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Short Communication

Organ-specific alterations in circadian genes by vertical sleeve gastrectomy in an obese diabetic mouse model

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Current therapies for obesity and related complications have been shown to have limited benefits, including unsatisfactory weight loss and poor metabolic improvement. With recent developments in bariatric surgery, promising advancements have been made in clinical and scientific research, particularly in the management of obesity and diabetes. Vertical sleeve gastrectomy (VSG) has become increasingly popular due to its safety, simplicity, and satisfactory outcomes [1]. However, the mechanisms through which VSG improves obesity and diabetes are not fully understood.

Accumulating evidence has suggested that disruption of circadian rhythms increases the risk of obesity, type 2 diabetes mellitus (T2DM), and other metabolic disorders [2]. Conversely, changes in feeding behavior also affect circadian rhythms [3], suggesting that circadian rhythm and metabolism may influence each other. *Bmal1*, *Clock*, and *Period* are the main circadian genes involved in regulating a variety of downstream metabolic targets. Although bariatric surgery has been shown to be successful for controlling body weight, shift workers showed unsatisfactory weight loss after bariatric surgery [4]. Thus, it is still unclear whether circadian genes are involved in mediating the beneficial effects of VSG on obesity and diabetes.

In order to investigate the relationship between VSG and circadian genes, we performed VSG in db/db mice, an obese model with T2DM (Supplementary Materials and Methods). Higher body weight, heavier fat depots, and impaired glucose tolerance observed in sham-operated db/db mice were alleviated after VSG (Fig. S1).

To the best of our knowledge, no studies have examined changes in the expression of circadian genes after VSG in diabetic obese rodents. Therefore, we examined the expression patterns of circadian genes in inguinal white adipose tissue (iWAT), epididymal white adipose tissue (eWAT), liver, and skeletal muscle of db/db and db/m control mice. Our results showed variations in the expression of circadian genes following VSG. For example, *Bmal1* was consistently decreased in iWAT, eWAT, liver, and skeletal muscle of db/db mice, but was all elevated by VSG (Fig. 1a and b). *Clock*, *Per2*, and *NR1D1* showed similar patterns, with decreased expression in iWAT, eWAT, and skeletal muscle of obese mice, but no changes in liver. VSG increased the expression of *Clock*, *Per2*, and *NR1D1* in WAT and skeletal muscle, but had no effect in liver. In contrast, the expression levels of *Cry1*, *Cry2*, *Per1*, *Per3*, and *RORα* were decreased in liver and skeletal muscle of obese mice, but were either elevated or unchanged in WAT. Similarly, VSG could partially reverse the changes in the expression levels of these five genes (Fig. S2). Previous studies reported the organ-specific roles of circadian genes, including *Bmal1* [5–7]. Thus, taken together, these findings suggested that circadian genes may have various roles in different organs and that VSG could alter the expression of these circadian genes in an organ-dependent manner.

Therefore, we next examined various metabolic profiles in different organs. Chronic inflammation and fibrosis are critical characteristics of obesity and are associated with insulin resistance [8,9]. The mRNA expression of *MCP-1* (a key inflammatory marker), *TNF-α*, and *IL-6* (important cytokines reflecting tissue inflammation) in WAT was higher in db/db + sham mice than in db/m + sham mice (Fig. 1c). Moreover, the expression of *Col6a1* (a fibrotic marker) showed similar changes. VSG significantly decreased the expression of *MCP-1*, *TNF-α*, *IL-6*, and *Col6a1* mRNAs in WAT. Our data demonstrated that inflammatory and fibrotic markers were

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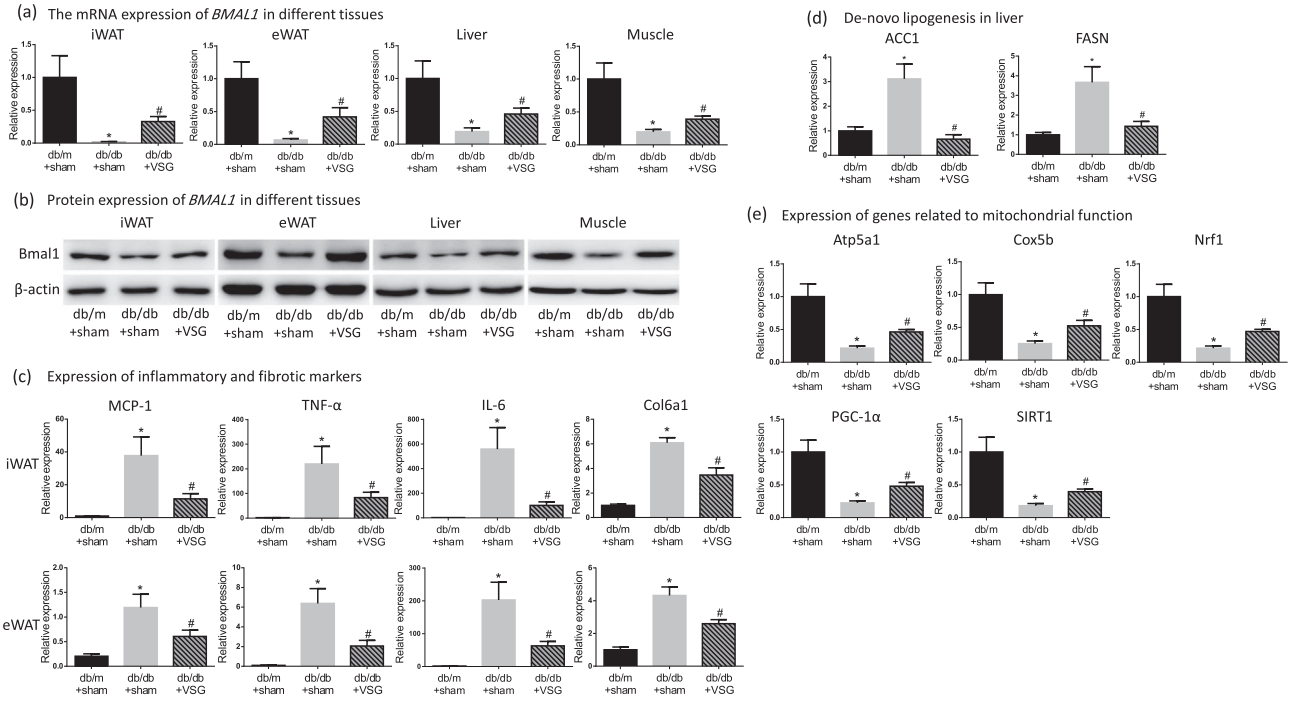

Fig. 1. VSG affected *Bmal1* expression and metabolic profiles in db/db mice. The expression levels of (a) *Bmal1* mRNA; (b) BMAL1 protein; (c) *MCP-1*, *TNF-α*, *IL-6*, and *Col6a1* mRNA; (d) *ACC1* and *FASN* mRNA; and (e) *Atp5a1*, *Cox5b*, *Nrf1*, *PGC-1α*, and *Sirt1* mRNA in skeletal muscle, WAT, and livers of db/db + sham and db/m + sham mice with or without VSG. Data are shown as means ± SEMs. $^{*}P < 0.05$, db/db + sham compared with db/m + sham; $^{\#}P < 0.05$, db/db + VSG compared with db/db + sham. $n = 4$ for db/m + sham and db/db + sham; $n = 5$ for db/db + VSG.

downregulated by VSG, suggesting that the VSG-induced weight-sparing effects were mediated by downregulation of these inflammatory or fibrotic genes. Indeed, our observations further supported the notion that suppression of inflammation could improve insulin resistance.

De novo lipogenesis in the liver is critical to lipid metabolism. In our study, genes related to lipogenesis (*Acc1* and *Fasn*) were highly expressed in livers of diabetic obese mice, and the effect was reversed by VSG (Fig. 1d). However, decreased de novo lipogenesis was found in Bmal1(-/-) primary mouse hepatocytes [6], which is contrary to our in vivo data. These conflicting results may be attributed to the different mouse models used. Thus, furthers studies are needed to elucidate the regulatory effects of circadian genes on hepatic lipogenesis.

Skeletal muscle is a critical tissue involved in energy consumption and metabolism. The mitochondrial function of skeletal muscle is critical for maintaining whole body metabolic homeostasis. Decreased mitochondrial function was found in db/db + sham mice, and this effect was reversed by VSG (Fig. 1e). Liu reported that peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α may stimulate the expression of *Bmal1* and regulate energy metabolism in the liver and skeletal muscle [10]. We found that VSG increased the expression of the *PGC-1α* and *Bmal1* genes in skeletal muscle of obese mice; thus, we speculated that in skeletal muscle, VSG may increase mitochondrial function by inducing the expression of *PGC-1α*, leading to the increased expression of *Bmal1*.

Although the beneficial effects of VSG on metabolism and altered expression of circadian genes were simultaneously observed in db/db mice, these results were not sufficient to conclude that circadian genes directly mediated the effects of VSG. To verify the specific impact of VSG, circadian genes, particularly global and organ-specific *Bmal1*-knockout mice, should be evaluated in further studies. In addition, we did not investigate the expression of these genes at different zeitgeber times. Reduction of brown adipose tissue was observed in our study. However, we could not detect *UCP-1* expression in db/db mice (data not shown). Further investigations are needed to reveal whether these changes were due to reduced ectopic fat deposits or consumption of brown adipose tissue.

In summary, our study found that VSG had beneficial effects on weight loss, controlling blood glucose, improving insulin resistance, decreasing adipose inflammation and fibrosis, and stimulating mitochondrial function in skeletal muscle, accompanied by tissue-specific alterations in circadian genes, as proposed in Supplementary Fig. S3. Thus, circadian genes may mediate the metabolic effects of VSG and could be potential targets for alleviating obesity and diabetes.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

This work was designed by YQY, ZMW, and ZZY. Surgery was performed by SQW, YYP, LWJ, and WM. YQY, HR, XB, and SYK provided technical guidance for VSG. Mice were sacrificed and tissues were collected by LWJ and SYK. The molecular experiments were performed by SQW and YYP. HE staining was performed by ZJJ. SQW and LWJ performed the statistical analysis. JWZ, WQH, SQW, and ZZY wrote the manuscript. ZXL, JBK, JWZ, HZY, and WQH were consultants for the study and helped with manuscript editing.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scib.2017.03.014.

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