Abstract

Alteration of peripheral clocks in development of fatty liver and obesity and ameliorative effects of soy protein β-conglycinin on fatty liver and obesity

Dongyang Li

Background

Diets high in fat and sucrose can result in obesity and non-alcoholic fatty liver disease (NAFLD). It is an important issue to clarify the molecular mechanism underlying the generation of obesity and NAFLD to provide new therapeutic target, and also prevent and improve obesity and NAFLD by diet component supplementation. Circadian rhythm is a physiological activity oscillating within a day, exists in almost all the cell, and is generated by transcriptional regulatory feedback loop that consists of clock genes. The disruption of circadian rhythm relates to the generation of obesity and NAFLD. The alteration of circadian rhythm in high-fat diet induced obese mice has been demonstrated. In addition, it has reported dietary component soybean protein β-conglycinin prevents obesity and NAFLD induced by high-fat diet in mice.

Objective

The aim of this study was to investigate the alteration of circadian rhythm in obese mice induced by HSD (high-sucrose diet) and compare the mechanism with that of circadian disruption in obesity induced by HFD (high-fat diet), and investigate the effects of β-conglycinin on the improvement of obesity and NAFLD in high-fat diet-induced obese (DIO) mice and clarify the underlying mechanism.

Methods and results

1. Alteration of peripheral clocks in the development of NAFLD and obesity

After one week of normal laboratory diet feeding, mice were divided into one of three groups: StD (starch diet; control diet), HFD, and HSD groups. After 8 weeks of experimental dietary manipulations, mice were killed every 4 h over a 24-h period. The body weight and WAT (white adipose tissue) weight in HFD and HSD feeding groups were significantly high comparing with that in StD feeding group. Mice in HFD and HSD groups showed higher liver TG (triglyceride) than mice in StD group. In mice fed

on HFD, the blood glucose and serum insulin level were extremely increased, and the serum insulin level were higher in HSD group comparing with StD group.

We investigated the diurnal expression of circadian rhythm of clock genes, nuclear receptors and metabolic related transcription factors in liver, WAT and BAT (brown adipose tissue) by real-time PCR. In liver of mice fed HFD, the mRNA expression of clock genes *Npas2*, *Clock*, *Bmal1*, *Per2*, *Per3*, *Cry1* and *Cry2,* nuclear receptors *Rorα, Rorγ and Dbp* were diminished*,* but that of nuclear receptor *Rev-erbβ* was increased. On the other hand, the effect of HSD feeding was opposite to HFD feeding, that is, the mRNA expressions of clock genes, *Npas2*, *Clock*, *Per2*, *Per3* and *Cry1*, nuclear receptors *Rorγ* were increased, but that of nuclear receptor *Rev-erbβ* was decreased. The transcription factor related to lipogenesis PPARγ2, showed higher expression in HFD group and transcription factor related to *de novo* lipogenesis SREBP-1c, showed higher expression in HSD group. Moreover, the phosphorylation of Akt was greatly induced by HSD feeding.

In the WAT, HFD feeding decreased the mRNA expressions of clock genes *Npas2*, *Bmal1*, *Per2*, *Per3*, *Cry1* and *Cry2,* and nuclear receptors *Rev-erbα and Rev-erbβ, Rorα, Rorγ, Dbp* and *E4bp4.* The effect of HSD feeding was similar to HFD feeding, that is, the mRNA expressions of clock genes *Npas2*, *Bmal1*, *Per2*, *Per3*, *Cry1* and *Cry2,* and nuclear receptors *Rev-erbα, Rev-erbβ, Rorγ* and *E4bp4* was decreased. The expression of *Pparγ2* and *Srebp-1c* was extremely increased by HFD feeding, and the expression of *Pparγ1* was increased by HSD feeding.

In the BAT, HFD feeding decreased the mRNA expressions of clock genes, *Npas2*, *Clock*, *Bmal1*, *Per1*, *Per2*, *Per3*, *Cry1* and *Cry2*, and nuclear receptors *Rev-erbα*, *Rev-erbβ*, *Rorα* and *Rorγ*. HSD feeding showed no effect on the circadian oscillation of clock genes and nuclear receptors, except increasing the expression of Dbp extremely. HFD feeding increased the expression of *Pparγ1, Pparγ2* and *Pparα*. HSD feeding increased the expression of *Srebp-1c*.

2. Ameliorative effects of soy protein β-conglycinin on NAFLD and obesity

The ddY mice at 7 weeks of age were fed a HFD for 4 weeks to generate DIO. DIO male ddY mice were divided into six groups: HFD, MFD (medium- fat diet) and LFD (low-fat diet) groups fed casein, and HFD, MFD and LFD groups in all of which the casein was replaced by β-conglycinin. A period of 5 weeks later, the

β-conglycinin-supplemented group resulted in lower body weight, relative weight of subcutaneous WAT, and hepatic TG content.

β-conglycinin suppressed the hepatic expression of *Pparγ2* in the HFD group, *Srebp-1c* and the target genes. The expressions of inflammation-related genes were significantly low in the epididymal and subcutaneous WAT from the mice fed β-conglycinin compared with those fed casein in the HFD group. Moreover, the expressions of *Pparγ1* and *Pparγ2* mRNA were suppressed in subcutaneous WAT in the HFD group but not in epididymal-WAT. Serum insulin and leptin concentrations were detected by enzyme-linked immune sorbent assay (ELISA). β-conglycinin suppressed serum insulin and leptin concentrations.

Conclusion and discussion

1. Alteration of peripheral clocks in development of NAFLD and obesity

We first demonstrated the circadian oscillation of clock genes in the liver, WAT and BAT in ddY mice and the alteration of clock oscillation induced by HSD. HFD induced the diminishing of circadian oscillation as reported previously, but HSD altered the circadian clock tissue specifically. In addition, for the interaction between clock genes and metabolic related transcription factors, the disrupted circadian rhythm accelerates the process of metabolic dysfunction. The circadian clock component RORα suppresses the *PPARγ* transcriptional activity by inhibiting the chromatin recruitment of PPARγ to its target genes in liver. The diminished oscillations of *Rorα* may release its inhibiting effect on *PPARγ* activity, which may stimulate the NAFLD generation process in HFD group. BMAL1 regulates the *de novo* lipogenesis in liver through up-regulating the insulin dependent SREBP-1c activation. The increased Bmal1 expression in HSD group resulted in the acceleration of insulin dependent *de novo* lipogenesis. PER2 inhibits the adipocyte differentiation by blocking the recruitment of PPARγ to the promoters in its target genes involved in the control of adipogenesis. The decreased expression of PER2 in HFD and HSD groups may induce the release the inhibition of PPARγ transcriptional activity to accelerate adipogenesis.

Our study first demonstrated that the alteration of circadian rhythm was different in HFD or HSD induced obese mice. Additionally, the alteration of circadian rhythm showed tissue specificity. Further study is needed to reveal the mechanism behind synchronization of peripheral clocks by food components and the mechanism behind the

metabolic regulatory effect of clock genes. Thus, the discovery of food components with circadian clock regulatory effect and the guidance for dietary intake according to the circadian clock oscillation are expected in the future.

2. Ameliorative effects of soy protein β-conglycinin on NAFLD and obesity

Dietary supplementation with β-conglycinin ameliorated NAFLD in DIO mice. β-Conglycinin suppressed the hepatic expression of *Pparγ2* in the HF dietary group and also that of *Srebp-1c*. β-Conglycinin also effectively improved obesity in DIO mice accompanied by decreases in the expression of *Pparγ1* in the HFD group and *Pparγ2* in the HFD and MFD groups and in the levels of serum insulin and leptin. β-Conglycinin may be a promising dietary protein for the amelioration of NAFLD and obesity.