Characteristics of Circadian Gene Expressions in Mice White Adipose Tissue and 3T3-L1 Adipocytes

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(Received August 6, 2004; Accepted September 23, 2004; Published online September 24, 2004)

Most, if not all, of physiological and behavioral processes exhibit circadian rhythms. Recently, molecular clocks similar to those operating in the suprachiasmatic nucleus (SCN) neurons have been found in several peripheral tissues. Among peripheral tissues, several recent studies have revealed that white adipose tissues secrete a number of biologically active molecules such as leptin, resistin, adiponectin, *etc*. Importantly, the levels of these cytokine-like molecules are associated with development of lifestyle-related diseases such as diabetic mellitus, cardiovascular diseases, and obesity. In this study, we attempted to characterize circadian gene expression in adipose tissue. Here, we show that the expression of several clock genes exhibits daily oscillation in mice white adipocytes. Circadian expression of adipocytes-related genes such as peroxisome proliferator-activated receptor (PPAR) γ2 was also observed. Interestingly, expression pattern of some clock genes in adipocytes is distinct from that in stromal-vascular fractions containing preadipocytes. In addition to these *in vivo* studies, we demonstrated that serum- or dexamethasone-shock induced the cyclic gene expression of clock genes in cultured adipocytes. Consequently, we are led to conclude that adipocytes contain the machineries necessary for circadian oscillation similar to that found in SCN. We then examined that whether the pharmacological effects of PPARγ2 ligand depend on the time of administration. Consist with its receptor levels, the pharmacological effects of PPARγ ligand administered during a dark period were more efficient than those during a light period in mice. These results suggest that chronotherapy targeted for adipocyte functions could be effective in improving of the symptoms of hyperlipidemia and other related diseases.

Key words —— adipocyte, circadian rhythm, chronotherapy

INTRODUCTION

Most, if not all, of physiological and behavioral processes exhibit circadian rhythms.[1,2] The master pacemaker of circadian rhythm resides in the suprachiasmatic nucleus (SCN) in mammals.[3,4] The SCN consists of multiple, autonomous single cell circadian oscillators that are generating a coordinated circadian rhythmic output in intact animals. Recently, molecular clocks similar to those operating in SCN neurons have been found in several peripheral tissues and in cultured fibroblast cells.[5–9] Analysis of circadian gene expression in liver and heart using DNA arrays revealed that peripheral circadian gene regulation is extensive, and distributions of circadian phases in the two tissues are markedly different.[9] Several recent studies have shown that various peripheral clocks are affected by metabolic aberration.[10,11] In addition, disturbance of the phase oscillation in several tissues has been demonstrated in diabetes mice induced by streptozotocin.[12] Consequently, nutritional effects such as diet may also regulate peripheral clock oscillation.

At the molecular level, the feedback loop in the transcriptional and posttranscriptional gene expression regulatory mechanism generates circadian oscillations.[13] Two transcription factors, brain and muscle Arnt like protein (BMAL) 1 and CLOCK, are identified as the positive limb in the feedback loop. BMAL1 and CLOCK form the heterodimer and bind to the E-box located in the regulatory region of several genes, including peroid (Per) 1 and cryptochrome (Cry). Per1 and Cry were identified as the genes regulated by BMAL1/CLOCK. Following translation of PER and CRY proteins, the PER/CRY complex translocates into the nucleus, where it inhibits gene expression driven BMAL1/CLOCK. At the same time, PER2 up-regulates Bmal1 mRNA

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level through an as yet uncharacterized mechanism leading to restart of the cycle.[14]

Among peripheral tissues, white adipose tissues (WAT) have been thought to be responsible only for storage of excess energy derived from diet. In addition to these passive roles in the regulation of homeostasis, several recent studies have revealed that mammalian WAT secretes a number of biologically active molecules such as leptin,[15] resistin,[16] plasminogen activator inhibitor-1 (PAI-1),[17] adiponectin,[18] *etc*. Importantly, the levels of these molecules are associated with the development of lifestyle-related diseases such as diabetic mellitus, cardiovascular diseases, and obesity.[15,16,19,20] The abundance of these adipocytokines varies throughout the day. For example, plasma leptin levels reach a low during light periods, peak during dark period, and surge significantly in response to feeding.[21] PAI-1 expression is elevated early in the morning and declines during the afternoon in humans.[22] Enhanced secretion of PAI-1 may contribute to both the increased incidence of acute atherothrombotic events and the decreased efficacy of thrombolytic therapy.[23] The levels of adiponectin exhibit a diurnal rhythm with a significant decline at night in healthy men.[24] Conversely, serum levels of adiponectin are diminished in humans and animals with insulin resistance and obesity.[20,25,26] The daily rhythms in serum-free fatty acid levels are well known.[27,28] Consequently, circumstantial evidence suggests that adipocytes possess a functional molecular clock.

In this study, we attempted to characterize circadian gene expression in adipose tissue. Here, we show that expression of several clock genes such as Bmal1 exhibit daily oscillations in mice white adipocytes. Rhythmic expression of adipocyte-related genes such as peroxisome proliferator-activated receptor (PPAR) γ was also observed. In addition to these *in vivo* studies, we demonstrated that serum- or dexamethasone-shock generated the cyclic gene expression of clock genes in cultured adipocytes, suggesting that adipocytes contain the machinery necessary for circadian oscillation that is as robust as that found in SCN. Consistent with its receptor levels, the pharmacological effects of PPARγ ligand administered during the dark period were more efficient than those during the light period in mice, indicating that chronotherapy targeted for adipocyte functions could be effective in the improvement of symptoms of hyperlipidemia and other related diseases.

MATERIALS AND METHODS

Animals —— The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Nihon University. Male C57Bl/6J mice (7-week-old) fed ad libitum were maintained at 23 ± 1°C with 50 ± 10% relative humidity, and 3–5 animals per cage. Animals were allowed to adapt to the lighting schedule [12 hr light/12 hr dark cycle (light: zeitgeber time (ZT) 0–12; dark: ZT 12–24)] for several weeks before the experiments. Obese mice were generated by feeding with a high fat diet (OYC, Tokyo, Japan) for 4 weeks. Rosiglitazone (RSG, LKT Laboratories, Inc., St. Paul, MN, U.S.A.) (10 mg/kg body weight) was daily administered either at ZT 6 or ZT 18 for 15 days by oral gavage as suspensions in 0.25% carboxymethylcellulose (CMC).

Fractionation of Mice Adipose Tissue —— Epididymal white adipose tissue was excised from mice and fractionated into an adipocyte and a stromal-vascular fraction as described previously.[29] Briefly, freshly excised fat pads from C57Bl/6J mice were rinsed in phosphate buffered saline (PBS), minced, and digested for 45–60 min at 37°C in Krebs-Ringer bicarbonate (pH 7.4) with 4% bovine serum albumin and 1.5 mg/ml type I collagenase (Worthington, Freehold, NJ, U.S.A.). The digested tissue was filtered through a 250-μm nylon mesh to remove undigested tissue and centrifuged at 500 × g for 5 min. The floating adipocyte fraction was removed, washed in the buffer, and re-centrifuged to isolate free adipocytes. The stromal-vascular pellet was resuspended in erythrocyte lysis buffer (154 mM NH_4Cl, 10 mM $KHCO_3$, and 1 mM EDTA), filtered through a 28-μm nylon mesh to remove endothelial cells, and pelleted at 500 × g for 5 min.

Analysis of mRNA Expression by RT-PCR —— Total RNA was extracted from tissues with TRI reagent (Sigma, St. Louis, MO, U.S.A.) according to the manufacturer's instructions. The RNA (1 μg) was then reverse transcribed using oligo dT primer. A portion of the cDNA (correspond to 0.1 μg of total RNA) was amplified by PCR in 50 μl mixtures consisting of 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 2 μCi of [α-^{32}P] dCTP (3000 Ci/mmol). PCR comprised 22 cycles for GAPDH and 25 cycles for others, with denaturing at 94°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 30 sec. The PCR products were analyzed by running reactions on 10% acrylamide gels, and the radioactivity was evaluated

Table 1. List of Gene Specific Primers

	Forward primer (5′-3′)	Reverse primer (5′-3′)
Bmal1	TTC TCC AGG AGG CAA GAA GA	TTG CTG CCT CAT CGT TAC TG
Clock	TGC CAG CTC ATG AAA AGA TG	CGC TGC TCT AGC TGG TCT TT
Per1	GGG AGC TCA AAC TTC GAC TG	TCG GAT GTG ATA TGC TCC AA
Per2	AGA GTG TGG TGT CCC TCA CC	ATG TGC AGC CAG AAC TTC CT
Per3	TGG CTG CAG GAG GAT TTA AG	TGC TGT GCT TAG CAG TGG AC
Dbp	ACC GTG GAG GTG CTA ATG AC	TTG TAC CTC CGG CTC CAG TA
Rev-Erbα	CTT CCG TGA CCT TTC TCA GC	CAG CTC CTC CTC GGT AAG TG
Adiponectin	AAG GAC AAG GCC GTT CTC T	TAT GGG TAG TTG CAG TCA GTT GG
Resistin	TCA TTT CCC CTC CTT TTC CT	CAA GAC TGC TGT GCC TTC TG
PAI-1	GCT GTA GAC GAG CTG ACA CG	ACG TCA TAC TCG AGC CCA TC
Leptin	TGA CAC CAA AAC CCT CAT CA	TCA TTG GCT ATC TGC AGC AC
TNFα	CGT CAG CCG ATT TGC TAT CT	CGG ACT CCG CAA AGT CTA AG
PPARα	ATG CCA GTA CTG CCG TTT TC	GGC CTT GAC CTT GTT CAT GT
PPARγ1	GAC GCG GAA GAA GAG ACC T	AAT GGC ATC TCT GTG TCA ACC
PPARγ2	GCT GTT ATG GGT GAA ACT CTG	ATA ATA AGG TGG AGA TGC AGG
aP2	CGC TCT AGA ATG TGT GAT GCC TTT GTG GGA AA	CGC CTC GAG TCA TGC CCT CTC ATA AAC TCT TGT GGA
ADRP	CCC TGT CTA CCA AGC TCT GC	CGA TGC TTC TCT TCC ACT CC
Adipsin	CTG CTG GAC GAG CAG TGG	GAT GAC ACT CGG GTA T
AEBP-1	AAT CCT CGC TCT GGG ACT TT	GAT GCC TTG CTC ATC TGT CA
LPL	AGG GCT CTG CCT GAG TTG TA	AGA AAT TTC GAA GGC CTG GT
IR	AAA GTT TGC CCA ACC ATC TG	GTG AAG GTC TTG GCA GAA GC
IRS1	GCT CTA GTG CTT CCG TGT CC	GTT GCC ACC CCT AGA CAA AA
IRS3	GGA GGG TGC CTG CAC TAT TA	GCG AAG ATC CAA GAC TCA GG
Glut1	GCT GTG CTT ATG GGC TTC TC	ACA CCT GGG CAA TAA GGA TG
Glut3	TGT CAC AGG AGA AGC AGG TG	GCT CCA ATC GTG GCA TAG AT
Glut4	GCT TTG TGG CCT TCT TTG AG	CGG CAA ATA GAA GGA AGA CG
Glut8	ATG TCA AGG GTG TGG CTA CC	AAT GGG CTG TGA CTT GTT CC
TfR	TGC AGA AAA GGT TGC AAA TG	AGG CAA CCC TGA TGA CTG AG
GAPDH	ACC ACA GTC CAT GCC ATC AC	TCC ACC ACC CTG TTG CTG TA

by autoradiography. The linearity of the quantitation of RT-PCR products was ascertained by the experiment using varied amounts of total RNA obtained from mice adipose tissue. The PCR products were also subcloned in pGEM-T vector (Promega, Madison, WI, U.S.A.), and the sequences were confirmed. Gene expression was quantitated using National Institutes of Health Image 1.61 software as described previously.[30] Table 1 lists the primer pairs used.

Cell Culture —— 3T3-L1 cells, obtained from the Human Science Research Resources Bank (Osaka, Japan), were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% calf serum. For induction of adipose differentiation, cells were grown to confluence. The cells were then fed with differentiation medium [a 3 : 1 mixture of DMEM and Ham's F12 containing 10% FBS, 1.6 μM insulin, 0.0005% transferrin, 180 μM adenine, 20 pM triiodothyronine, 0.25 μM dexamethasone (DEX), and 500 μM isobutylmethylxanthine (IBMX)]. After 3 days, the cells were refed with fresh differentiation medium without DEX and IBMX and maintained over the following days.

***In Vitro* Adipocytes Synchronization** —— To synchronize the molecular clock in differentiated 3T3-L1 cells, the cells were treated with 50% horse serum or DEX as described previously.[5,7,8] Briefly, at time = 0, the medium was exchanged with serum-rich medium (DMEM supplemented with 50% horse serum) or DEX-rich medium (DMEM supplemented with 250 μM DEX). After 2 hr, the medium was replaced with serum-free OPTI-MEM (Invitrogen, Carlsbad, CA, U.S.A.). At the indicated times, total RNA was isolated with TRI reagent.

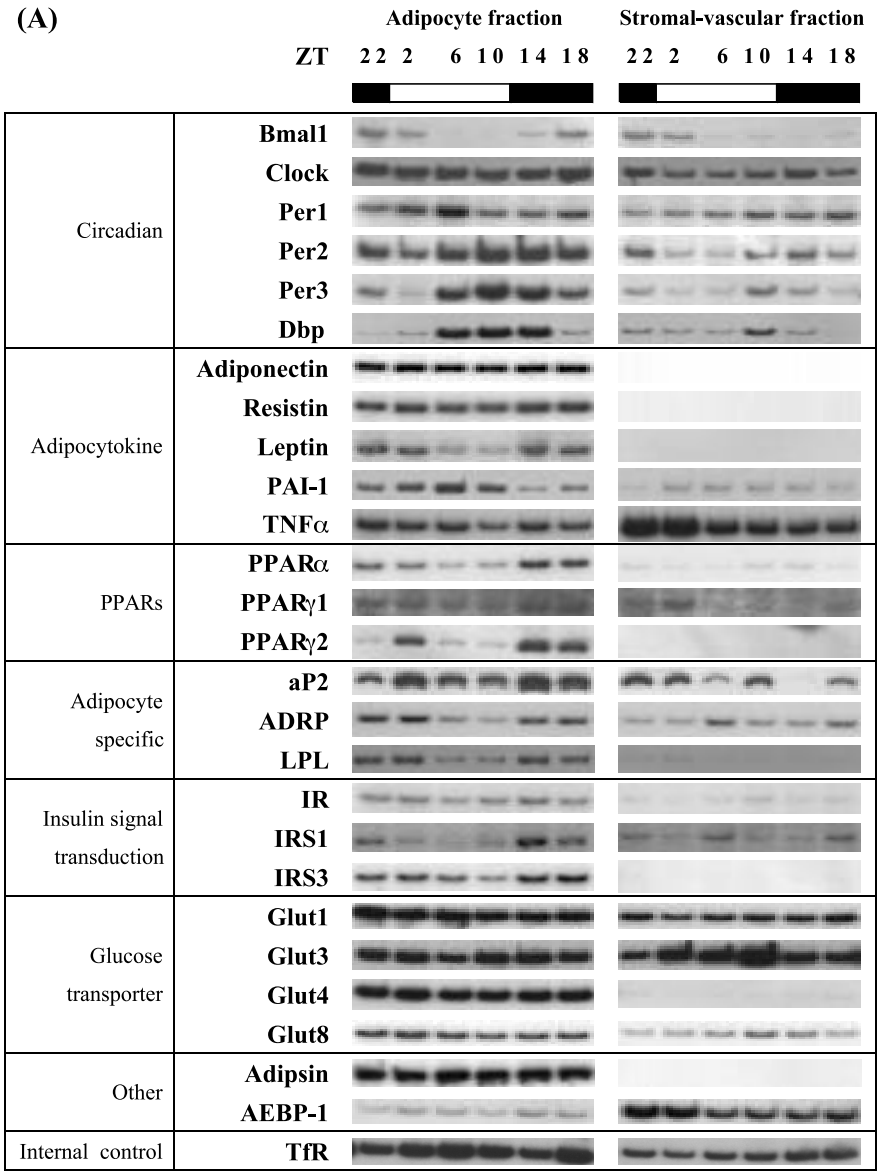

Fig. 1. Analysis of Circadian Gene Expressions in Mice WAT

(A) Mice white adipose tissues isolated from five mice were mixed, and fractionated into adipocyte and stromal-vascular fractions at the indicated times as described in MATERIALS AND METHODS. Total RNA was isolated, and the expression of clock genes was determined by RT-PCR using specific primers. Expression of transferrin receptor (TfR) was measured as an internal control to evaluate sample-to-sample differences in RNA concentration. The experiment was performed three times with similar results. (B) Expression levels of clock genes in each fraction are assigned as arbitrary units based on the band intensity assessed by densitometry. Solid line and dashed line represent levels in adipocytes and stromal-vascular fraction, respectively.

Measurement of Level of Plasma Triacylglycerol (TG) and Non-Esterified Fatty Acids (NEFA) ——— Blood samples (1 ml) were withdrawn from the heart and centrifuged for 5 min, and the resulting supernatant was used for the assays. Plasma levels of TG and NEFA were measured spectrophotometrically with a commercially available kit (Wako, Osaka, Japan).

RESULTS

Circadian Gene Expressions in Mice WAT

In a first set of experiments, we examined the expression level of clock genes in adipocytes isolated from mice epididymal WAT. WAT was fractionated into adipocyte and stromal-vascular fractions. Preadipocytes were collected into the stromal-vascular fraction. The purity of the fractions was ascertained by measuring the expression of adipocytes-specific gene such as adipsin (Fig. 1). The results

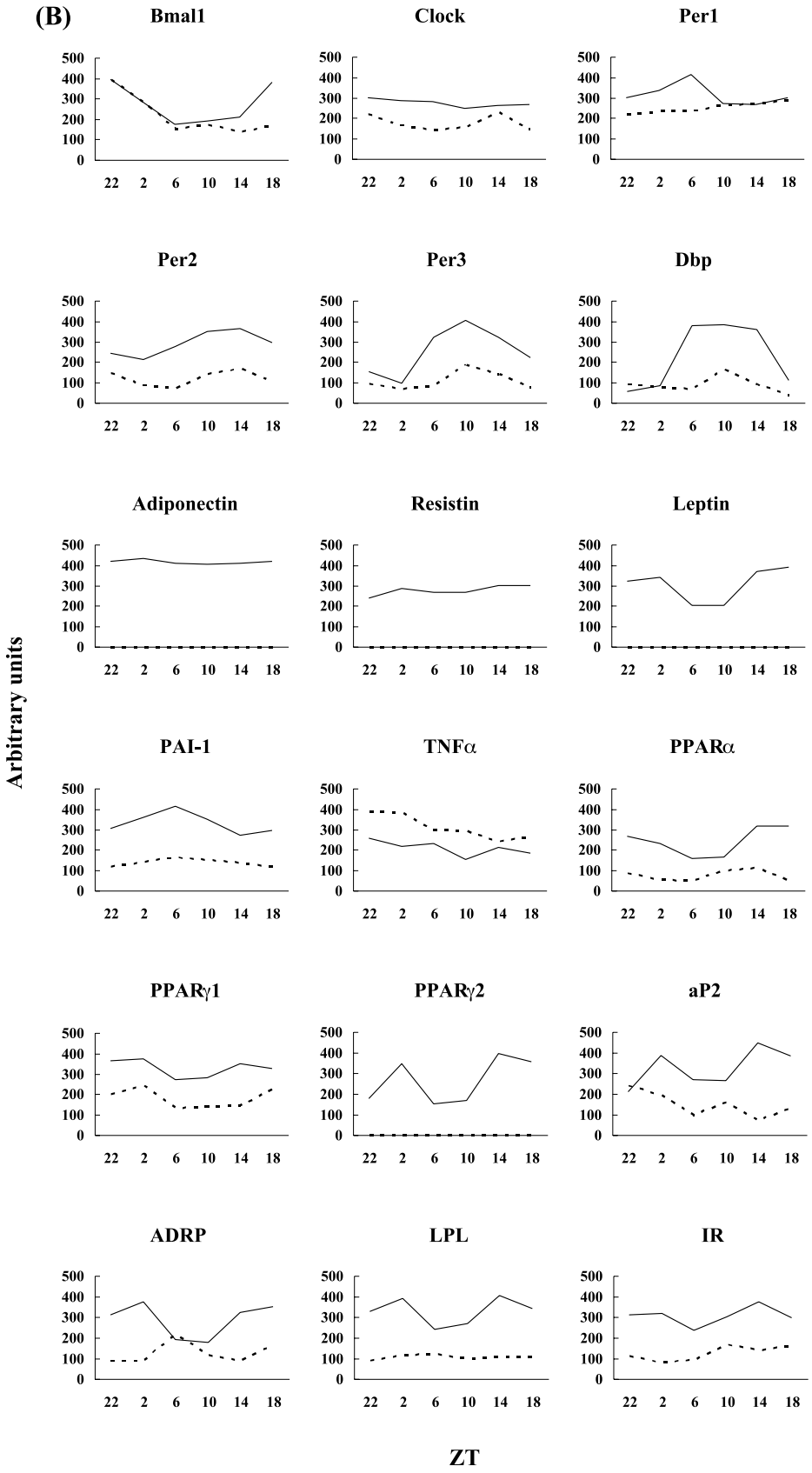

Fig. 1. Continued

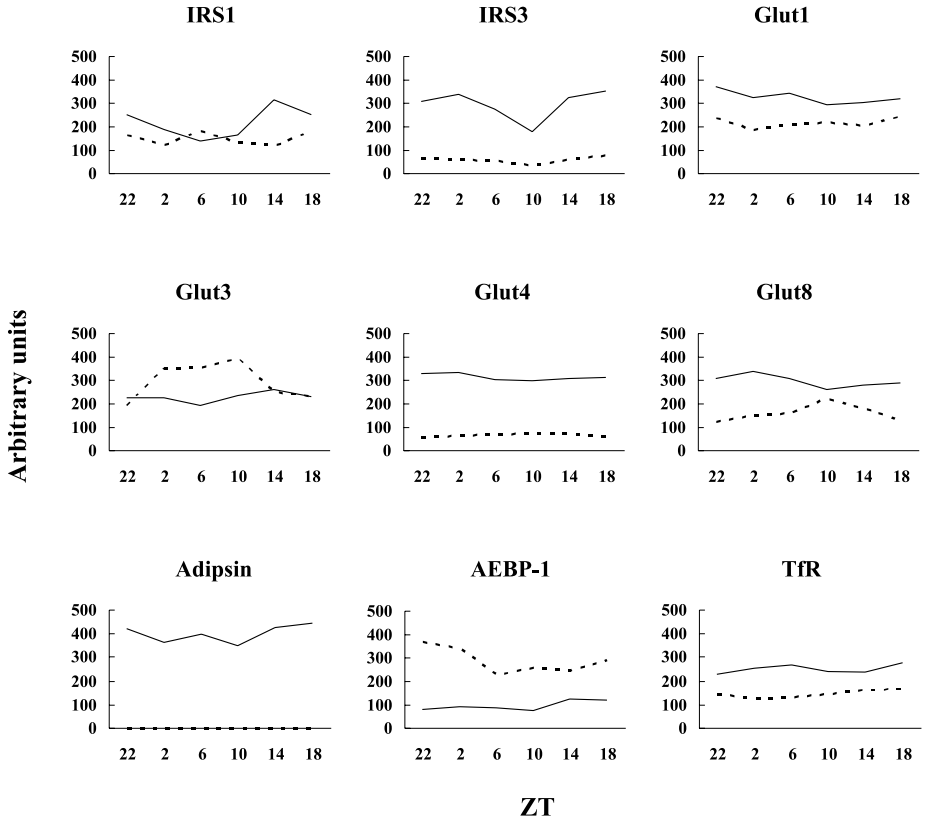

Fig. 1. Continued

shown in Fig. 1 demonstrated that the clock genes examined were expressed in both the adipocyte and stromal-vascular fractions. Interestingly, these factors were expressed preferentially in adipocytes rather than the stromal-vascular fraction. Expression of Bmal1, a core transcription factor of the circadian clock, exhibited similar amplitude of circadian oscillation in the adipocyte fraction and stromal-vascular fractions. However, the expression phase in adipocytes was advanced to that in the stromal-vascular fraction (Fig. 1). Expression of Per1 was rhythmic in adipocytes but less rhythmic in the stromal-vascular fraction (Fig. 1). In contrast to Per1 gene expression, Clock gene showed circadian expression in the stromal-vascular fraction but not in adipocytes (Fig. 1). The expression pattern of Per2, Per3, and Dbp showed robust circadian rhythms in both adipocytes and the stromal-vascular fraction (Fig. 1).

With regard to adipocytokines, expression of adiponectin, resistin, and leptin was detected only in adipocytes (Fig. 1). Expression of adiponectin and resistin was maintained to almost constant during the day (Fig. 1). As reported previously,[21,31–33] expression of leptin was raised during the dark period and lowered during the light period. PAI-1, which is highly associated with the development of cardiovascular diseases, was preferentially expressed in adipocytes compared with the stromal-vascular fraction (Fig. 1). The expression pattern of PAI-1 showed clear circadian oscillations in both the adipocyte and stromal-vascular fractions (Fig. 1). Tumor necrosis factor (TNF) α, which causes insulin resistance in target tissues, was expressed with weak oscillations in both the adipocyte and stromal-vascular fractions (Fig. 1). The expression of PPARα and PPARγ1 was lowered during the light period and elevated during the dark period (Fig. 1). Interestingly, expression of PPARγ2 in adipocytes showed a dual peak at ZT 2 and ZT 14 (Fig. 1). Expression of aP2, and LPL, known as the target gene of PPARγ2, also exhibited a dual peak during the day (Fig. 1). Factors involving insulin signaling such as IR, IRS1 and IRS3 are predominantly expressed in adipocytes compared with the stromal-vascular fraction (Fig. 1). While IRS1 expression exhibited a dual peak at ZT 6 and ZT 18 in the stromal-vascular fraction, the expression in adipocyte peaked only at ZT 14 (Fig. 1). The expression of IR and IRS in adipocytes showed mod-

erate oscillation in adipocytes. The glucose transporters examined were expressed in adipocytes constantly throughout the day (Fig. 1). In the stromal-vascular fraction, expression of Glut3 and Glut8 displayed circadian oscillations; the expression levels of these factors were relatively higher during the light phase than the dark phase (Fig. 1).

Circadian Gene Expressions in 3T3-L1 Adipose Cells

Several recent studies revealed that cultured organs or cells possess the machinery for circadian oscillation.[5–9] Stimulation of cultured fibroblasts with serum-rich medium induces cyclic expression of several genes implicated in mammalian clockwork.[5] Although the precise mechanism by which treatment of cells with serum-rich medium or other chemicals such as DEX synchronize cellular clock is still unknown, it is hypothesized that rhythmic gene expression elicited by serum treatment could be the result of either a synchronization of already existing cycles in desynchronized cells, or an induction of oscillations in arrhythmic cells.[5] The molecular makeup of the peripheral oscillator in cultured cells resembles that of the core oscillator in the SCN.[5] As a consequence, cultured cells have been used as a model system for the study of circadian rhythms in peripheral mammalian tissue.[7,8,34,35] Thus, to confirm the existence of a peripheral clock in adipocytes, 3T3-L1 mature adipocytes were stimulated with either 50% horse serum or DEX for 2 hr, and the subsequent clock gene expression was determined. Both serum and DEX stimulation triggered cyclic expression of Clock, and Per1 genes in cultured adipocytes (Fig. 2). While serum shock also generated robust oscillation of Bmal1 expression, DEX treatment induced lesser cyclic amplification of Bmal1 expression. Expression of the Dbp and Rev-Erbα genes also oscillated in response to serum and DEX, but the magnitude of amplification was weaker than that in the expression of other clock genes (Fig. 2).

Pharmacological Effects of PPARγ Ligand are More Effective when it is Administered at Night

The above data indicate that the PPARγ(1 + 2) gene is expressed rhythmically in white adipose tissue (Fig. 1), suggesting that the pharmacological effects of PPARγ ligand may vary according to the time of administration. To test this hypothesis, rosiglitazone was administered to obese mice at ZT 6 or ZT 18 for 15 days and the pharmacological effects of the drug were compared (Table 2). Treatment of mice with rosiglitazone lowered plasma TG levels, as reported previously (Table 2).[36] Administration of rosiglitazone at ZT 18 more efficiently lowered plasma TG levels than administration at ZT 6 (Table 2). More interestingly, while administration of rosiglitazone at ZT 6 had no effects on plasma non-esterified fatty acid (NEFA) levels, rosiglitazone treatment at ZT 18 lowered the plasma NEFA levels to 70% of the control (CMC alone) treatment (Table 2).

DISCUSSION

A growing body of evidence suggested that biological activity in adipocytes is strongly related to the development of several diseases, including diabetes, hyperlipidemia, and cardiovascular disease.[15,16,19,20] Importantly, the frequencies of onset of these diseases show marked circadian variations.[37,38] Therefore, in this study, we examined if adipocytes possess the machinery for a molecular clock.

As in other peripheral tissues such as liver and heart, the expression of several clock genes exhibits circadian oscillations in adipose tissue (Fig. 1). In addition, stimulation of cultured adipocytes with serum or DEX resulted in the generation of cyclic gene expression of clock genes (Fig. 2). Thus, we are led to conclude that adipocytes possess the molecular machinery for a biological clock. A comparison of the expression patterns of clock genes between adipocyte and stromal-vascular fractions containing preadipocytes revealed unsuspected features of clock gene expression in adipocytes. First, clock genes are more preferentially expressed in adipocytes than in the stromal-vascular fraction. Interestingly, the expression of Bmal1 in adipocytes advanced to that in the stromal-vascular fraction (Fig. 1). Also, expression of IRS1 peaked twice in the stromal vascular-fraction, while IRS1 expression peaked once in adipocytes (Fig. 1). Finally, expression of Per1 was strongly oscillated observed in adipocytes but less oscillated in the stromal-vascular fraction (Fig. 1). In contrast, Clock gene expression was rhythmic in the stromal-vascular fraction but arrhythmic in adipocytes fraction (Fig. 1). At this stage, it is not clear how adipocytes acquire specific regulation machinery of clock gene expression during differentiation process. Recent studies using DNA arrays revealed that several hundreds of gene

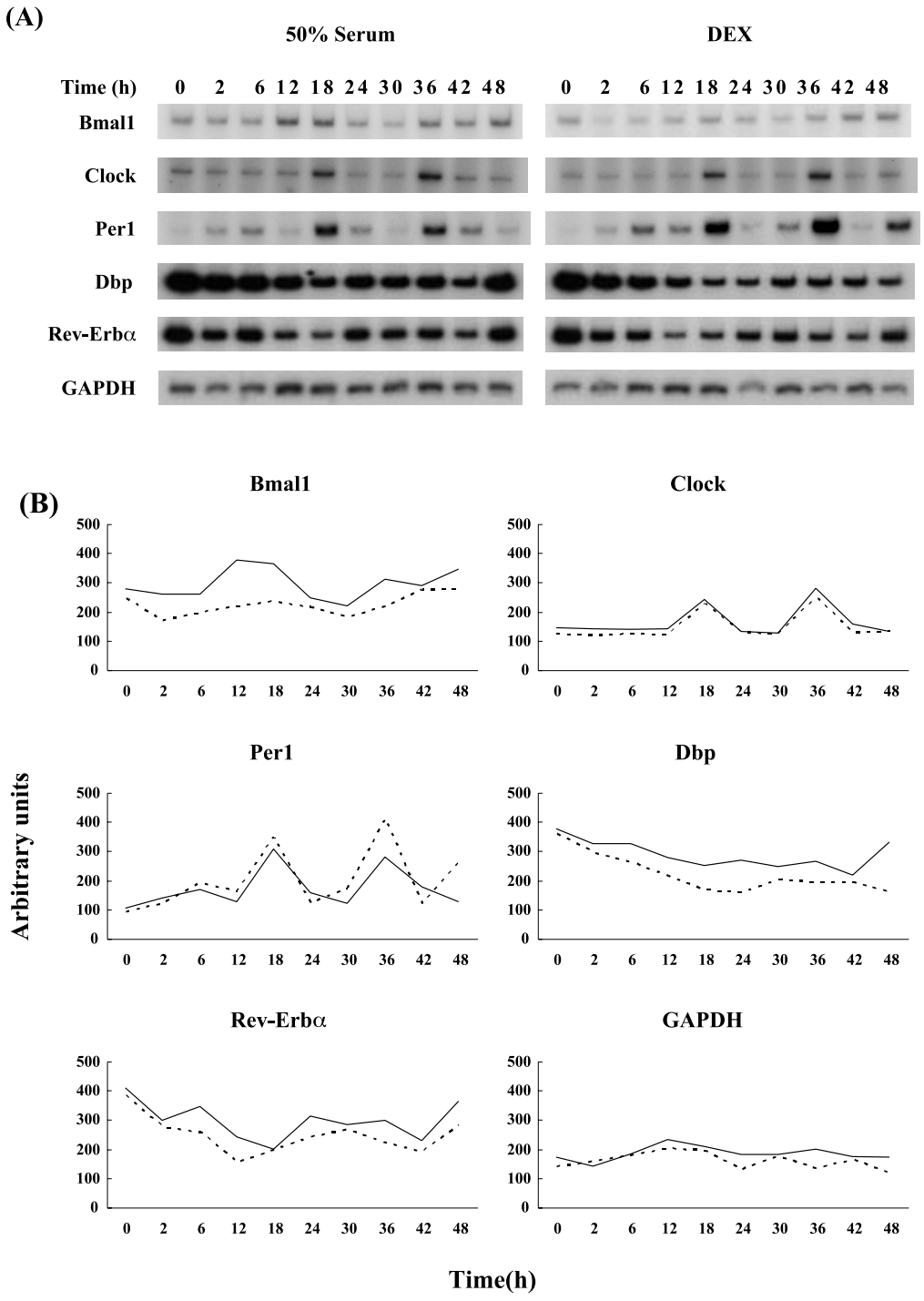

Fig. 2. Induction of Circadian Gene Expression in 3T3-L1 Mature Adipose Cells

(A) 3T3-L1 mature adipocytes were treated with either 50% serum or DEX. After 2 hr, the medium was replaced with serum-free OPTI-MEM medium and cultured for the following days. Total RNA was isolated at the indicated time, and the expression of clock genes was determined by RT-PCR using specific primers. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was measured as an internal control to evaluate sample-to-sample differences in RNA concentration. The experiment was performed three times with similar results. (B) Expression levels of clock genes are assigned as arbitrary units based on the band intensity assessed by densitometry. Solid line and dashed line represent levels in 50% serum-treated cells and DEX-treated cells, respectively.

Table 2. Pharmacological Effects of Rosiglitazone on Obese Mice

Time	CMC	RSG
	Plasma TG level (mg/dl)	
ZT 6	56.09 ± 6.62	29.67 ± 4.15[a)]
ZT 18	48.32 ± 6.38	15.46 ± 4.49[a)]
	Plasma NEFA level (mEq/l)	
ZT 6	0.94 ± 0.06	0.90 ± 0.11
ZT 18	0.92 ± 0.07	0.65 ± 0.07[a)]

Obese mice received oral doses of rosiglitazone (RSG, 10 mg/kg body weight) in 0.25% carboxymethylcellulose (CMC) at ZT 6 or ZT 18. After 15 days of consecutive administration, blood samples were collected at ZT 8. Values are the means of five mice samples. *a*) significant differences ($p < 0.01$) from the value of the CMC treatment.

expression and signaling pathway are altered during adipogenesis.[39)] This process is induced by DEX and cyclic AMP while these inducers are unnecessary for maintaining the status of adipocytes. Since DEX and cAMP also induce circadian gene expression, one can speculate that signal pathways responding to DEX and cAMP in adipocytes are distinct from that in preadipocytes. Taken together, unique clock gene expression in adipocytes may reflect cell-specific signaling pathway for activation of the molecular clock and effecter genes carry out this regulation.

To gain the insight of the adipocyte-specific activation pathway for clock gene expression, *in vitro* model is an essential tool. 3T3-L1 adipocytes have been extensively used for characterization of adipocytes as an *in vitro* model.[40–42)] We therefore used 3T3-L1 adipocytes to establish an *in vitro* model for studying the circadian rhythm in adipocytes. The serum or DEX shock of 3T3-L1 adipocytes induced the circadian expression of various genes whose transcription also oscillates in white adipose tissue (Fig. 2), indicating that the cultured adipocytes possess a molecular clock similar to that operating in adipocytes in the tissues. Nevertheless, some differences are evident between *in vivo* and *in vitro* conditions. While the average period length of several clock genes expressed in adipocytes *in vivo* is 24 hr, that in cultured adipocytes is 18–24 hr (Figs. 1 and 2). Also, the expression of Dbp in adipocytes displayed robust oscillations *in vivo* but not *in vitro* (Figs. 1 and 2). One of possible explanations of this difference is that the magnitude of circadian gene expression in adipocytes is influenced by exogenous factors. This hypothesis could be supported by the fact that the cyclic expression of Bmal1 gene was strongly induced by serum shock but weakly by DEX shock (Fig. 2). Interestingly, fasting alters circadian gene expression in various tissues.[43)] Also, streptozotocin-induced diabetic rat showed phase shift and/or upswing of clock genes expressions.[12)] Therefore, plasma level of insulin and glucose may be the candidates of modulator of circadian gene expression in adipose tissue. The availability of this *in vitro* system may greatly facilitate identification of factors involved in the regulation of adipocyte-specific circadian gene expression.

PPARγ2 plays a crucial role in regulation of the biological activity of adipocytes such as expression and secretion of cytokines.[44–47)] Therefore, PPARγ2 ligand such as rosiglitazone and pioglitazone are now clinically used for therapeutics of type II diabetics. The present data indicated that expression of PPARγ2 and the target genes oscillates in adipocytes (Fig. 1). Expression of PPARγ2 showed dual peak during the day. This expression pattern is similar to that of plasma glucose level reported by Bailey *et al.*[48)] Also, plasma glucose level is highly associated with PPARγ2 expression in adipocytes.[49)] Therefore, it could be possible that dual expression pattern of PPARγ2 is induced by glucose, although the detailed mechanism is remained to be uncovered. Based on diurnal variation of PPARγ2 expression, we examined whether the effectiveness of pharmacological actions of PPARγ2 ligands depend on the time of administration. As shown in Table 2, rosiglitazone administered at ZT 18 more efficiently decreased the plasma levels of TG and NEFA compared with that administered at ZT 6, while neither day nor night treatment with CMC had no effects on these markers. Several previous studies on pharmacokinetics of rosiglitazone reported that peak plasma concentration of rosiglitazone is observed within 1 hr and elimination half-life is approximately 4 hr after oral administration.[50,51)] The result in Fig. 1 showed that expression level of PPARγ2 is downregulated between ZT 6 to ZT 10. The consistence in expression pattern between PPARγ2 and the target genes such as aP2 (Fig. 1) suggests that expression level of PPARγ2 mRNA may reflect the protein level. Consequently, these results suggest that less pharmacological effects of rosiglitazone administered at ZT 6 may be due to the lower expression level of PPARγ2 during ZT 6–10 in adipocytes.

Several recent studies have shown that metabolic activity is closely linked to circadian rhythms in cells.[11)] Therefore, diseases in metabolic active tis-

sues such as brain, liver, adipose tissue, and tumor tissues could be a target for chronotherapy. In this study, we characterized circadian rhythms in adipose cells *in vivo* and *in vitro* (Figs. 1 and 2). In addition, a preliminary approach of chronotherapy for diabetics was demonstrated (Table 2).

In conclusion, the present data should provide opportunities to study the possibility of the chronotherapy for syndrome X and the related diseases.

Acknowledgments This work is supported in part by a grant from the Ministry of Education, Sciences, Sports, and Culture, Japan (S. S. & M. T.), by a General Individual Research Grant from Nihon University (S. S.), by a grant from the Ministry of Education, Culture, Sports, Science, and Technology to promote multi-disciplinary research projects, and by an Interdisciplinary General Joint Research Grant from Nihon University (M. T.).

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