mammals, energy expenditure is correlated with core body temperature, which in turn has been reported to be modulated by food components[38]. The core body temperature is associated with diet-induced thermogenesis[39]. In this study, the core body temperature rose sharply during the first active phase in the control groups along with diet-induced thermogenesis (Fig. 1(b)). However, the core body temperature in the SBR group rose slightly during the first active phase and sharply rose immediately after feeding (ZT 16), suggesting that thermogenesis was delayed by SBR. In the present study, the body temperature in control mice increased sharply at ZT 12 (Fig. 1(b)). However, the body temperature of SBR mice increased slightly at ZT 12 and further increased sharply at ZT 16 (Fig. 1(b)). These results suggest that both light and diet regulate the circadian oscillations in body temperature. We speculate that a delay in rising body temperature led to the suppression of increased energy expenditure in the active phase, and energy expenditure would contribute to an increase in body weight associated with fat accumulation in adipose tissues.

Hepatic circadian oscillations are regulated by both the suprachiasmatic nucleus and diet[40,41]. In this study, the peak time of clock gene expression in the liver was delayed by 4 h by SBR (Fig. 3). The peak time of serum NEFA, insulin and corticosterone levels was delayed by 4 h by SBR (Fig. 2). These results suggest that serum NEFA, insulin and corticosterone levels are synchronised with hepatic clock gene expression. We have demonstrated that insulin plays an important role in synchronising the circadian clock in the liver[42]. Interestingly, the circadian rhythms of Igf-1 (insulin-like growth factor-1) have sexual dimorphism[43]. Although we have not examined sex differences in the present study, sex of mice is an important modulator of circadian oscillations and glucose/lipid metabolism. Further experiments are required to reveal the effects of NEFA and corticosterone on the circadian clock.

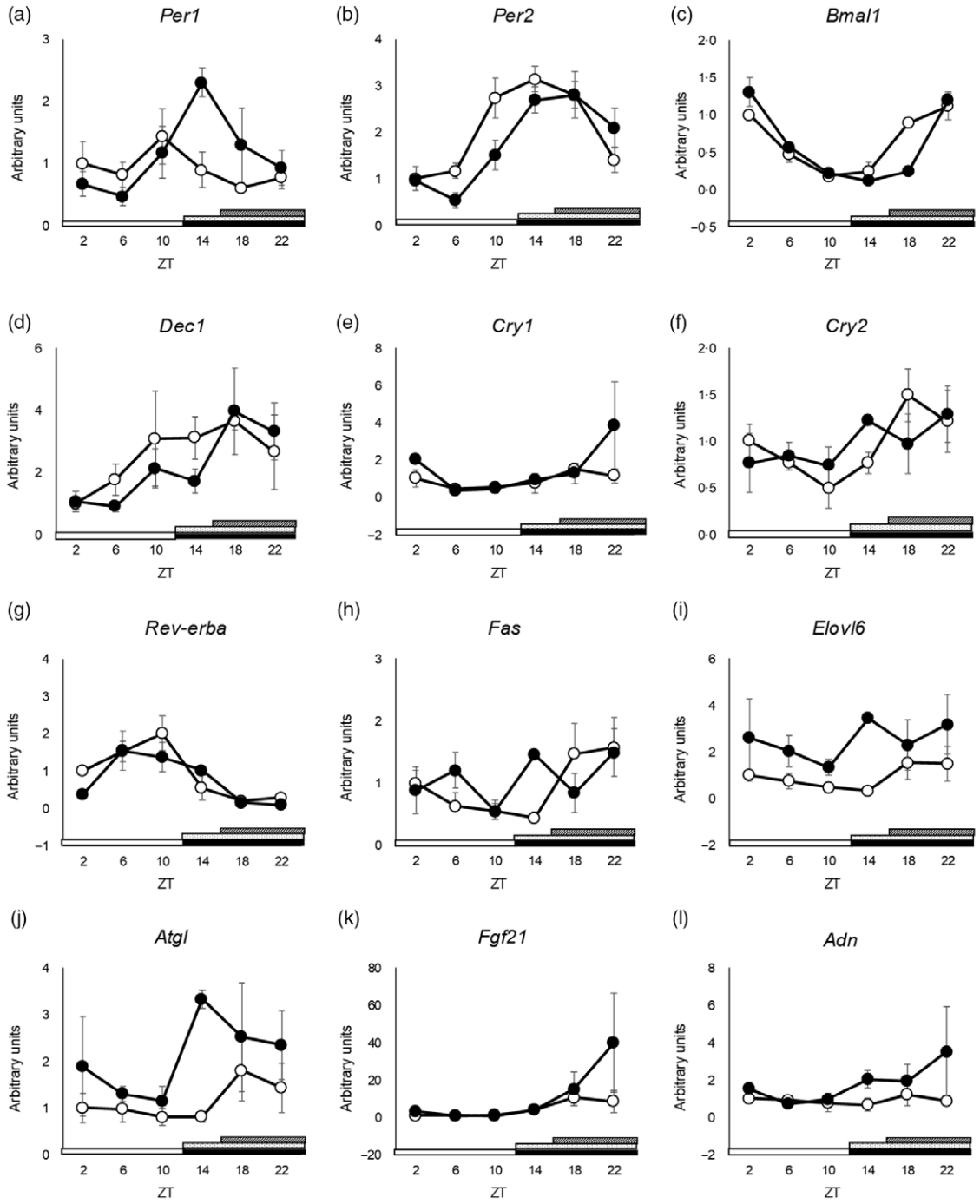

Fig. 5. Oscillation patterns of clock and lipid metabolism-related gene expression in adipose tissue. (a) *Per1*, (b) *Per2*, (c) *Bmal1*, (d) *Dec1*, (e) *Cry1*, (f) *Cry2*, (g) *Rev-erba*, (h) *Fas*, (i) *Elovl6*, (j) *Atgl*, (k) *Fgf21* and (l) *Adn* mRNA expression in the epididymal adipose tissues was analysed using RT-qPCR. *18S* rRNA was used for normalisation in the RT-qPCR. The open circles represent the control group, and the closed circles represent the SBR group. The light periods are shown with open bars (ZT 0–12), and the dark periods are shown with closed bars (ZT 12–24). The dot pattern bar represents the control feeding time (ZT 12–24), and the striped bar represents the SBR feeding time (ZT 16–24). Each value in the ZT points is means with their standard error of the mean; *n* 3. The rhythmicity was analysed by JTK_CYCLE. The results of the rhythmicity are shown in online Supplementary Table S5. SBR, skipping breakfast regimen; ZT, zeitgeber time.

Several reports have discussed the functions of clock genes[44,45]. Clock genes are known to regulate lipid metabolism-related genes, such as *Ppara*, *Pparg* and *Srebp1c*[9,33,46]. In this study, the peak time of lipid metabolism-related gene expression was delayed by 4 h in both the liver and epididymal adipose tissue in SBR mice (Figs. 4 and 5). These results suggest that the expression of clock genes and lipid metabolism-related genes is synchronised and controlled in both the liver and adipose tissues. It has been reported that lipogenic enzymes are mainly regulated at the transcriptional level[47] and are correlated with both protein levels and enzyme activities[48]. Several mRNA involved in lipid metabolism as well as their metabolites exhibit

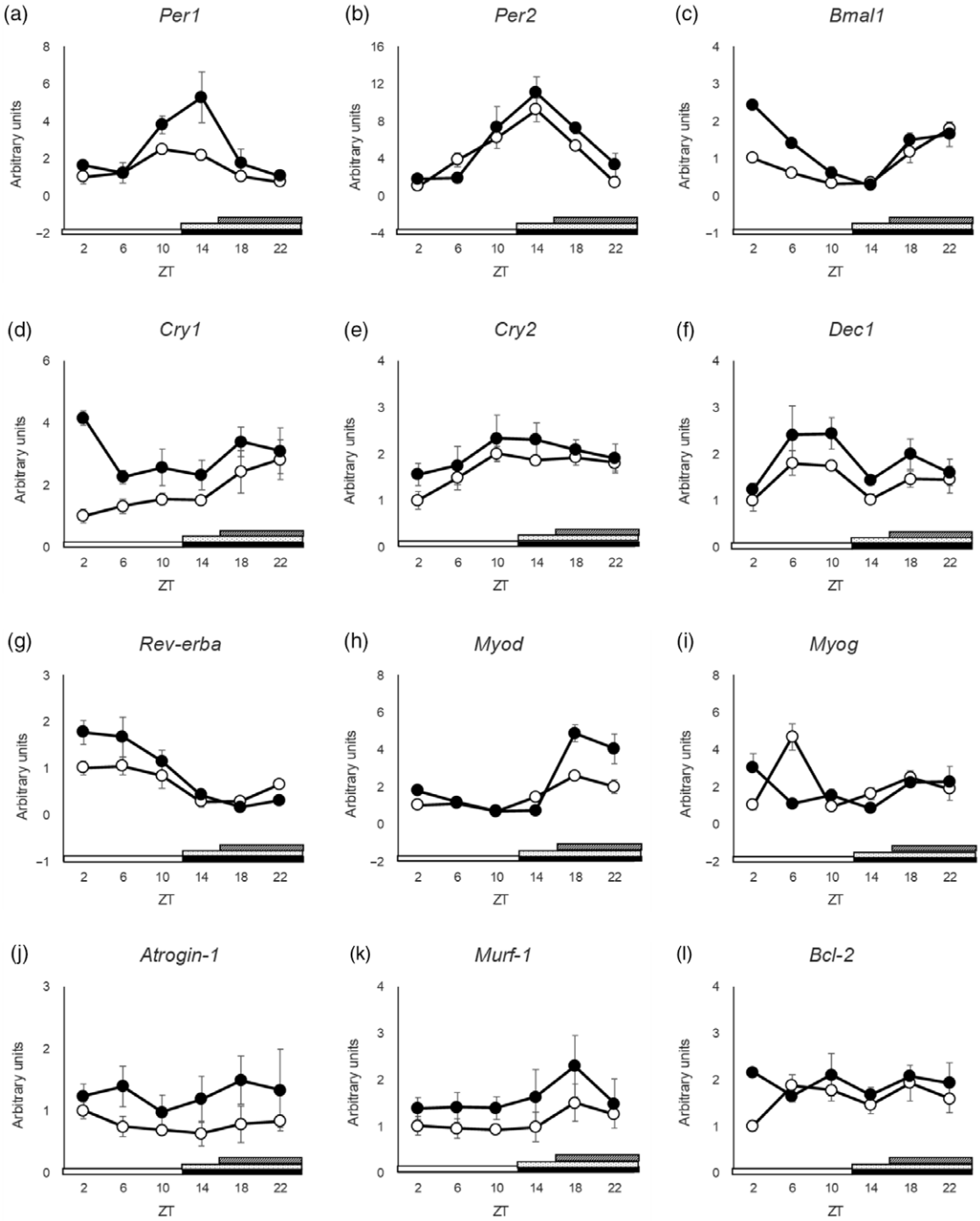

Fig. 6. Oscillation patterns of clock and muscle metabolism-related gene expression in muscle. (a) *Per1*, (b)*Per2*, (c) *Bmal1*, (d) *Cry1*, (e) *Cry2*, (f) *Dec1*, (g) *Rev-erba*, (h) *Myod*, (I) *Myog*, (j) *Atrogin-1*, (k) *Murf-1* and (l) *Bcl-2* mRNA expression in the plantar muscle tissues was analysed using RT-qPCR. *18S* rRNA was used for normalisation in the RT-qPCR. The open circles represent the control group, and the closed circles represent the SBR group. The light periods are shown with open bars (ZT 0–12), and the dark periods are shown with closed bars (ZT 12–24). The dot pattern bar represents the control feeding time (ZT 12–24), and the striped bar represents the SBR feeding time (ZT 16–24). Each value in the ZT points is means with their standard error of the mean; *n* 3. The rhythmicity was analysed by JTK_CYCLE. The results of the rhythmicity are shown in online Supplementary Table S6. SBR, skipping breakfast regimen; ZT, zeitgeber time.

circadian oscillations[49]. Therefore, it is possible that altered gene expression profiles may influence lipid metabolism. Clock genes are reported to regulate the circadian modulation of lipogenesis, lipolysis and adipokine secretion[9,50,51]. It has been reported that clock gene deficiency mice showed dyslipidaemia and was suggested that clock genes involved in the regulation of lipid metabolism[52]. In the present study, the gene expression of *Acly* and *Fas* was delayed, and the rhythmicity of *Acc* and *Srebp1c* expression was suppressed in the liver by SBR (Figs. 3 and 4); furthermore, the expression of several clock

genes was delayed by SBR in the epididymal adipose tissues (Fig. 5). These results suggest that altered gene expression caused by SBR leads to abnormal lipid metabolism. The alteration of clock gene expression in adipose tissue and suppressed energy expenditure may be related to increased body weight gain. We showed that SBR leads to an increase in body weight (Fig. 1(a)), and the disruption of the expression of clock genes and lipid metabolism-related genes would contribute to lifestyle-related diseases such as obesity. In the present study, 2 weeks of SBR induced abnormal lipid metabolism in mice. Since clock gene oscillations are flexible and rapid depending on the diet[53], it is possible that recovery from SBR may cause the oscillations in clock gene expression to return to normal again at an earlier stage. Recovery from SBR may be expected to abolish the effect of SBR on abnormal lipid metabolism. Since we only evaluated the expression of lipid metabolism-related genes in the present study, there is insufficient evidence for a conclusive result. Further investigations are necessary to elucidate the relationship between gene alterations and lipid metabolism.

In the present study, plantar muscle weight was significantly decreased by SBR (Table 1). Muscle not only regulates locomotion but also controls nutritional homoeostasis. The disruption of clock gene expression in muscles induces disorders of metabolism and energy homoeostasis[54]. Muscle metabolism is regulated by diet and exercise[55]. *Myod* is a master regulator of myogenesis and is a circadian oscillator that is regulated by clock genes[56]. *Rev-erba*-deficient mice showed increased expression of muscle atrophy-related genes and reduced muscle mass[57]. Choi *et al.* suggested that the disturbance in circadian rhythm caused by shift work leads to an increased risk of sarcopenia[58]. The oscillations in the expression of clock genes and *Myod* in the plantar muscle were delayed by SBR (Fig. 6). The expression of *Myod* increased from ZT 18 to ZT 22 in SBR mice. This result suggested that the conditions for muscle breakdown, such as SBR, trigger a compensatory increase in the expression of *Myod* (Fig. 6). E3 ubiquitin ligases, such as *Murf-1* and *Atrogin-1*, are regulators of protein degradation in skeletal muscle[59]. In the present study, the expression of *Murf-1* and *Atrogin-1* tended to increase in every time period in SBR mice (Fig. 6). The expression of *Murf-1* and *Atrogin-1* is known to increase after fasting[60]. In the present study, SBR mice were exposed to longer fasting periods than control mice. These results indicated that muscle protein breakdown was boosted by SBR. It has been suggested that the SBR-inducible disruption of circadian rhythm increases the risk of muscle atrophy and sarcopenia.

The present study demonstrated that SBR increased body weight and fat mass in adipose tissue. Moreover, skeletal muscle weight was reduced by SBR. The expression of clock genes was delayed in the liver, adipose tissue and muscle of SBR mice. SBR also leads to the delayed or fluctuating expression of several lipid and muscle metabolism-related genes. On the other hand, skipping meal in latter half of the active phase is reported to improve lipid metabolism[61]. The findings of their study support the result of the present study. Thus, we speculate that the disruption in the expression of clock, lipid metabolism-related and muscle metabolism-related genes caused by SBR led to body weight gain and muscle-mass loss. The mechanism of SBR-induced lipid abnormalities was suggested to be due to alterations in clock and lipid metabolism genes, as well as suppression of rising body temperature by nutrient depletion. The phenomena of the SBR-induced delays can be partially explained by our previous findings that insulin is a major factor in clock control[41]. Since the peripheral tissues evaluated in this study are the target tissues of insulin, we speculate that the alterations of several clock genes were caused by insulin.

In this study, several genes showed different results in the JTK_CYCLE analysis and cosinor analysis. These results can be explained by differences in the principles of the analysis methods. When the rhythm is complex or the waveform is not clear, it is difficult to fit actual curves by parametric analysis. This is because cosinor analysis tries to fit the results to a cosine curve, which does not fit the actual curve. However, the results and trends of the cosinor analysis are mostly similar to those of the JTK_CYCLE analysis. The results of this study indicate that dietary restriction, such as SBR, may not only lead to abnormal lipid metabolism but also deplete nutrients and suppress muscle synthesis. The combination of abnormalities in lipid and muscle metabolism may contribute to more serious health problems such as sarcopenic obesity. Our study suggests the importance of a breakfast regimen to prevent obesity and sarcopenia. Further studies are necessary to determine the relationship between obesity, sarcopenia and skipping breakfast.

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Supplementary material

For supplementary materials referred to in this article, please visit https://doi.org/10.1017/S0007114522000356

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