Published in final edited form as:

Curr Opin Clin Nutr Metab Care. 2012 July ; 15(4): 336–341. doi:10.1097/MCO.0b013e3283548629.

Clock regulation of dietary lipid absorption

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Abstract

Purpose of review—To summarize the new knowledge about the regulation of dietary lipid absorption by circadian locomotor output cycles kaput (Clock) and Nocturnin.

Recent findings—Recent findings have shown that Clock and Nocturnin, proteins involved in circadian regulation, play an important role in the regulation of dietary lipid absorption. Clock deficiency increases, whereas Nocturnin deficiency decreases lipid absorption. Clock plays a role in turning off the genes involved in lipid absorption at the onset of the day. Molecular studies revealed that Clock binds to the promoter of small heterodimer partner to enhance its transcription. When levels are high, small heterodimer partner interacts with the transcription factors associated with the promoter of microsomal triglyceride transfer protein to repress transcription. Reduced microsomal triglyceride transfer protein levels are correlated with low intestinal lipid absorption and plasma lipid levels. In contrast, Nocturnin assists in lipid absorption by regulating their partitioning in different intracellular compartments.

Summary—Clock and Nocturnin regulate lipid absorption involving different mechanisms. It is likely that other clock genes also modulate lipid absorption and plasma lipid levels.

Keywords

circadian regulation; diet; lipids; regulation

INTRODUCTION

Light and food are two dominant stimuli that affect daily life. Animals respond to light by turning on or off certain transcription factors, known as ‘core clock genes’ [circadian locomotor output cycles kaput (Clock), Bmal1, Cry1, Cry2, Per1, Per2, Per3], in the suprachiasmatic nuclei (SCN) of the brain [1–3]. In addition to their auto-regulation, clock genes also modulate the expression of different transcription factors that control various metabolic pathways and are referred to as ‘clock-controlled genes’ [1–4]. Recent studies draw attention to the importance of Clock and a clock-controlled gene, Nocturnin, in intestinal lipid absorption. Here, we will discuss the spatial and temporal expression of clock

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Conflicts of interest

There are no conflicts of interest.

genes in the intestine and their regulation by food and light. Further, we will review the recent studies that highlight the importance of Clock and Nocturnin in lipid absorption.

CIRCADIAN RHYTHMS AND INTESTINE

Several behavioral, physiological and biochemical activities of life circle around sunrise and sundown. Eyes transmit information about light to the SCN. This information is processed resulting in increased transcription of the *Bmal1* gene representing the turning on of the biological clock. Bmal1 interacts with Clock and this heterodimer binds to specific *cis*-elements in the promoter regions of *Cryptochrome* (Cry) and *Period* (Per) genes. Cry and Per proteins form heterodimers, interact with *Bmal1* promoter to reduce expression representing turning off of the clock. This transcriptional–translational feedback loop recurs with an approximate interval of 24h and is considered circadian, that is, with daily intervals. The description of the above loop is an oversimplification of several events involved in the control of the biological clock; more details can be found elsewhere [1–4]. An important feature of these biological clocks is that they are dependent on constant training by external clues. Thus, daily exposure (entrainment) to light helps maintain the periodicity of the central SCN clock.

A major behavioral activity associated with sunrise and sundown is wakefulness and sleep. Even though some animals go to sleep while others wake up with sunrise, a consistent activity associated with wakefulness is eating indicating that it is controlled by circadian clocks. Food is another major external stimulus that also modulates various behavioral, biological and biochemical activities. Therefore, perpetual eating at times that are out of sync with sunrise and sunset profoundly alters circadian patterns of various biochemical activities including the expression of circadian clock genes in peripheral tissues, but not in the SCN [5].

The main task of the intestine is to digest food and absorb its constituents. Additionally, intestine elicits several humoral and vagal signals that could inform, instruct and entrain other tissues about the availability of food. In addition to these mechanisms, intestine also uses dietary constituents and their hydrolyzed products as messengers and signal transmitters. Thus, intestine not only can process food, but also can transmit information about the availability of food and can potentially train and prepare other organs to receive and assimilate energy.

It is well documented that several intestinal functions exhibit characteristic circadian rhythms [4,6■]. Moreover, many adverse events associated with shifting circadian clocks, for example, transcontinental flights, night shift occupations, space flights manifest as intestinal dysfunctions such as food aversion and intestinal discomfort. Further gastrointestinal complications associated with chemotherapies and radiotherapies for colon cancer have been associated with the time of administration [7]. Because of the circadian nature of various intestinal functions and pathologies associated with their disruptions, studies are being conducted to elucidate the role of clock genes in the control of intestinal function.

Expression and regulation of clock genes in the intestine

Circadian expression of clock genes in the colon has been well documented [8,9]. Further, temporal feeding restrictions reset circadian expression of these genes. We studied the expression of clock genes in different regions of the intestine with major focus on jejunum [10]. In general, maximum expression of core clock genes was in the colon followed by ileum, jejunum and duodenum. Specific studies related to Bmal1 expression in the jejunum revealed that it is mainly expressed in the villi with lesser levels in the crypt. Thus, intestinal expression of clock genes appears to increase along the jejunum-to-colon and crypt-to-villus axes.

Not only were the clock genes expressed in the intestine, but they also showed robust diurnal variations. The peak expressions of intestinal core clock genes were in phase with the expression of these genes in other tissues but were phase delayed compared to their expression in the SCN [8,9]. This might indicate neuronal or humoral entrainment of intestinal clock genes by the SCN. Intestinal clock genes were also responsive to restricted food entrainment protocols; peaks in the expression of these genes occurred around the time of food availability, indicating that signals from the SCN are overridden by food entrainment. These significant shifts suggest that food might be a dominant regulator of their expression. But, when these food entrained mice were placed in constant light or constant dark to disrupt light-induced entrainment, changes seen as part of the food-entrainment response were dampened or lost [10]. These studies suggest that 12h light and dark entrainment is critical in order to respond to food entrainment. Thus, it appears that clock genes integrate both light and food entrainment stimuli for optimum lipid absorption.

To address the molecules involved in the integration of light and food entrainment, we studied the need and role of Clock in the circadian and food-entrained regulation of clock genes using C57Bl6 Clock$^{\Delta 19/\Delta 19}$ mice [10] that express a dominant negative form of Clock. The circadian expression of different clock genes was dampened or not present in the jejunum of Clock$^{\Delta 19/\Delta 19}$ mice. Moreover, food-entrained temporal changes in the expression of different clock genes was significantly dampened or not seen in these mice. Therefore, normal expression of Clock is critical for the diurnal and food-entrained regulation of different core clock genes in the intestine.

Regulation of intestinal functions by clock genes

To understand the role of different clock genes in intestinal function, investigators have used different mouse models with altered expression of different clock gene. These include various gene-ablated mouse models and Clock$^{\Delta 19/\Delta 19}$ mice. Hoogerwerf *et al*. [6 ■] showed that diurnal colonic motility requires normal period expression. Colonic motility mainly occurs at night in wildtype mice, but this rhythmicity is not seen in Per1$^{-/-}$/Per2$^{-/-}$ mice [6■].

To examine the role of Clock in macronutrient absorption, we studied the absorption of α-methyl-glucopyranoside (a glucose analog), α-glycylsarcosine (a peptide), cholesterol and triolein (lipids) using in-situ intestinal loops and isolated primary enterocytes from Clock$^{\Delta 19/\Delta 19}$ mice and their wildtype siblings [10,11■■]. Absorption of macronutrients was

high at night and low in the daytime in wildtype mice, whereas Clock$^{\Delta 19/\Delta 19}$ mice absorbed similar amounts in the day and night indicating loss of rhythmicity [10]. The amounts of carbohydrates and lipids absorbed by Clock$^{\Delta 19/\Delta 19}$ mice at all times were similar to those seen at night in wildtype mice. Thus, in the presence of Clock mutant protein, mice absorb more carbohydrates and lipids at all times and this might be a reason for hyperlipidemia and hyperglycemia found in these mice [12]. In contrast, the amounts of peptides absorbed at all times were significantly lower in Clock$^{\Delta 19/\Delta 19}$ mice and were similar to amounts absorbed by wildtype mice in the daytime [10]. Therefore, overall peptide absorption might be reduced in Clock$^{\Delta 19/\Delta 19}$ mice. These studies revealed that Clock plays an important regulatory role in the absorption of macronutrients.

To identify the molecules involved in macronutrient absorption that are regulated by Clock, we measured changes in the mRNA levels of several candidate genes [10]. Glucose absorption by enterocytes depends on three transporters. Sodium/glucose cotransporter 1 (SGLT1) and glucose/fructose transporter type 5 (GLUT5) are expressed on the apical side of enterocytes and are involved in the uptake of glucose from intestinal lumen. Glucose transporter 2 (GLUT2), expressed mainly on the basolateral side, is involved in the export of glucose from enterocytes to circulation. Several investigators have shown that these transporters show rhythmic expression with peak levels at night. Further, restricted feeding has been shown to shift the expression of these genes [13]. In contrast to wildtype mice, SGLT1, GLUT2 and GLUT5 mRNA did not show diurnal changes in Clock$^{\Delta 19/\Delta 19}$ mice, suggesting that Clock plays a role in circadian regulation of these genes. To recognize the role of Clock in peptide absorption, we measured changes in mRNA levels of proton-coupled oligopeptide transporter 1 (PEPT1) in the jejunum of wildtype and Clock$^{\Delta 19/\Delta 19}$ mice. The peptide transporter PEPT1 also showed circadian expression in wildtype mice but not in Clock$^{\Delta 19/\Delta 19}$ mice, indicating that Clock also plays a role in the diurnal regulation of PEPT1. Clock was not only important for diurnal regulation of these transporters, but it was also critical for their food-entrained regulation. Food entrainment experiments showed that several genes involved in macronutrient absorption robustly respond to this stimulus in wildtype mice [10]. Expression of genes involved in carbohydrate and peptide absorption were high before the mealtime suggesting an anticipatory response. Similar food-entrained changes in the expression of these transporters were severely curtailed or absent in Clock$^{\Delta 19/\Delta 19}$ mice. These studies underscore the importance of Clock in diurnal as well as food-entrained regulation of several macronutrient transporters.

Regulation of lipid absorption by Clock

Dietary lipids are broken down in the lumen of the intestine and the hydrolyzed products, free fatty acids and monoacylglycerols, are taken up by the enterocytes [14,15]. Detail studies related to several genes involved in lipid absorption by the intestine identified two sets of genes that respond to either ‘food and light’ or ‘food’ only [10]. Genes that respond to light and food show diurnal variations, phase shift their peak expression at mealtime after food entrainment, and their circadian as well as food entrainment responses are dampened in Clock$^{\Delta 19/\Delta 19}$ mice. Genes that only respond to food do not show diurnal variations but show robust upregulation after food entrainment. In general, their response to food is not altered in Clock$^{\Delta 19/\Delta 19}$ mice.

Lipids taken up by enterocytes are packaged into chylomicrons and transported to plasma. In addition, liver synthesizes very low density lipoproteins to transport endogenous fat. Assembly of lipoproteins occurs around a large structural protein, apolipoprotein B, with the help of an intracellular chaperone MTP [16–18]. Plasma lipoproteins show diurnal variations; they are high in the day and night in humans and rodents, respectively. These diurnal variations were absent in Clock$^{\Delta 19/\Delta 19}$ mice, indicating that Clock plays a role in the control of daily changes in plasma lipoproteins [10,11■■,19]. As lipoproteins are transport vehicles for dietary fat by the intestine in the form of chylomicrons and endogenous lipids by the liver as very low density lipoproteins, it is anticipated that availability of food would be the major determining stimuli for their mobilization. Indeed, synthesis and secretion of these lipoproteins is increased at the time of food availability in wildtype mice [10,11■■, 19]. These increases at mealtime were dampened when mice were kept in constant dark and light. Moreover, these changes were significantly less in Clock$^{\Delta 19/\Delta 19}$ mice. Thus, light entrainment and Clock appear to be necessary for optimum food entrainment of plasma lipoproteins.

Our studies have shown that changes in plasma lipids coincide with the variations in MTP expression [19]. Further, changes in plasma lipoproteins after restricted feeding were associated with peak expression of MTP at mealtime. Both diurnal and food-entrained changes were dampened or absent for plasma lipids and MTP expression in mice exposed to constant dark or light [19] and in Clock$^{\Delta 19/\Delta 19}$ mice [11■■]. Thus, there is a significant correlation between changes in MTP expression and variations in plasma triglyceride-rich lipoproteins, and Clock appears to be important for these changes.

Molecular mechanisms in the regulation of MTP by Clock have been elucidated [11■■]. These studies showed that Clock modulates MTP expression by regulating the expression of small heterodimer partner (Shp) [11■■]. Clock was shown to interact with the E-box present in the Shp promoter leading to increased transcription and protein levels (Fig. 1). When Shp levels were high, there was increased association of Shp with different transcription factors that bind to MTP. This increased association of Shp with various transcription factors represses their activity reducing MTP expression. Thus, Clock responds to both light and food cues, and increases Shp expression. This leads to reduced MTP expression and lower lipid absorption in the daytime.

Role of Nocturnin in lipid absorption

Nocturnin is an exonuclease that degrades poly(A) tails of specific target mRNAs leading to their degradation and suppression of translation. Its expression shows circadian rhythms with high levels found at night [20]. Interest in this gene was enhanced with the observation that Nocturnin-deficient mice are resistant to diet-induced obesity and hepatosteatosis [21]. In the intestine, its expression decreases from duodenum to colon. Thus, high levels of Nocturnin in the jejunum correlate with maximum lipid absorption by the intestine. Further, its expression is significantly increased after an olive oil gavage indicating regulation by fat. Thus, Nocturnin is regulated by both light and food-entrained cues.

To understand how Nocturnin promotes obesity, Douris *et al.* [22■■] studied lipid absorption in Nocturnin-deficient (Noc$^{-/-}$) mice and observed that these mice exhibit

attenuated response to an oral fat tolerance test. To explore the reasons for the reduced postprandial response, radiolabeled lipid absorption studies were performed in wildtype and $Noc^{-/-}$ mice. In wildtype mice, lipids are taken up by the proximal jejunum and are subsequently secreted to plasma so that very small amounts of radiolabeled lipids are present in the intestine. In contrast to wildtype mice, transport of radiolabeled triglyceride and cholesterol from the intestinal lumen to the plasma compartment was significantly curtailed in $Noc^{-/-}$ mice, and increased amounts of these lipids were found associated throughout the intestine with higher amounts predominating in the distal portions. These studies indicated that $Noc^{-/-}$ intestinal cells take up lipids but are unable to transport them to the plasma compartment. This conclusion was supported by the studies performed in isolated primary enterocytes. $Noc^{-/-}$ enterocytes took up radiolabeled lipids and retained them resulting in their reduced secretion into the media.

Increased cellular accumulation of lipids could be because of defects in the assembly and secretion of chylomicrons. As MTP is an essential chaperone for their assembly and secretion, we looked at its expression with the hypothesis that increased cellular retention and decreased lipid secretion might be secondary to reduced MTP expression. Surprisingly, we found increased expression of MTP. Apolipoprotein B is another protein that is required for lipoprotein assembly and its expression was moderately reduced. Oil Red O staining and transmission electron microscopy studies showed that enterocytes from $Noc^{-/-}$ mice contain significant amounts of large lipid droplets. Thus, it appears that in the absence of Nocturnin, lipids are preferentially targeted for storage in the cytoplasm as lipid droplets, and assembly and secretion of lipoproteins is curtailed. Hence, accumulation of lipids in enterocytes and reduced secretion are not related to defects in MTP expression. Instead, these studies point to a novel unidentified mechanism that is regulated by Nocturnin and is involved in the partitioning of lipids in the cytoplasm and endoplasmic reticulum.

CONCLUSION

It has been proposed that intestinal gene regulation might involve lumenal signals, vagal communications and humoral directives [23]. Studies summarized here point to the fact that the expression of genes involved in macronutrient absorption are also regulated by circadian genes. Particularly, Clock and Nocturnin appear to play a significant role in the regulation of genes involved in food absorption. Thus, clock and clock-controlled genes also play an important role in the intestinal gene expression and nutrient absorption.

Although light entrainment appears necessary for the optimal regulation of gene expression at the time of food availability, it is unclear how the light and food-entrainments are integrated to obtain maximum behavioral shift to assimilate food at mealtimes. Possible mechanisms for this could be the regulation of intestinal Clock by the SCN clock involving humoral factors or neural connections. However, these factors might not be simply entraining the Clock transcription, as Clock mRNA itself does not show significant diurnal and food entrained shifts. Therefore, other events such as association with Bmal1 or chromatin remodeling via its histone acytyltransferase activity [24,25] might be important in transmitting light and food cues to regulate intestinal function. Hence, there is a need to

identify mechanisms that integrate light and food stimuli to optimize intestinal lipid absorption.

Acknowledgments

None.

This work was supported in part by a grant from NIH DK-81879 to M.M.H. and a Scientist Development Grant (2300158) from AHA to X.P.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

■■ of outstanding interest

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KEY POINTS

- Core clock genes are expressed in the intestine and respond to light and food cues.
- Clock plays an important role in the diurnal and food-entrained regulation of lipid absorption.
- Clock turns off *microsomal triglyceride transfer protein (MTP)* gene involved in lipoprotein assembly to lower lipid absorption at daybreak in mice.
- Nocturnin promotes lipid secretion by unknown mechanisms.

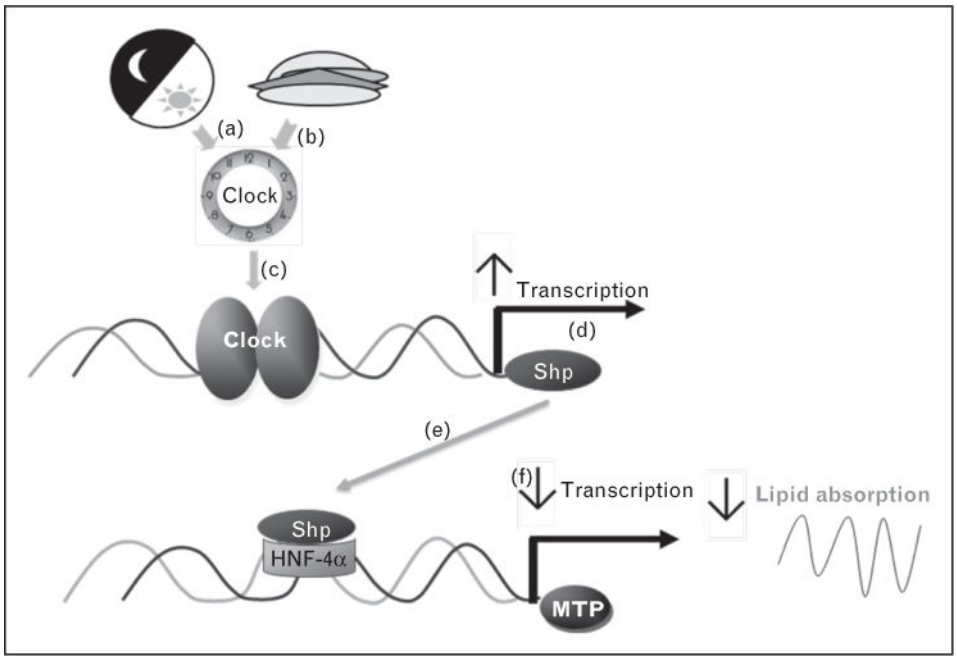

FIGURE 1.

Molecular events in the regulation of plasma lipids by light and food involving circadian locomotor output cycles kaput (Clock). Both light (a) and food (b) entrainment cues are transmitted through Clock to regulate plasma lipids, as light and food mediated changes are dampened or absent in Clock$^{\Delta 19/\Delta 19}$ mice. One mechanism proposes that Clock reduces plasma lipids in mice at daybreak by upregulating small heterodimer partner (Shp) that leads to downregulation of MTP [11■■]. In the daytime Clock interacts with *Shp* promoter (c) to increase transcription (d). When levels are high, Shp interacts with transcription factors, such as HNF-4α, that are associated with the *MTP* promoter (e). This interaction leads to repression of *MTP* gene transcription (f) and lower levels of MTP. Reduced MTP levels are associated with attenuated lipid absorption. In Clock$^{\Delta 19/\Delta 19}$ mice, Clock and Shp levels are low, whereas MTP levels are high at all times. This might be a mechanism contributing to hyperlipidemia in these mice [12]. Modified with permission from [15].