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Effects of Fasting and Re-Feeding on the Expression of *Dec1*, *Per1*, and Other Clock-Related Genes

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To elucidate the food-entrainable oscillatory mechanism of peripheral clock systems, we examined the effect of fasting on circadian expression of clock genes including *Dec1* and *Dec2* in mice. Withholding of food for 2 days had these effects: the expression level of *Dec1* mRNA decreased in all tissues examined, although *Per1* mRNA level markedly increased; *Per2* expression was reduced in the liver and heart only 42–46 h after the start of fasting; and expression profiles of *Dec2* and *Bmal1* were altered only in the heart and in the liver, respectively, whereas *Rev-erbα* mRNA levels did not change significantly. Re-feeding after 36-h starvation erased, at least in part, the effect of fasting on *Dec1*, *Dec2*, *Per1*, *Per2*, and *Bmal1* within several hours, and restriction feeding shifted the phase of expression profiles of all examined clock genes including *Dec1* and *Dec2*. These findings indicate that short-term fasting and re-feeding modulate the circadian rhythms of clock genes to different extents in peripheral tissues, and suggest that the expression of *Dec1*, *Per1*, and some other clock genes was closely linked with the metabolic activity of these tissues.

Key words: circadian rhythms, clock, DEC1, DEC2, PER1.

Abbreviations: SCN, suprachiasmatic nucleus; ZT, zeitgeber time; RF, restriction feeding; ANOVA, analysis of variance.

The generation of both circadian rhythmicity in mammalian behavior and homeostasis is maintained by the central clock system in the suprachiasmatic nucleus (SCN) (1), and that various gene products are involved in this central clock system (2). Recent studies have shown that the clock genes are also expressed in several peripheral tissues (3), and these genes may directly contribute to the regulation of daily rhythms in each peripheral tissue. Expression of some clock genes—such as *Per1, Per2, Dec1, Dec2, Rev-erbα*, and *Dbp*—are regulated through E boxes in their promoters (4), which are target sites for the transcriptional activator CLOCK and BMAL1 heterodimer. The mRNA expression of *Bmal1* and *Clock* shows the opposite phase of circadian rhythm from that of the other clock genes, and REV-ERBα/ROR binding elements (RORE) in their promoter regions are responsible for this phase of rhythmic expression. In the core feedback loop of the clock system, PER1 and PER2, along with CRY1 and CRY2, suppress CLOCK/BMAL1 activity, and DEC1 and DEC2—basic helix-loop-helix transcription factors—repress transcription from the CLOCK/BMAL1-activated promoter by binding to BMAL1 and/or E boxes (5–8).

By restricting daily food access to two or three hours during daytime (restriction feeding: RF), the phase of circadian rhythm of *Per1, Per2, Bmal1, Rev-erbα*, and *Dbp* expression—as well as that of behavior activity—shifted up to 12 h in mouse and rat peripheral tissues—such as liver, heart, retina, and kidney—although it had little effect on circadian expression of the clock genes in the SCN (9–14). In *Per1*-luciferase transgenic rats, the phase of circadian rhythm of luciferase expression in the colon, stomach, and esophagus was also affected by RF (15). On the other hand, while short-term fasting altered the expression levels of *Per1, Per2*, and *Dbp* in the liver and heart, it had little effect on the phase of their rhythms (16). Despite all these findings, the mechanisms underlying peripheral clock modulation by RF remain unknown, and information on the effects of fasting on circadian gene expression is known for only a few clock genes in the liver and heart. In the present study, we examined the effect of fasting on the expression profiles of *Dec1, Dec2, Per1, Per2, Rev-erbα*, and *Bmal1* in several peripheral tissues, including the liver, submandibular gland, heart, and small intestine. We were particularly interested in the effect of fasting and restriction feeding on the circadian expression of *Dec1* and *Dec2*, and we found that fasting had the greatest effect on the circadian rhythms of *Dec1* and *Per1* expression, while it had the least effect on those of *Dec2* and *Rev-erbα* expression. Expression levels of *Dec1, Per2*, and *Bmal1* mRNA were enhanced by re-feeding in some tissues, while *Per1* expression was reduced in all tissues examined. We also found that RF for 19 days shifted the circadian phases of *Dec1* and *Dec2* as well as *Per2* and *Rev-erbα*. These findings suggest that in peripheral tissues, short-term fasting modifies the molecular circadian sys-

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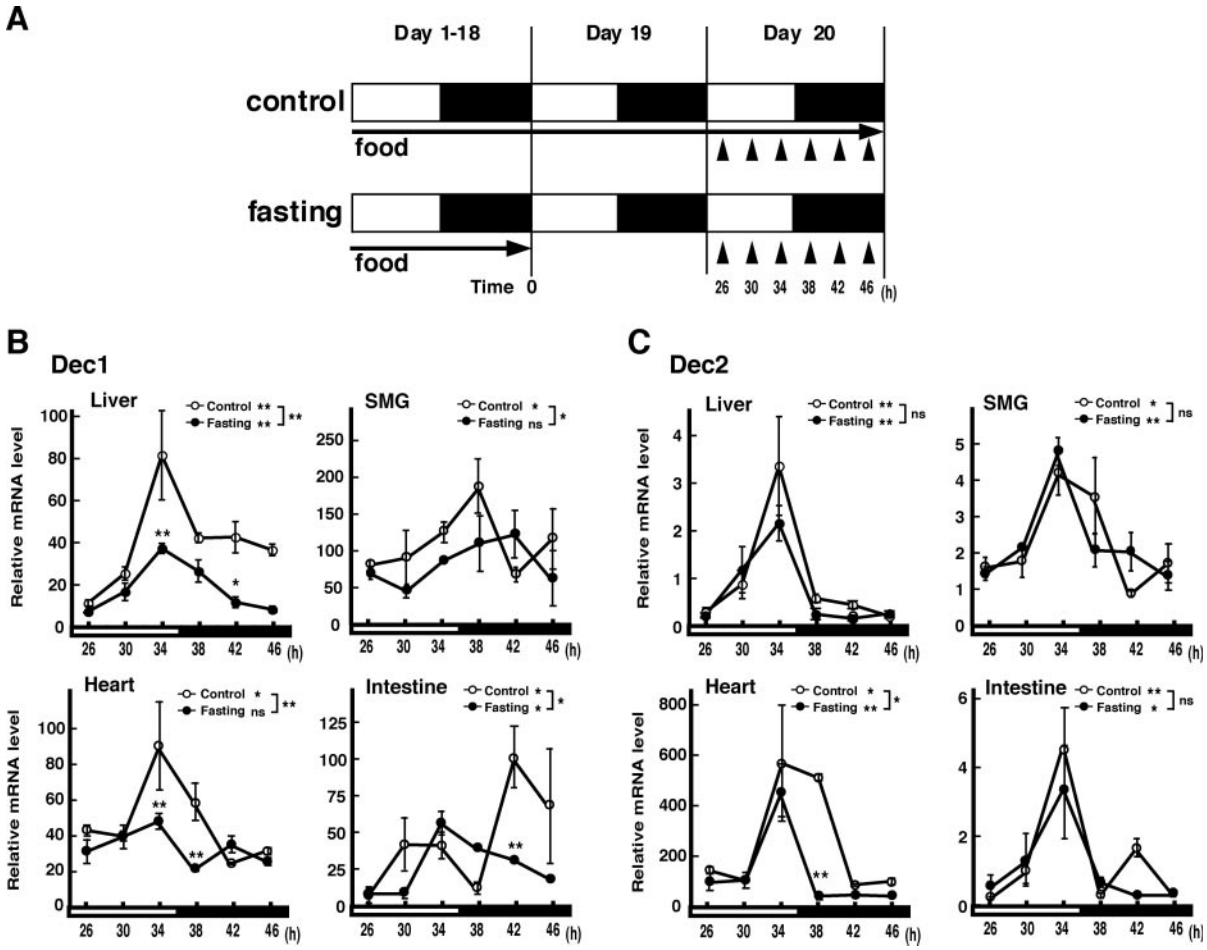

Fig. 1. **Daily expression profiles of *Dec1* and *Dec2* mRNAs in the liver, submandibular gland (SMG), heart, and small intestine under fasting conditions.** (A) Experimental schedule of fasting. Mice were housed under 12:12-h light-dark cycle for 18 days. Food of the fasting group was withheld at ZT0 on day 19 and the mice were sacrificed at the indicated hours after fasting. Feeding period is indicated by an arrow and sampling points are shown by arrow heads. Total RNA was isolated form various tissues and subjected to real-time RT-PCR analysis. (B and C) Expression profiles of mRNAs for *Dec1* and *Dec2*. Open circle, control mice; closed circles, fasting mice. *Dec1* and *Dec2* mRNA levels (mean ± SE, $n = 3$) are shown. *P*-values of one-way ANOVA for control and fasting mice are indicated in each panel. *P*-values of two-way ANOVA for control vs. fasting are also shown in the figure. $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not significant. Significantly changed points (Bonferroni/Dunn post hoc tests) are also indicated by asterisks.

tems through a decrease in *Dec1* expression or an increase in *Per1* expression.

MATERIALS AND METHODS

Feeding Schedule—Six-week-old male C57/BL6 mice (Crea Japan, Tokyo) were housed under a 12:12-h light-dark cycle for 18 days at constant temperature and given food and water ad libitum. Following this, food for the fasting group was withheld at zeitgeber time (ZT) 0 on day 19. The mice were sacrificed at the indicated hours after the start of fasting: The experimental schedule of fasting is shown in Fig. 1A. In another experiment, mice were re-fed after 36 h fasting. For the RF experiment, 8-week-old mice were allowed access to food for 3 h from ZT4 to ZT7 for 3 weeks. Food for the RF-fasting group was withheld after 3 weeks restriction feeding, and the mice were then sacrificed at the indicated hours after the start of

Table 1. **Expression levels of clock and clock-controlled genes in the liver, submandibular gland (SMG), heart, and small intestine altered by fasting or re-feeding.**

	Dec1	*Dec2*	*Per1*	*Per2*	*Bmal1*	*Rev-erbα*
Effect of fasting						
Liver	**,↓	ns	**,↑	**,↓	**,↓	ns
SMG	*,↓	ns	**,↑	ns	ns	ns
Heart	**,↓	*,↓	**,↑	*,↓	ns	ns
Intestine	*,↓	ns	*,↑	ns	ns	ns
Effect of re-feeding on starved mice (fasting → re-feeding)						
Liver	**,↑	**,↓	**,↓	**,↑	**,↑	ns
SMG	ns	ns	**,↓	ns	**,↑	**,↓
Heart	**,↑	*,↑	**,↓	ns	ns	ns
Intestine	*,↑	ns	**,↓	**,↑	ns	ns

P-values were calculated by two-way ANOVA. $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not significant. ↓, down-regulated; ↑, up-regulated.

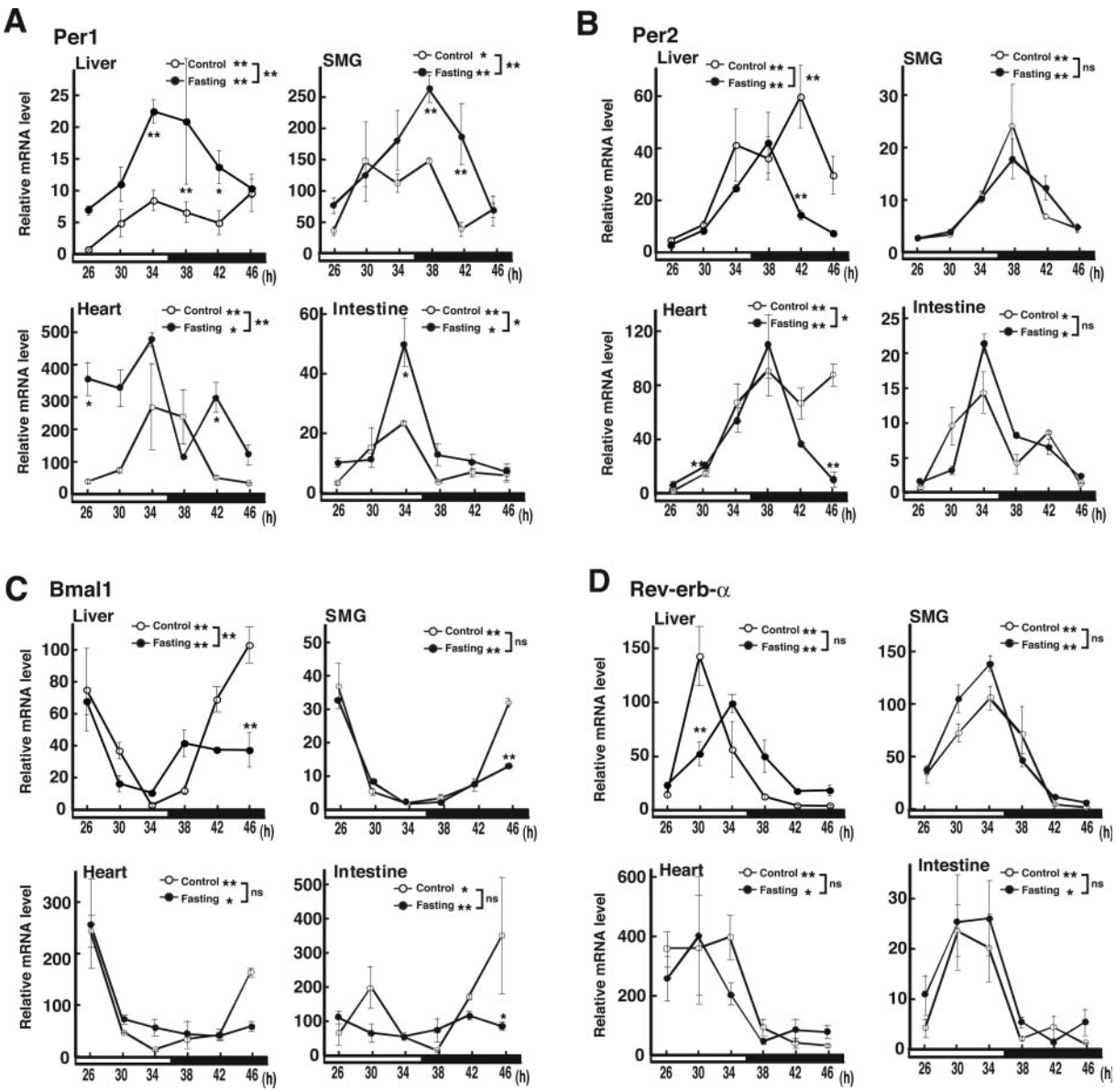

Fig. 2. **Daily mRNA expression profiles of various clock and clock-controlled genes under fasting conditions.** Expression levels (mean ± SE, $n = 3$) of *Per1* (A), *Per2* (B), *Rev-erbα* (C), and *Bmal1* (D) mRNA in the liver, submandibular gland (SMG), heart, and small intestine were determined. Open circle, control mice; closed circles, fasting mice. *P*-values of one-way ANOVA for control and fasting mice are indicated in each panel. *P*-values of two-way ANOVA for control vs. fasting are also shown in the figure. $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not significant. Significantly changed points (Bonferroni/Dunn post hoc tests) are also shown by asterisks.

fasting. All procedures were performed in compliance with standard principles and guidelines for the care and use of laboratory animals, Hiroshima University Graduate School of Biomedical Sciences and Hokkaido University Graduate School of Medicine.

RNA Extraction and Real-Time Quantitative RT-PCR—Total RNA was extracted from various mouse tissues, and mRNA levels were determined by real-time quantitative RT-PCR analysis using ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, CA). Sequences of TaqMan probe and primers for *Dec1, Dec2, Per1, Per2, Rev-erbα,* and *Bmal1* were previously described (17, 18).

Statistical Analysis—Circadian rhythmicity during a 24 h period was statistically analyzed by one-way analysis of variance (ANOVA). Differences between fasting and control groups were analyzed by two-way ANOVA, and the significance of difference at each time point was analyzed by Bonferroni/Dunn post hoc tests.

RESULTS

Effect of Fasting on Dec1 *and* Dec2 *Expression in Peripheral Tissues*—To investigate whether fasting affects expression of clock genes including *Dec1* and *Dec2* in peripheral tissues, mice were starved for 26 h, and then sacrificed at 4-h intervals as shown in Fig. 1A. The mRNA expression of *Dec1* and *Dec2* in control mice, fed ad libitum, showed circadian rhythms in the liver, submandibular

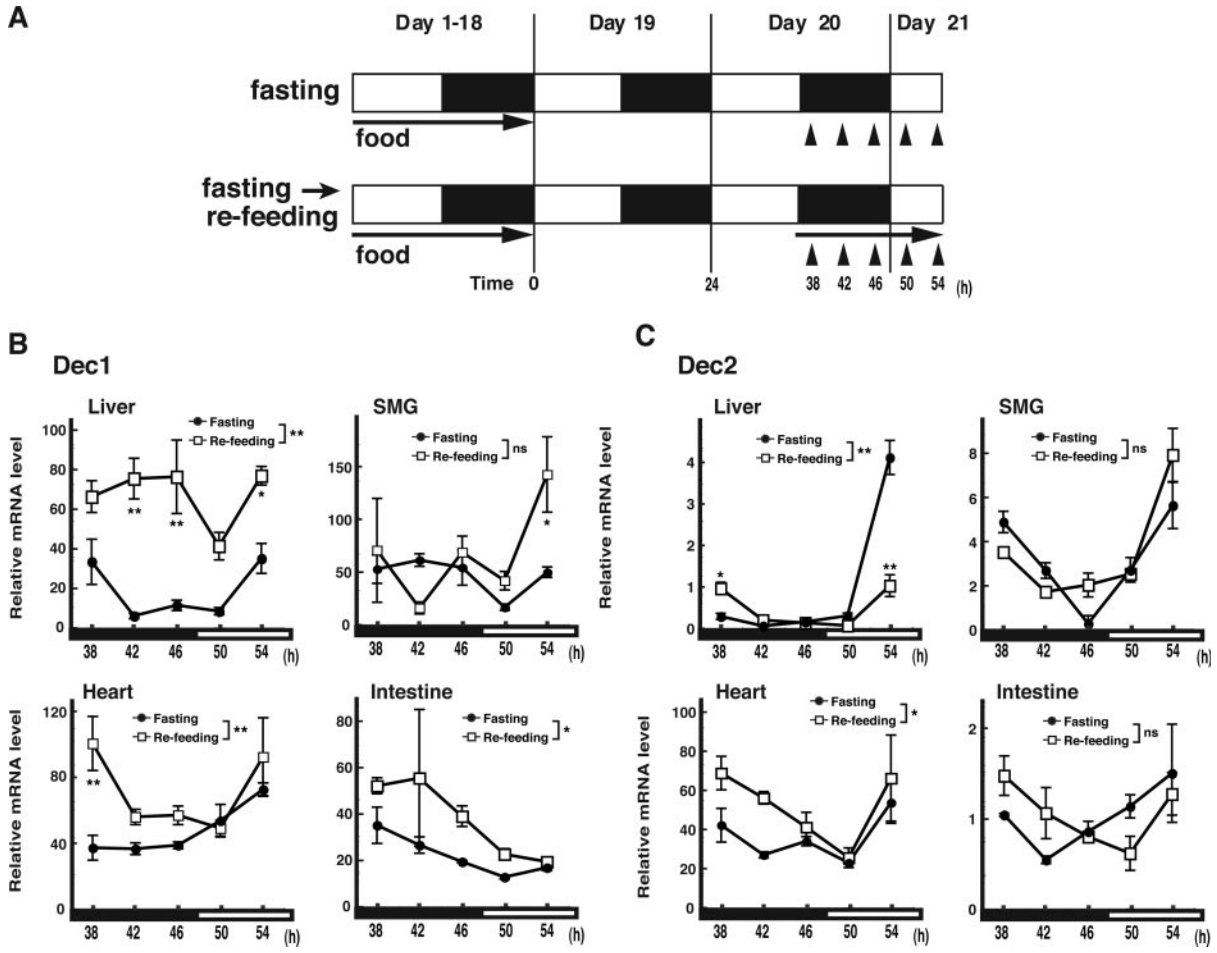

Fig. 3. **Effect of re-feeding on expression of *Dec1* and *Dec2* mRNAs.** (A) Experimental schedule of fasting and re-feeding. Food was withheld at ZT0 on day 19 and then given to mice of the re-feeding group at ZT12 on day20. Mice were sacrificed at the indicated hours after the start of fasting. Feeding period is indicated by an arrow and sampling points are shown by arrow heads. Total RNA was isolated from various tissues and subjected to real-time RT-PCR analysis. (B and C) Expression profiles of mRNAs for *Dec1* and *Dec2*. Closed circles, fasting mice; open square, re-feeding mice. *Dec1* and *Dec2* mRNA levels (mean ± SE, $n = 3$) are shown. *P*-values of two-way ANOVA for fasting *vs.* re-feeding are shown in the figure. $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not significant. Significantly changed points (Bonferroni/Dunn post hoc tests) are also indicated by asterisks.

gland, heart, and small intestine (one-way ANOVA, $P < 0.05$) (Fig. 1, B and C). Fasting reduced the mRNA level of *Dec1* in all tissues examined (two-way ANOVA, $P < 0.05$) (Fig. 1B and Table 1), and disrupted the rhythmicity of *Dec1* expression in the submandibular gland and heart. In contrast, fasting had a marginal effect on the rhythmicity and mRNA levels of *Dec2* in these tissues except for the expression level in the heart, which decreased significantly at 38 h after the start of fasting (Fig. 1C and Table 1).

Effect of Fasting on Per1, Per2, Bmal1*, and* Rev-erbα *Expression in Peripheral Tissues*—Expression profiles of other clock genes were also analyzed in the presence or absence of food. In the control mice, mRNA levels of *Per1, Per2, Rev-erbαα*, and *Bmal1* showed circadian rhythmicity in all tissues examined (one-way ANOVA, $P < 0.05$) (Fig. 2), and fasting increased *Per1* mRNA levels in the liver, submandibular gland, heart, and small intestine (two-way ANOVA, $P < 0.05$) (Fig. 2A and Table 1). In contrast, fasting decreased *Per2* mRNA levels 42–46 h after start of fasting in the liver and heart (two-way ANOVA, $P < 0.05$), but did not do so in the submandibular gland or small intestine (Fig. 2B and Table 1). Fasting significantly down-regulated the expression of *Bmal1* in the liver but not in the submandibular gland, heart, or small intestine (Fig. 2C and Table 1), although it reduced *Bmal1* mRNA levels 46 h after start of fasting in the submandibular gland and small intestine as well as the liver (Bonferroni/Dunn post hoc tests, $P < 0.05$). The expression profile of *Rev-erbα* was not significantly altered by fasting (Fig. 2D and Table 1),

Effect of Re-Feeding on Dec1 *and* Dec2, Per1, Per2, Bmal1*, and* Rev-erbα *Expression after 36 h Fasting*—To examine whether re-feeding would restore the altered expression profiles of these clock genes, we re-fed mice after 36 h fasting and sacrificed them at 4-h intervals as shown in Fig. 3A: Re-feeding significantly increased the *Dec1* mRNA level in the liver, heart, and small intestine (two-way ANOVA, $P < 0.05$) immediately after the start of re-feeding (Fig. 3B and Table 1), while *Dec2* expression changed only in the liver and heart (Fig. 3C and Table 1).

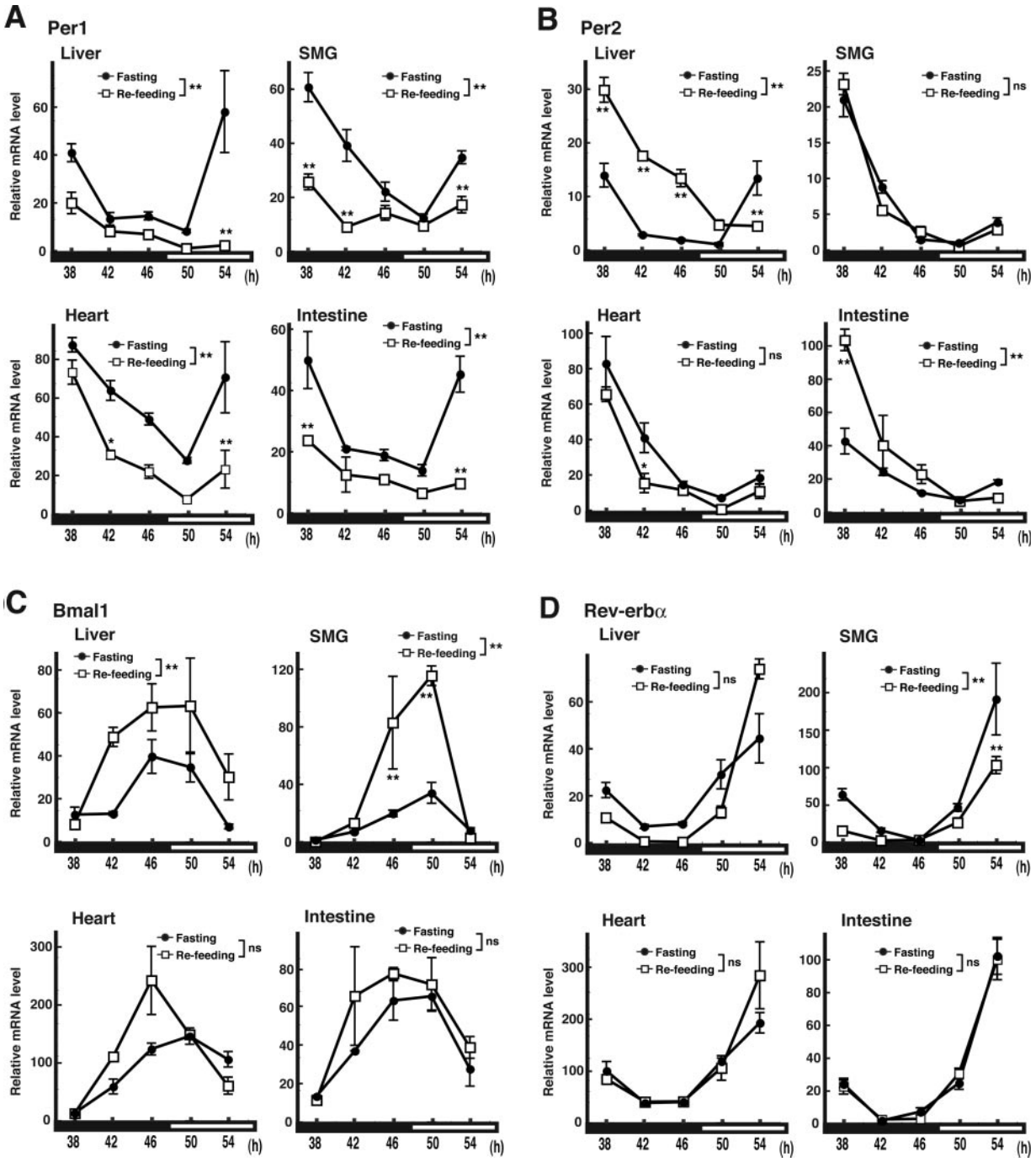

Fig. 4. **Effect of re-feeding on expression of *Per1, Per2, Rev-erbα,* and *Bmal1* mRNAs in the liver, submandibular gland (SMG), heart, and small intestine under fasting conditions.** Expression levels (mean ± SE, $n = 3$) of *Per1* (A), *Per2* (B), *Rev-erbα* (C), and *Bmal1* (D) mRNA in the liver, submandibular gland (SMG), heart, and intestine were determined. Closed circles, fasting mice; open square, re-feeding mice. *P*-values of two-way ANOVA for fasting *vs.* re-feeding are shown in the figure. $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not significant. Significantly changed points (Bonferroni/Dunn post hoc tests) are also shown by asterisks.

On the other hand, *Per1* mRNA levels were down-regulated in all tissues examined after re-feeding (two-way ANOVA, $P < 0.01$) (Fig. 4A and Table 1), although *Per2* mRNA levels were up-regulated in the liver and small intestine (two-way ANOVA, $P < 0.05$) (Fig. 4B and Table 1). *Bmal1* expression significantly increased in the liver and submandibular gland (two-way ANOVA, $P < 0.05$) (Fig. 4C and Table 1), whereas *Rev-erbα* expression slightly decreased only in the submandibular gland (Fig. 4D and Table 1).

Effect of Restriction Feeding (RF) on Dec1, Dec2, Per2*, and* Rev-erbα *Expression in the Liver*—In the next experiment, we investigated whether RF for 3 weeks would alter the expression profiles of clock genes including *Dec1* and

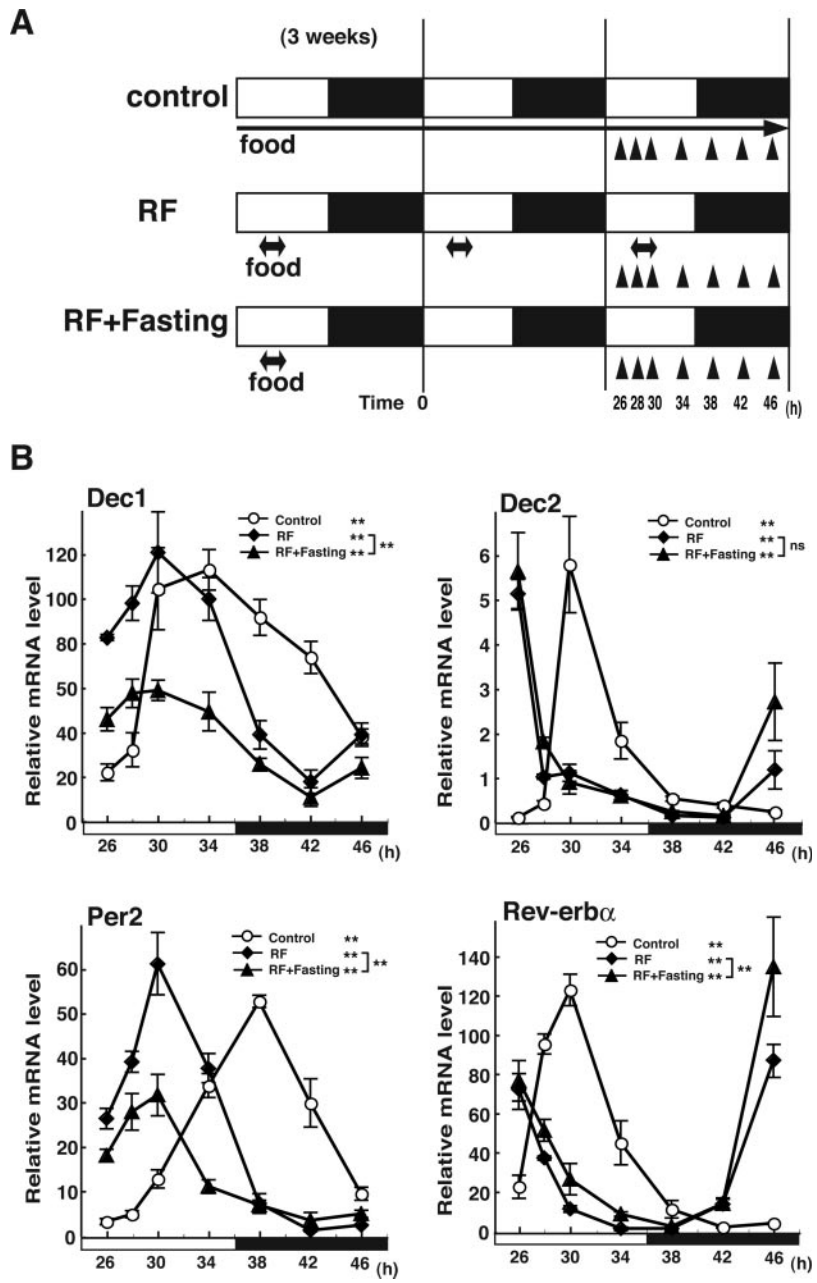

Fig. 5. **Effect of restriction feeding (RF) on daily expression of *Dec1, Dec2, Per2*, and *Rev-erbα*.** (A) Experimental schedule. Mice were housed under 12-h light-on (ZT0–ZT12) and 12-h light-off (ZT12–ZT24) cycle. Mice from the RF and RF+fasting groups were allowed access to food for 3 h from ZT4 to ZT7 for 3 weeks, after which food of mice from the RF+fasting group was withheld. The mice were sacrificed at the indicated hours after the start of fasting. Feeding period is indicated by an arrow and sampling points are shown by arrow heads. Total RNA was isolated form the liver and subjected to real-time RT-PCR analysis. (B) Expression profiles of mRNAs for *Dec1, Dec2, Per2*, and *Rev-erbα* (mean ± SE, $n = 3$). *P*-values of one-way ANOVA are indicated in each panel. *P*-values of two-way ANOVA for RF *vs.* RF+fasting are also shown in the figure. $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not significant.

Dec2: RF shifted the phase of expression of *Dec1, Dec2, Per2*, and *Rev-erbα* forward by 4–8 h (Fig. 5); fasting after RF markedly decreased the expression of *Dec1* and *Per2*, only slightly increased that of *Rev-erbα*, and did not affect *Dec2* expression.

DISCUSSION

In the present study, we examined the expression profiles of *Dec1, Dec2, Per1, Per2, Bmal1*, and *Rev-erbα* after an entire day of fasting, and we found that fasting had varying effects on the circadian rhythms of these clock genes: For example, fasting up-regulated *Per1* expression in the all tissues examined, whereas it down-regulated *Dec1* expression in these tissues. Recently, Kobayashi *et al.* (16) reported that *Per1* expression gradually increased in the liver and heart after withdrawal of food, while *Per2* expression decreased in the liver: We confirmed their observations for *Per1* and *Per2* expression, and further demonstrated that fasting also increased the expression of *Per1* in other tissues—the submandibular gland and small intestine—while it had little effect on *Per2* expression in these tissues (Fig. 2, A and B). In contrast to *Per1*, we found that fasting reduced *Dec1* circadian expression in all tissues examined (Fig. 1B): The rhythmicity of *Dec1* expression, in particular, was abolished in the submandibular gland and heart.

As shown in the present study, fasting decreased mRNA levels of *Dec1, Per2*, and *Bmal1* in tissue- and time-dependent manners, and it increased *Per1* mRNA levels in all tissues examined (Figs. 1 and 2). In addition, re-feeding canceled, at least in part, the effect of fasting:

mRNA levels of *Dec1, Per2*, and *Bmal1* in some tissues were enhanced by re-feeding and *Per1* mRNA levels in all tissues were reduced (Figs. 3 and 4). These results indicate that feeding readily affects the circadian expression of some clock genes in the peripheral tissues, although short term fasting/re-feeding may not immediately affect circadian rhythms of whole animal activity: Perhaps only in some peripheral tissues, the expression of some clock genes is linked with metabolic activity.

Recently we reported that DEC1 and DEC2—like PER and CRY—are negative regulators in the circadian clock system: These negative factors repress the CLOCK/BMAL1-activated promoters, including their own promoters. In addition, the expression profiles of *Dec1* and *Per1* were remarkably similar in the SCN and in other areas of brain, and light pulses induced expression of both *Dec1* and *Per1* in the SCN (5, 19). However, fasting had contrasting effects on their mRNA levels: *Dec1* expression decreased after fasting, whereas *Per1* expression increased, which suggests that these two genes play different roles in the metabolic activity–sensitive peripheral clock system. On the other hand, *Dec2* and *Per2* showed little responsiveness to fasting, so not all clock genes respond to short-term withdrawal of food.

In the previous study (17), we demonstrated that the mRNA levels of *Cry1* and *Amy1* showed two peaks in daily expression profiles in the submandibular gland, with the second peaks of these genes at ZT22 abolished by fasting. In the present study, we also found that the expression profiles of *Dec1, Dec2* (Fig. 1, B and C), and *Cry1* (data not shown) showed two peak patterns in the small intestine: The marked second peak at ZT18 was also abolished by fasting, suggesting that these second peaks in digestion-related organs may be associated with food intake at night.

Scheduled RF during daytime has been reported to alter the expression profiles of *Per1, Per2, Bmal1, Rev-erbα*, and *Dbp* in the liver, heart, retina, and kidney (*9–14*). In the present study, we demonstrated that RF shifted the circadian phases of *Dec1* and *Dec2* in addition to *Per2* and *Rev-erbα* (Fig. 5), indicating that the food-entrainable oscillatory mechanisms for *Dec1* and *Dec2* are similar to those of other well-characterized clock genes. The shift of the circadian phase of these clock genes may account for the subsequent coordination of the entire molecular clock system entrained by limited feeding over an extended time. Although RF altered the phase of the all clock genes examined, more than 43 h fasting after RF only reduced the expression levels of *Dec1* and *Per2* (Figs. 1B and 2B), which coincide with the down-regulation of these genes after fasting without RF. Since the central clock in the SCN is not affected by RF (10), the mechanisms underlying the alteration of each peripheral clock system in response to RF or fasting remain elusive. We theorize that *Dec1* and *Per1* play important roles in the responses of the peripheral clock system to fasting/feeding: Fasting/feeding alters plasma insulin levels, and *Dec1* in the liver is a target gene for insulin (20), so it is possible that *Dec1* links fasting/feeding status to the peripheral clock system. Cross-talk between circadian rhythms and metabolism has already been reported on *Bmal1* expression, which is induced during adipogenic differentiation of 3T3-L1 and embryonic fibroblast cells (21). In contrast, *Dec1* expression is down-regulated during adipogenic differentiation (22). Homozygous *Clock* mutant mice are obese and develop a metabolic syndrome of hyperleptinemia, hyperlipidemia, hepatic steatosis, hyperglycemia, and hypoinsulinemia (23), which suggests that circadian regulators such as BMAL1, DEC1, and CLOCK also play a role in some aspects of metabolism to maintain the energy balance in specific tissues.

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REFERENCES

1. Dunlap, J.C. (1999) Molecular bases for circadian clocks. *Cell* **96**, 271–290
2. Gekakis, N., Staknis, D., Nguyen, H.B., Davis, F.C., Wilsbacher, L.D., King, D.P., Takahashi, J.S., and Weitz, C.J. (1998) Role of the CLOCK protein in the mammalian circadian mechanism. *Science* **280**, 1564–1569
3. Panda, S., Antoch, M.P., Miller, B.H., Su, A.I., Schook, A.B., Straume, M., Schultz, P.G., Kay, S.A., Takahashi, J.S., and Hogenesch, J.B. (2002) Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* **109**, 307–320
4. Ueda, H.R., Hayashi, S., Chen, W., Sano, M., Machida, M., Shigeyoshi, Y., Iino, M., and Hashimoto, S. (2005) System-level identification of transcriptional circuits underlying mammalian circadian clocks. *Nat. Genet.* **37**, 187–192
5. Honma, S., Kawamoto, T., Takagi, Y., Fujimoto, K., Sato, F., Noshiro, M., Kato, Y., and Honma, K. (2002) Dec1 and Dec2 are regulators of the mammalian molecular clock. *Nature* **419**, 841–844
6. Kawamoto, T., Noshiro, M., Sato, F., Maemura, K., Takeda, N., Nagai, R., Iwata, T., Fujimoto, K., Furukawa, M., Miyazaki, K., Honma, S., Honma, K., and Kato, Y. (2004) A novel autofeedback loop of Dec1 transcription involved in circadian rhythm regulation. *Biochem. Biophys. Res. Commun.* **313**, 117–124
7. Hamaguchi, H., Fujimoto, K., Kawamoto, T., Noshiro, M., Maemura, K., Takeda, N., Nagai, R., Furukawa, M., Honma, S., Honma, K., Kurihara, H., and Kato, Y. (2004) Expression of the gene for Dec2, a basic helix-loop-helix transcription factor, is regulated by a molecular clock system. *Biochem. J.* **382**, 43–50
8. Sato, F., Kawamoto, T., Fujimoto, K., Noshiro, M., Honda, K.K., Honma, S., Honma, K., and Kato, Y. (2004) Functional analysis of the basic helix-loop-helix transcription factor DEC1 in circadian regulation. Interaction with BMAL1. *Eur. J. Biochem.* **271**, 4409–4419
9. Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F., and Schibler, U. (2000) Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev.* **14**, 2950–2961
10. Hara, R., Wan, K., Wakamatsu, H., Aida, R., Moriya, T., Akiyama, M., and Shibata, S. (2001) Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Genes Cells* **6**, 269–278
11. Minami, Y., Horikawa, K., Akiyama, M., and Shibata, S. (2002) Restricted feeding induces daily expression of clock genes and Pai-1 mRNA in the heart of Clock mutant mice. *FEBS Lett.* **526**, 115–118
12. Oishi, K., Miyazaki, K., and Ishida, N. (2002) Functional CLOCK is not involved in the entrainment of peripheral clocks to the restricted feeding: entrainable expression of mPer2 and BMAL1 mRNAs in the heart of Clock mutant mice on Jcl:ICR background. *Biochem. Biophys. Res. Commun.* **298**, 198–202

13. Oishi, K., Sakamoto, K., and Ishida, N. (2003) Bimodal circadian expression of serotonin N-acetyltransferase mRNA in the retina of rats under restricted feeding. *Neurosci. Lett.* **351**, 21–24
14. Oishi, K., Kasamatsu, M., and Ishida, N. (2004) Gene- and tissue-specific alterations of circadian clock gene expression in streptozotocin-induced diabetic mice under restricted feeding. *Biochem. Biophys. Res. Commun.* **317**, 330–334
15. Davidson, A.J., Poole, A.S., Yamazaki, S., and Menaker, M. (2003) Is the food-entrainable circadian oscillator in the digestive system? *Genes Brain Behav.* **2**, 32–39
16. Kobayashi, H., Oishi, K., Hanai, S., and Ishida, N. (2004) Effect of feeding on peripheral circadian rhythms and behaviour in mammals. *Genes Cells* **9**, 857–864
17. Furukawa, M., Kawamoto, T., Noshiro, M., Honda, K.K., Sakai, M., Fujimoto, K., Honma, S., Honma, K., Hamada, T., and Kato, Y. (2005) Clock gene expression in the submandibular glands. *J. Dent. Res.* **84**, 1193–1197
18. Noshiro, M., Kawamoto, T., Furukawa, M., Fujimoto, K., Yoshida, Y., Sasabe, E., Tsutsumi, S., Hamada, T., Honma, S., Honma, K., and Kato, Y. (2004) Rhythmic expression of DEC1 and DEC2 in peripheral tissues: DEC2 is a potent suppressor for hepatic cytochrome P450s opposing DBP. *Genes Cells* **9**, 317–329
19. Butler, M.P., Honma, S., Fukumoto, T., Kawamoto, T., Fujimoto, K., Noshiro, M., Kato, Y., and Honma, K. (2004) Dec1 and Dec2 expression is disrupted in the suprachiasmatic nuclei of Clock mutant mice. *J. Biol. Rhythms* **19**, 126–134
20. Yamada, K., Kawata, H., Shou, Z., Mizutani, T., Noguchi, T., and Miyamoto, K. (2003) Insulin induces the expression of the SHARP-2/Stra13/DEC1 gene via a phosphoinositide 3-kinase pathway. *J. Biol. Chem.* **278**, 30719–30724
21. Shimba, S., Ishii, N., Ohta, Y., Ohno, T., Watabe, Y., Hayashi, M., Wada, T., Aoyagi, T., and Tezuka, M. (2005) Brain and muscle Arnt-like protein-1 (BMAL1), a component of the molecular clock, regulates adipogenesis. *Proc. Natl. Acad. Sci. USA* **102**, 12071–12076
22. Iwata, T., Kawamoto, T., Sasabe, E., Miyazaki, K., Fujimoto, K., Noshiro, M., Kurihara, H., and Kato, Y. (2006) Effects of overexpression of basic helix-loop-helix transcription factor Dec1 on osteogenic and adipogenic differentiation of mesenchymal stem cells. *Eur. J. Cell Biol.* **85**, 423–431
23. Turek, F.W., Joshu, C., Kohsaka, A., Lin, E., Ivanova, G., McDearmon, E., Laposky, A., Losee-Olson, S., Easton, A., Jensen, D.R., Eckel, R.H., Takahashi, J.S., and Bass, J. (2005) Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* **308**, 1043–1045