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Chrononutrition

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Summary Well-regulated eating habits are said to be important for health. A major breakthrough was the discovery of the negative regulatory feedback for transcription via the binding of Clock/Bmal1 to E-box, which forms the basis of biological clocks. Well-regulated eating habits normalize the liver clock gene, the rhythm of CYP7A1 gene, and blood cholesterol levels through insulin secretion. Moreover, well-regulated eating habits actively contribute to better lipid metabolism such as obesity, even if animals ingest a high-fat diet. From reported results so far, chrononutition has two important functions: 1) meal timing is important for our health, and 2) meal timing entrains our body clock.

Key Words chrononutrition, meal timing, clock gene, liver clock, lipid metabolism

Well-regulated eating habits are said in both the East and the West to be important for health. The importance of breakfast is emphasized in ancient Japanese books (*1*). We seem to have recognized the importance of the timing of meals empirically. It is generally understood that people who work at night suffer from coronary disease and obesity more frequently (*2*). Furthermore, there is a relationship between shift work and cancer (*3*). However, the vast majority of these people are unaware that irregular eating habits are a major factor leading to health problems, because the mechanisms leading to the effects of the timing of meals on health are unclear. I here review how meal timing is important for our health and how chrononutrition works. I also review two functions of chrononutrition: 1) meal timing affects our health, and 2) meal timing entrains our body clock.

Why Is Chrononutrition Receiving Attention Now?

According to the National Health and Nutrition Survey, Japan, 2007, one-sixth to one-fifth of the Japanese are suspected to have diabetes (*4*). This issue is becoming more common worldwide. Obesity is particularly increasing in men. However, caloric intake is decreasing slightly in Japan. Although it is thought that fat intake and decreasing physical activity are contributing to this problem, many people are health conscious and do exercise regularly. Then, what factor(s) are causing this problem? People generally think of what they should eat when they hear the word "meal." However, we realize now it is better to think eating habits might be more important for our health than what we eat ("Meal Style"). Nutritional sciences have traditionally prioritized what we eat. The "5 W's and 1 H (5W1H) of meals" should be considered (i.e., what, when, where, who, why, and how); in other words, the way meals are eaten should be considered to better understand nutrition.

A field called "chronobiology," which has been widely recognized as one of the basic phenomena of life for a long time, includes the clinically serious subject of sleep disorders. A major breakthrough was the discovery of the negative regulatory feedback for transcription via the binding of Clock/Bmal1 to E-box, which forms the basis of biological clocks (*5*). In 2005, the first instance of the term "Chrononutrition" appeared in a nutrition textbook in Japanese edited by us (*6*). The world's first book on the subject, entitled "Chrononutrition," was published in 2009 (*7*). Chrononutrition, a relatively new field of nutritional science, is gaining recognition because it is regarded as the key that will help in understanding why there is an increase in the number of patients with obesity or metabolic syndrome in spite of a decrease in energy intake.

Intrinsic Circadian Clocks in Our Body

The human body has a diurnal rhythm (a phenomenon with daily periodicity) (*8*) (Fig. 1). Children who sleep well are said to grow up strong, maybe because growth hormone is secreted at night. Stomach ulcers tend to worsen early in the morning. On the other hand, there are periods called danger times in which sudden death occurs frequently in the morning (*9*). Physiological phenomena leading to myocardial and cerebral infarction often occur in the morning. Diurnal rhythms are observed not only with respect to when diseases occur, but also when death occurs, which varies depending on the cause of death. The human body is regulated by an internal clock more than we expected.

During evolution, circadian clocks began with the rhythm of simple biochemical reactions (*9*). Cyanobacteria possess a 24-h clock that functions via enzymatic reactions. However, these cases are exceptions. Circadian clocks mainly function through the negative regulatory feedback of transcription via the clock genes (*5*). Organisms are thought to need a circadian clock because of the "predictability" and "functional division of labor". Predictability is essential for obtaining food and escaping from predators. Functional division of labor enables temporal division of biochemical labor for many complicated cell functions.

Clock Genes and Synchronizer

As mentioned above, circadian rhythms involve a clock regulated by transcriptional negative feedback. Clock/Bmal1, transcription factors, bind to E-box hexanucleotides to activate the transcription of *Per* and *Cry*, which are the clock genes (Fig. 2). The complex consisting of *Per* and *Cry* inhibits transcriptional activation by Clock/Bmal1. Subsequently, decreased *Per* and *Cry* activation in turn causes transcriptional activation. This cycle takes about 24 h. Although small gaps among cells occur, these gaps are adjusted by synchronizers.

Biological clock studies originally focused on the clock of the brain. They found that there is a master clock in the suprachiasmatic nucleus (SCN) of the brain. Consequently, it was understood that the brain clock, which is stimulated by light, controls the entire body. However, at present, it is understood that all cells have their own autonomous 24-h clocks that function together as organ clocks, which collectively form an integrated clock through factors that synchronize organs. Therefore, synchronizers are important for a regulated body clock. The rhythm of the digestive system is reversed when the meal timing is reversed, indicating that the synchronizer of the digestive system clock is stronger than light. Meals have come to be understood as the strongest synchronizer of all organs. Therefore, meals synchronize the clocks of all organs present below the neck. Furthermore, a study reports that meals synchronize the brain clock, too (*10*).

The clocks of the organs cooperate to control the functions of the entire body, which can be defined as good health. Experiments using clock gene knockout mice shows that the loss of clock gene causes not only behavioral disorders, but also metabolic disorders. A report which showed *Clock* gene knockout mice exhibited obesity and metabolic syndrome published in 2005 received much attention (*11*). In addition to dysrhythmia, which was originally predicted, metabolic disorders in knockout mice revealed that the circadian clock is strongly linked to peripheral metabolism. On the other hand, there is a report on familial advanced sleep phase syndrome

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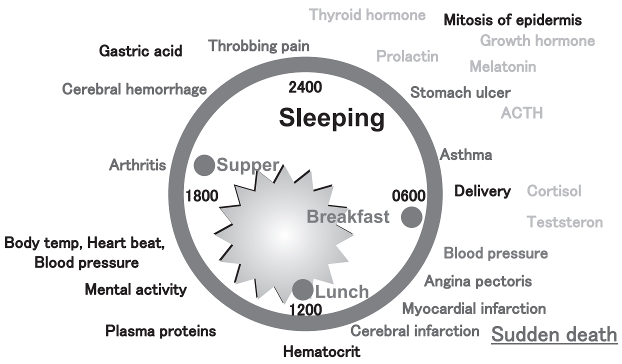

Fig. 1. Diurnal rhythm of physiological function diseases.

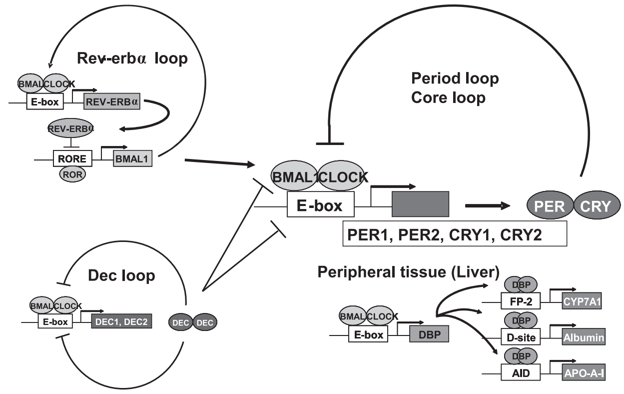

Fig. 2. Negative feedback regulation of circadian oscillation of transcriptions.

due to mutations of *Per2* in humans. However, no associations between mutations of clock genes and metabolic disorders in humans have been reported. Mutations in human clock genes are uncommon, although many SNPs in clock genes have been reported.

Meal Timing and Lipid Metabolism

It is well known that midnight snacking leads to obesity. It is thought that the influx of extra energy in rest time converts easily to lipid accumulation in adipose tissue. But now we understand midnight snacking brings about liver clock abnormalities and lipid metabolism. It is common to use a high-fat diet to create an obesity animal model. Although it is easy to understand the phenomena, its molecular mechanism has not been well understand. As I discuss later, it is understood that abnormality in the body clock by feeding a high-fat diet is what makes mice obese (*12*). Moreover, it is also known that one meal in a day leads to human obesity. This eating habit also changes the liver clock and the hepatic rhythmicity of lipid metabolism. The influence of meal timing on lipid metabolism is not considered as significant, while the important of well-regulated eating habits is recognized. Therefore we examined the influence of meal timing using non-genetically modified animals. We developed a feeding protocol, in which the animals ate continually irrespective of time; although restricted feeding (e.g., feeding during the daytime only) causes day/night inversion in nocturnal rats (*13*), they get used to it. In 2009, we reported for the first time that irregular meals cause abnormalities in the circadian clock of the liver and increase blood cholesterol (*14*). It indicated that differences in meal timing cause cholesterol metabolism abnormalities even if the same quantity of food is provided. Hypercholesterolemia was caused by the advanced shift of the circadian rhythm of the gene expression of *CYP7A1*, a rate-limiting enzyme involved in bile acid synthesis. Thus, orchestrated cholesterol metabolism did not occur and bile acid excreted in the feces decreased. Moreover, at that time, the rhythmicity of the clock gene *DBP* in the liver was advanced, which might be a major cause. These results indicate that well-regulated eating habits normalize the liver clock gene, and normalize the rhythm of *CYP7A1*, and that blood cholesterol levels are normalized due to normalized secretion of VLDL. In addition, apolipoprotein A-I, the main constituent protein of HDL (high density lipoprotein), is also under the control of *DBP* (unpublished results). This result indicates that irregular eating habits may reduce HDL. In other words, regular eating habits may decrease "bad" cholesterol and increase "good" cholesterol.

The mice and rats with ad lib feeding eat 80% of the food during their active period in the dark phase and the other 20% during their rest period. This raises the question of how mice and rats will react if they are forced to have tightly regulated eating habits, in which they eat no food during their rest period. A recent report indicates that diet-induced obesity with a high-fat diet is reduced only by tightly regulated eating habits (*15*). Although it has been reported that a hig-fat diet changed the body clock (*12*). Well-regulated eating habits ameliorate obesity induced by feeding a high-fat diet. This report importantly demonstrates that well-regulated eating habits actively contribute to good health rather than demonstrating that irregular eating habits are unhealthy.

Eating Signals Synchronizing Peripheral Clocks

The peripheral clocks are synchronized by meals independent of the brain clock, which is synchronized by light. In other words, there are presumably several factors that synchronize the peripheral clocks, including the nervous system, endocrine system (i.e., hormones), exercise (i.e., activity), body temperature, and eating behavior. To determine which factors synchronize the liver clock, rat primary cultured hepatocytes were treated with various hormonal factors. Three dimensional primary cultured hepatocytes obtained from rats kept a 24-h rhythm because the hepatocytes recognized the time even if the organism was dead (*16*).

We then focused on insulin, which is thought to be the most closely involved in the synchronization associated with meal timing. Insulin is a well-known hormone that fluctuates according to the meal timing. However, experimental conditions and results vary widely among studies. Therefore, we aimed to resolve these discrepancies by performing a considerably large-scale experiment. The results show that insulin is a strong factor that synchronizes the liver clock (*16*). The procedures of this experiment are described. First, insulin was added to hepatocytes with desynchronized clock gene expression, and synchronization by insulin was confirmed to ensure they had the same rhythm. The 3-D hepatocytes exhibited an obvious phase response curve to insulin. The phase response curve is a schematization to show that a given stimulus has a particular effect on clock resetting. If a phase response curve is found, it will demonstrate that the agent in question is a synchronizer. The effect of insulin in individual animals was examined using rats with streptozotocin-induced type I diabetes mellitus. The results revealed that the liver clocks advanced in those diabetic rats due to insulin deficiency. Insulin administration exhibited a phase response curve, which presumably suggests that the abnormalities of the liver clocks in diabetic rats might be improved if treatment was performed during the active period when insulin was secreted. Considering these findings, we concluded that insulin is the synchronizer of the liver clock. Insulin also synchronizes the clocks of adipose tissue (*16*). In other words, the clocks of the organs contributing to metabolic syndrome are entrained by insulin.

Food Factors as Synchronizers (*17*)

As mentioned above, some nutrients act as synchronizers. Glucose is the most important energy source that synchronizes circadian rhythms. Glucose also directly synchronizes the rhythm of cultured cells. It is reported that carbohydrate and protein act as strong synchronizers of the liver. Another study also demonstrates that the rhythms of not only the liver, but also the SCN, are synchronized when glucose and amino acids are administered to rats (*10*).

On the other hand, lipids have not been though to be syn-

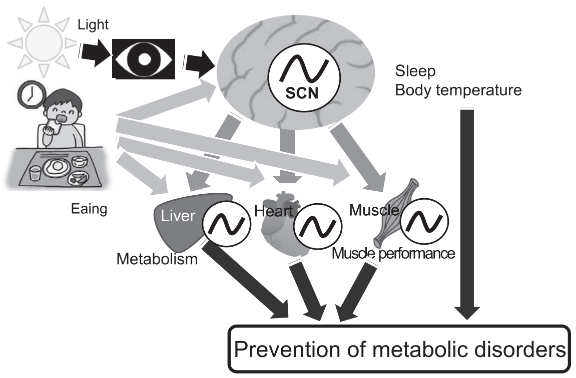

Fig. 3. Function of tissue clock and integration of tissue clock for health.

chronizers, although high-fat diets seem to change the length (i.e., frequency) of one period (i.e., day) (*12*). A drug called clofibrate, which promotes lipid catabolism, counteracts this effect. A pathway through PPARα seems to control the period of the rhythm. Salt and some vitamins also synchronize the clocks. Non-nutrients, such as resveratrol affects the clocks. Thus, there are many ingredients in food that control circadian clocks. Our everyday meals include many synchronizers. Consequently, daily meals provide synchronization stimuli.

Serum concentrations of glucocorticoid hormone (cortisol in humans and corticosterone in rats and mice) secreted by the adrenal cortex show circadian rhythm, and they are high just before the active period. The circadian rhythm of corticosterone in rats is maintained when meals are administered orally, while the oscillations disappear when nutrients are administered parenterally (i.e., not via the intestinal tract). These results show that food passing through the digestive organs or that getting absorbed *per se* synchronizes the clocks of some organs.

Smart Meal Styles from an Aspect of Chrononutrition

Considering the findings mentioned above, we must determine what kinds of meal styles are good for our health, e.g., well-regulated eating habits that differ between daytime and nighttime (i.e., only eating during the active period but not during the rest period). Insulin will be secreted 3 times if 3 meals are eaten; the first insulin secretion is the most important (i.e., after long fasting). Eating breakfast after insufficient fasting may provide a weak reset effect. Midnight snacks alter the liver clock, making the metabolism function suboptimally. As mentioned above, meals act as bundles of synchronizers. Therefore, it may be sufficient if daily meals include carbohydrate and protein. Thus, it can be thought that we may take the meals we usually eat without any specific limitation.

A lack of coordination among the organ clocks also seems to lead to an unhealthy condition, independent of the effects due to disturbances in the liver clock (Fig. 3). The orchestration of tissue clocks would contribute to our health, although each tissue clock has its own physiological functions (e.g. the brain clock for sleep, liver clock for metabolism, muscle clock for performance and so on).

A major problem faced when discussing eating habits in humans from the perspective of chrononutrition is that details of one's own body clock cannot be understood. As long as we have no way to measure the biological clock, we cannot evaluate it, making it difficult to use in clinical settings. The body clock was recently measured using hair follicle cells; however, this is also impractical for use with the public. We are currently developing a smartphone application that estimates a person's body clock called "chrononutrition clock."

Conclusion and Perspective

There is one important issue I do not discuss in this review, namely that the liver is a central organ of drug metabolism; disturbances to the liver clocks induced by irregular eating habits may cause abnormalities in drug metabolism, inhibiting expected efficacy and increasing unexpected side effects. It may be possible to improve the efficacy of drugs by having patients have regular meal timings, which would normalize the liver clock. We expect that the importance of chrononutrition as the basis for treatment timing, including chronotherapy and chronopharmacology, will become more important. I would like to emphasize again that chrononutition has two important functions, 1) meal timing is important for our health, and 2) meal timing entrains our body clock.

Our future goal is to establish a well-regulated smart meal style called the "*Smart Nutri Style*" (*SNS*), which considers the way people eat or the meal style used (i.e., "5W1H of meals").

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