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Original Research

Involvement of adenosine monophosphate–activated protein kinase in the influence of timed high-fat evening diet on the hepatic clock and lipogenic gene expression in mice

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A B S T R A C T

A high-fat diet may result in changes in hepatic clock gene expression, but potential mechanisms are not yet elucidated. Adenosine monophosphate–activated protein kinase (AMPK) is a serine/threonine protein kinase that is recognized as a key regulator of energy metabolism and certain clock genes. Therefore, we hypothesized that AMPK may be involved in the alteration of hepatic clock gene expression under a high-fat environment. This study aimed to examine the effects of timed high-fat evening diet on the activity of hepatic AMPK, clock genes, and lipogenic genes. Mice with hyperlipidemic fatty livers were induced by orally administering high-fat milk via gavage every evening (19:00-20:00) for 6 weeks. Results showed that timed high-fat diet in the evening not only decreased the hepatic AMPK protein expression and activity but also disturbed its circadian rhythm. Accordingly, the hepatic clock genes, including *clock*, *brain-muscle-Arnt-like 1*, cryptochrome 2, and period 2, exhibited prominent changes in their expression rhythms and/or amplitudes. The diurnal rhythms of the messenger RNA expression of *peroxisome proliferator–activated receptor α*, *acetyl-CoA carboxylase 1α*, and *carnitine palmitoyltransferase 1* were also disrupted; the amplitude of *peroxisome proliferator–activated receptor γ coactivator 1α* was significantly decreased at 3 time points, and fatty liver was observed. These findings demonstrate that timed high-fat diet at night can change hepatic AMPK protein levels, activity, and circadian rhythm, which may subsequently alter the circadian expression of several hepatic clock genes and finally result in the disorder of hepatic lipogenic gene expression and the formation of fatty liver.

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Abbreviations: ACC-1α, acetyl-CoA carboxylase 1α; AMPK, adenosine monophosphate–activated protein kinase; ATP, adenosine triphosphate; Bmal1, brain-muscle-Arnt-like 1; CPT-1, carnitine palmitoyltransferase 1; Cry2, cryptochrome 2; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; p-AMPK, phosphorylated AMPK; PGC-1α, peroxisome proliferator–activated receptor γ coactivator 1α; Per2, period 2; PPARα, peroxisome proliferator–activated receptor α; Real-time, PCR real-time polymerase chain reaction; TG, triglyceride.

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1. Introduction

Mammalian homeostatic systems have adapted to environmental 24-hour day/night cycles via the development of the endogenous circadian clocks, which are located in the suprachiasmatic nucleus of the anterior hypothalamus [1] and some peripheral tissues, such as liver, intestine, and adipose tissue [2-4]. These clock genes mainly consist of positive (clock and brain-muscle-Arnt-like 1 [Bmal1]) and negative components (periods [Pers] and cryptochromes [Crys]). The genes may control some physiologic functions by regulating clock-controlled genes, such as nuclear receptors including peroxisome proliferator–activated receptor α (PPARα) [5]. If the integrity and temporal coordination between these clock genes are disrupted, the risks for developing metabolic disorders are increased [6,7]. Some external stimuli, such as overnutrition, may influence clock function. For example, a long-term high-fat diet across the 24-hour light/dark cycles in mice may lead to alterations in the rhythmic expression of circadian clock genes clock, Bmal1, and Per2 in the liver [8]. Thus, energy metabolism may be closely related to the clockwork system, and further investigation is needed to clarify the pathogenesis of metabolic disorders.

In many countries, usual dietary habits include an evening meal. Currently, few studies have investigated the effects of feeding time on the hepatic circadian clock genes. Our previous studies showed that timed high-fat diet in the evening may result in the changes of hepatic clock gene expression and formation of fatty liver [9]. However, the potential molecular link between energy metabolism and clock genes is not yet elucidated completely. Adenosine monophosphate–activated protein kinase (AMPK) is a serine/threonine protein kinase that is recognized as a key regulator of energy metabolism and is activated by the increment of the AMP/adenosine triphosphate (ATP) ratio [10]. Several works demonstrated that AMPK may be involved in the regulation of some clock genes [11,12]. More recent research data have shown that metformin, a therapeutic drug for diabetes mellitus and an activator of AMPK, may influence the AMPK circadian gene expression in muscle cells [13]. These research findings suggest that the interaction of energy metabolism and clock genes may be mediated by AMPK.

Basing on the aforementioned data, we hypothesized that AMPK may be involved in the alteration of hepatic clock gene expression in mice receiving timed high-fat diet, but findings on this mechanism have not been reported to date. In the present study, we investigated the following in Kunming mice: the effects of a timed high-fat evening diet on the hepatic AMPK and phosphorylated-AMPK (p-AMPK) expression; the relationship between hepatic AMPK and clock genes; and the rhythmic variations of clock-controlled gene PPARα and several lipid metabolism-related genes, including PPAR γ coactivator 1α (PGC-1α), acetyl-CoA carboxylase 1α (ACC-1α), and carnitine palmitoyltransferase 1 (CPT-1).

2. Methods and materials

2.1. Reagents

The assay kit for triglyceride (TG) was obtained from Beijing Beihua Kangtai Clinical Reagent Company (Beijing, China). The assay kit for free fatty acid (FFA) was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The enzyme-linked immunosorbent assay (ELISA) kit for mouse AMPK was the product of Shanghai Xitang Biotechnology Co Ltd (Shanghai, China). Anti-AMPK, anti–p-AMPK, and anti–β-actin antibodies were purchased from Cell Signaling Technology Company (Boston, MA, USA). Anti-Cry2 and anti-Per2 antibodies were purchased from Abcam (Cambridge, United Kingdom) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. Trizol was obtained from Invitrogen (Carlsbad, CA, USA). The primers (Table 1) used for amplification by real-time polymerase chain reaction (PCR) were synthesized by Shanghai Sangon Gene Company (Shanghai, China). All other reagents used in this study were of analytic grade.

2.2. Animals and treatments

Male Kunming mice weighing 20 to 22 g were purchased from Shanghai SLAC Laboratory Animal Co Ltd (Shanghai, China) and maintained in regular cages in a room with controlled humidity and temperature with a 12-hour light (8:00-20:00)/ 12-hour dark (20:00-8:00) cycle. The mice were allowed free access to standard pellet diet (Table 2) and water. They were also allowed to acclimatize to the laboratory environment for 3 days before the study. The animal study was approved by the university ethics committee and conducted according to the regulations for the Use and Care of Experimental Animals at Soochow University.

Seventy-two mice were randomly divided into 2 groups: a high-fat diet group (n = 36) and a control group (n = 36). All mice were allowed free access to standard pellet diet for the entire duration of each 24-hour light/dark cycle during the experiment. Simultaneously, the high-fat diet mice were given high-fat milk at 0.2 mL (containing 1.37 kilojoules from fat and carbohydrate)/10 g body weight per day orally by gavage every evening (19:00-20:00) for 6 weeks. The milk contained 20% lard, 10% cholesterol, 5% saccharose, 0.2% propylthiouracil, 20% propylene glycol, and 20% Tween 80. The control mice were given an equal volume of distilled water in the same manner. The components of fatty acids in the fat milk were 2.5% myristic acid, 26.6% palmitic acid, 10.6% octadecanoic acid, 2.7% palmitoleic acid, 43.7% oleic acid, and 13.9% linoleic acid. After 6 weeks, all of the mice were weighed and euthanized followed by cervical dislocation at a time (6 mice per group) for each of the following time points: 8:00, 12:00, 16:00, 20:00, 24:00, and 4:00. Liver samples were collected for parameter measurements, and partial hepatic tissues were flash-frozen in liquid nitrogen and stored at −80°C for real-time PCR and Western blot analyses.

2.3. Measurement of hepatic TG, FFA, and AMPK levels

Hepatic tissues were obtained and homogenized (10% wt/vol) in cold normal saline. Afterward, tissue homogenates were mixed with a solution of chloroform/methanol (2:1 vol/vol) to a ratio of 1:1 (vol/vol). The prepared samples were then centrifuged at 3000*g* for 10 minutes. The substrata obtained were used to measure TG and FFA contents according to the manufacturer's methods. For the hepatic AMPK measurement, partial hepatic tissues were excised and placed immediately in ice-cold normal saline containing 50 U/mL

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