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EGCG ameliorates diet-induced metabolic syndrome associating with the circadian clock

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ABSTRACT

In response to the daily light-dark (LD) cycle, organisms on Earth have evolved with the approximately 24-h endogenous oscillations to coordinate behavioral and physiological processes, including feeding, sleep, and metabolism homeostasis. Circadian desynchrony triggered by an energy-dense diet rich in fats and fructose is intimately connected with a series of metabolic disorders. Previous studies revealed that (−)-Epigallocatechin-3-gallate (EGCG) could mitigate metabolic misalignment; however, only a few reports have focused on its potential effect on directly manipulating circadian rhythms to ameliorate metabolic syndrome. Our goal was to investigate the regulating effect of EGCG treatment on metabolic misalignment triggered by a high-fat and high-fructose diet (HFFD) associating with the circadian clock. Our results indicated that HFFD treatment partially exhibited poor circadian oscillations of the core clock gene and the clock-controlled gene in the liver and fat relative to the control group. EGCG administration may ameliorate the diet-dependent decline in circadian function by controlling the Sirt1-PGC1αloop, implying the existence of an EGCG-entrainable oscillator. Subsequently, reducing fatty acid synthesis and elevating β-oxidation in the liver coupled with the increasing brown adipose tissue (BAT) energy expenditure observed in the EGCG group of mice prevented the adipocyte hypertrophy and fat accumulations common to BAT and white adipose tissue (WAT) derived from the HFFD mice. This study is the first to provide compelling evidences that EGCG may ameliorate diet-induced metabolic misalignment by regulating the rhythmic expression of the circadian clock genes in the liver and fat.

1. Introduction

Obesity, caused by the energy imbalance between calorie intake and consumption, has become a major international health burden all over the world. In recent decades, high dietary intake of fats and sugars has been identified as one of the main causes for obesity [1]. Population-based studies have indicated that obesity is correlated with a wide spectrum of metabolic syndrome including type 2 diabetes mellitus, hypertension, cardiovascular diseases, and hyperlipidemia. Growing evidence revealed that insulin resistance (IR), inducing a progressive reduction in the responsiveness of peripheral tissue to insulin, was the common basis of metabolic syndrome [2]. In diabetes, the liver and adipose tissue, the critical organs for insulin actions, are damaged, which prevents the ability of insulin to trigger downstream metabolic actions, resulting in insulin resistance (IR) [3].

Circadian rhythms are the approximately 24-h endogenous oscillations of physiology and behavior that coordinate biological processes to maintain synchrony with the environmental cycles of light and nutrients [4]. Circadian rhythms in mammals are generated by a central clock located in the hypothalamic suprachiasmatic nucleus (SCN) that receives direct photic input from the retina and synchronize the phases of other central and peripheral tissue via humoral factors or autonomic innervation [5]. The cell-autonomous molecular oscillator is operated by the transcription/translation feedback loops of circadian genes driven by heterodimeric transcription factors of Clock/Bmal1 or Npas2/Bmal1, which motivate transcription of their own repressors Cryptochromes (Cry1–2) and Period (Per1–3) via E-box sequences in gene promoters. In contrast, competing nuclear receptors RORα and

Abbreviations: EGCG, Epigallocatechin gallate; CLOCK, Circadian locomotor output cycles kaput; BMAL1, Brain and muscle arnt-like protein-1; CRY, Cryptochromes; PER, Period; SIRT1, Sirtuin 1; PGC1α, Peroxisome proliferator-activated receptor coactivator 1-α; IR, Insulin resistance; PPARα, Peroxisome proliferator activated receptor α; NAMPT, Nicotinamide phosphoribosyl transferase; ROREs, ROR enhancer elements; RER, respiratory exchange ratio; H & E, Hematoxylin and eosin stained; ACC, Acetyl-Coenzyme A carboxylase; PPARγ, Peroxisome proliferator activated receptor gamma; FASN, Fatty acid synthase; SREBP1c, Sterol regulatory element-binding protein-1; Cpt1/2, Carnitine palmitoyltransferase 1/2; GCK, Glucokinase; G6pc, Glucose-6-phosphatase; Pepck, Phosphoenolpyruvate kinase; CYP7A1, Cholesterol 7a1-hydroxylase gene; CYP7B1, cholesterol 7b1-hydroxylase gene; UCP1, Uncoupling protein 1; ABCG5/8, ATP-binding cassette sub-family G member 5/8; Tnfα, Tumor necrosis factor alpha; IL1β, Interleukin 1β; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme-A reductase; SQLE, squalene epoxidase gene

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REV-ERBα regulate Bmal1 expression to confer rhythm stability and robustness [6,7]. The intrinsic rhythmicity of the molecular clock is coordinated by the rhythmic interaction of the core clock genes over a 24-h period [6].

Evidence accumulated during recent years reveals that circadian rhythms are intimately associated with metabolic homeostasis via the regulation of a series of metabolic processes [8], and plenty of metabolites, including lipids and glucose, and some metabolism-related hormones, such as insulin and leptin, oscillate in a circadian manner in blood [9]. Epidemiological studies show circadian misalignment can contribute to a wide variety of metabolic syndrome, including hypertension, obesity and insulin resistance (IR), which are serious threats to human health [10]. Mouse models with mutations of the core clock gene such as *Clock* [11], *Bmal1* [12], *Per2* [13], and *Rorα* [14] have demonstrated various metabolic disorders, such as diabetes, obesity, and cardiovascular disease [15], suggesting the key role of clock genes in metabolic regulation. At the molecular level, the Clock/Bmal1 heterodimer regulates the transcription of metabolic genes or clock-controlled genes (CCGs) by binding to E-box (CACGTG) sites within their promoters, such as *Glut2*, *Pparα*, and *Nampt*, which are related to many biochemical and metabolic processes [16]. Reciprocally, many metabolic systems also cycle and may in turn affect the function of clock genes and circadian systems [17]. Emerging evidence revealed Rev-erbα [18], a transcription factor regulated by adipogenesis, and Rorα [19], a nuclear receptor involved in both lipogenesis and lipid storage, alternates repression and activation of Bmal1 expression via binding to the ROR enhancer elements (ROREs) within the Bmal1 promoter.

Mounting evidence indicated that food components, such as protein, vitamins and glucose, are capable of resetting or phase-shifting circadian rhythms, especially the peripheral clock [20]. As mentioned above, an energy-dense diet rich in fats and fructose, as part of an unwholesome lifestyle, is partially responsible for diet-induced obesity. Several studies in mice identified that a high-fat diet leads to the disruption of the normal feeding cycle and metabolic cycles. Mice with a high-fat diet displayed dampened diurnal rhythms in their food intake and whole-body respiratory exchange ratio (RER) and also decreased oscillations of the circadian clock and metabolic genes, such as *Bmal1*, *Per1*, and *Fasn* [21]. Intriguingly, emerging evidence has indicated that resveratrol, a natural antioxidant polyphenol compound in red wine and grapes, could restore the circadian desynchrony of lipid metabolism induced by a high-fat diet [22].

(−)-Epigallocatechin-3-gallate (EGCG), the major catechin found in green tea (*Camellia sinensis*), has been demonstrated to possess antioxidant, anticarcinogenic, anti-inflammatory, and cardioprotective bioactivities [23]. Moreover, EGCG was also reported to alleviate high fat diet-induced symptoms and body fat accumulation by decreasing lipid absorption and reducing levels of inflammatory cytokines [24]. However, it remains unclear whether EGCG directly impacts the circadian desynchrony triggered by a western diet, which coordinates nutritional inputs and hormonal signaling with downstream metabolic outputs.

The current study investigates the effectiveness of EGCG against circadian misalignment triggered by high-fat and high-fructose diets in the oscillations of circadian-clock genes, clock-controlled metabolism and insulin sensitivity in C57BL/6J mice. Moreover, from a translational perspective for obese humans, EGCG reversed circadian misalignment, obesity and metabolic syndrome and can potentially serve as an additional therapeutic intervention in the arsenal against obesity.

2. Materials and methods

2.1. Animals and diet

3 month-old C57BL/6J mice were purchased from Xi'an Jiaotong University (Xi'an, Shaanxi, China). EGCG (purity of > 95%) was purchased from Yuanye Biotechnology, Ltd. (Shanghai, China). Fructose (99%, F0127) was obtained from Sigma-Aldrich (St Louis, MO, USA). Mice were housed in the animal facility under standard conditions (12/12 light-dark cycle, humidity at 50 ± 15%, temperature 22 ± 2 °C) and assigned to three groups based on diet (n = 30/group): control group fed with a standard diet (AIN-93M), high-fat and high fructose diet (HFFD) group fed with high-fat diet (45% kcal from fat, TP230100, purchased from TROPHIC Animal Feed High-tech Co., Ltd. Nantong, China) and 10% fructose in drinking water, and HFFD plus EGCG group fed with HFFD and 2 g/L EGCG in drinking water for 16 weeks. Body weight and food intake were recorded every week. After 16 weeks intervention, 24 mice in each group were used for collecting tissues such as the liver and adipose at four time points (ZT1, ZT7, ZT13, ZT19, n = 6/ZT), while the remaining 6 mice were used for GTT and ITT assays. All of the experimental procedures followed the Guide for the Care and Use of Laboratory Animals: Eighth Edition, ISBN-10: 0-309-15396-4, and the animal protocol was approved by the animal ethics committee of Xi'an Jiaotong University. All surgeries were performed under anesthesia and all efforts were made to minimize suffering. All of the experimental procedures followed by Guide for the Care and Use of Laboratory.

2.2. Assays of glucose tolerance test (GTT) and insulin tolerance test (ITT)

Mice were fasted in advance for 12 h or 6 h for GTTs or ITTs, respectively. Glucose (2 g/kg body weight) and insulin (0.75 U/kg body weight) were injected intraperitoneally. Plasma glucose levels were measured using OneTouch Ultra glucometer (Lifescan Benelux, Beerse, Belgium) before and 15, 30, 60, and 120 min after injections. HOMA-IR was calculated as follows: [fasting insulin concentration (mU/L) × fasting glucose concentration (mg/dL) × 0.05551] / 22.5 [25].

2.3. Analyses of plasma contents and hepatic lipids

The plasma insulin, leptin, adiponectin, andresistin were measured using ELISA kits (Anhui Joyee Biotechnics Co., Ltd., Anhui, China). The total plasma cholesterol (TC) (A111-1), HDL cholesterol (HDLC) (A112-1), LDL cholesterol (LDLC)(A113-1) levels were detected using enzymatic assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), triglyceride (TG) level was examined by enzymatic assay kits (Applygen Technologies Inc., Beijing, China).

2.4. H & E and immunohistochemical staining

For H & E staining, the liver and adipose tissue were fixed in 4% (v/v) paraformaldehyde/PBS and embedded in paraffin for staining with hematoxylin and eosin. For inmmunohistochemical (IHC) staining, the liver and adipose tissue embedded in paraffin were cut into 5 μm sections using a microtome, which were dehydrated through xylene. Use Tris-EDTA Buffer Epitope Retrieval Method. Endogenous peroxidase activity was quenched by incubating the samples in 3% hydrogen peroxide in PBS for 10 min. The liver and adipose tissue sections were blocked for 20 min in normal goat serum blocking solution to block non-specific binding of immunoglobulin and incubated overnight at 4 °C with a rabbit polyclonal antibody against CLOCK (ab93804) (1:400; Abcam, Inc., Cambridge, MA, USA), BMAL1 (ab93806) (1:8000; Abcam, Inc., MA, USA) and a mouse polyclonal antibody against Sirt1 (23411) (1:200, Cell Signaling Technology, MA, USA). After washing, liver and adipose tissue sections were incubated for 20 min at 37 °C with the biotinylated goat anti-rabbit or goat anti-mouse diluted in secondary antibody dilution buffer. Incubate sections with HRP-Streptavidin diluted in HRP-streptavidin dilution buffer (Streptavidin Peroxidase link Detection Kits, Zhongshan Golden Bridge biotechnology Co. Ltd., Beijing, China) for 20 min at room temperature. Liver and adipose tissue sections were washed three times with PBS for each 5 min and visualized by chromogen DAB (DAB kit,