Protective effects of prebiotic beta-glucans on non-alcoholic steatohepatitis aggravated by circadian disruption in C57BL/6J mice

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Introduction

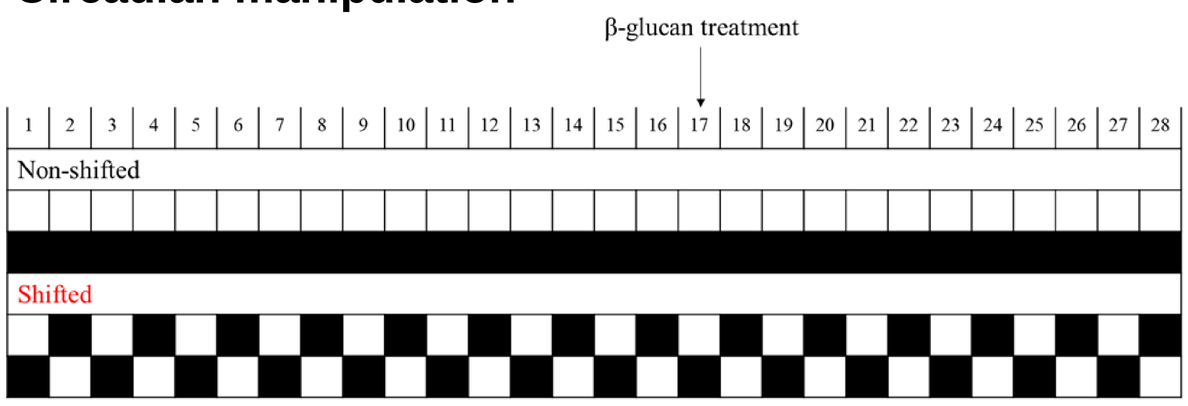
Non-alcoholic steatohepatitis (NASH) is becoming the most common cause of chronic liver diseases. Circadian disruption (CD), a common phenomenon among shift workers, has been shown to promote hepatic steatosis in rodent model. Recent studies have shown an association between gut microbiota and liver function. Anti-inflammatory beta-glucan (BG) with prebiotic potential is found in cereal and fungi. We hypothesize that oral supplementation of BG with structural differences results in differential alleviation of NASH mediated by gut microbiota and short-chain fatty acids. Our results showed that CD can accelerate the progression of NASH but it can be reversed by BG supplementation.

Objectives

1. To establish the fructose, palmitate, cholesterol (FPC) diet-induced NASH mouse model aggravated by CD
2. To compare the protective effects of structurally characterized β-1,3/1,4-glucan isolated from oat (OBG) and β-1,3/1,6-glucan from *Aureobasidium pullulans* (APBG) (fungal origin) on NASH at dose level of 500mg/kg

Experimental Design

Circadian manipulation



- Open and filled bars represent light and dark periods of the day, respectively
- Weekly shifted light-dark cycle was adopted to mimic chronic shift work to induce circadian disruption

Feeding protocol

- Non-shifted light-dark cycle + Standard diet (NSC)
- Non-shifted light-dark cycle + FPC diet (NSFPC)
- Shifted light-dark cycle + Standard diet (SC)
- Shifted light-dark cycle + FPC diet (SFPC)
  - + INU (Inulin as positive control)
  - + APBG
  - + OBG

- Male C57BL/6J mice were randomly divided into 7 groups (n = 8-9)
- FPC diet-fed mice were given drinking water containing 42 g/L glucose and fructose (55%/45%, w/w)

Results

Characterization of beta-glucans

	Oat BG	*Aureobasidum pullulans* BG
BG content	76.13%	50.59%
Protein content	2.10%	0.61%
Molecular weight	83,531 kDa	159,093 kDa
Monosaccharide composition	Glc (major), Ara, Xyl	Glc (major) Man, Gal
Glycosidic linkages	β-1,3/1,4	β-1,3/1,6

Locomotor activity of mice

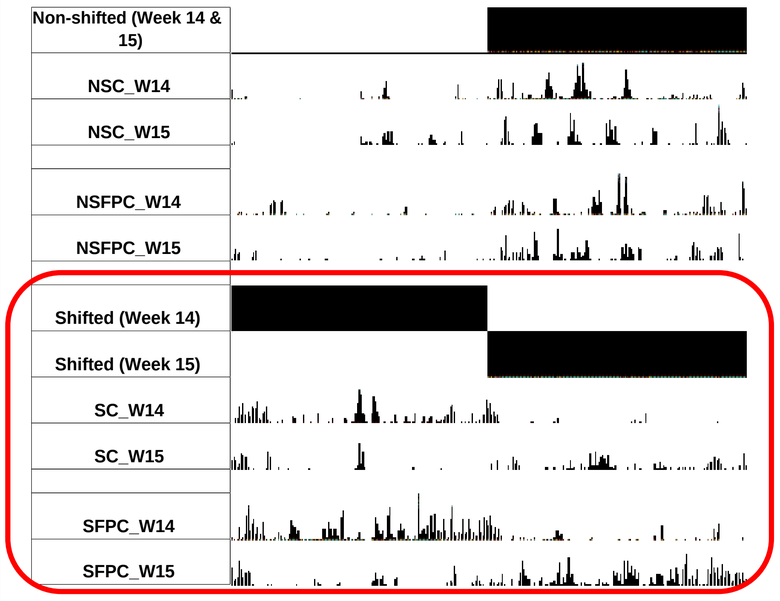


Fig. 1. Representative actograms of non-shifted and shifted group mice on a 24-hr cycle in week 14–15. Waveforms represent the activity as total distance travelled over 24-hr in 5-min time-bins.

Metabolic parameters of mice

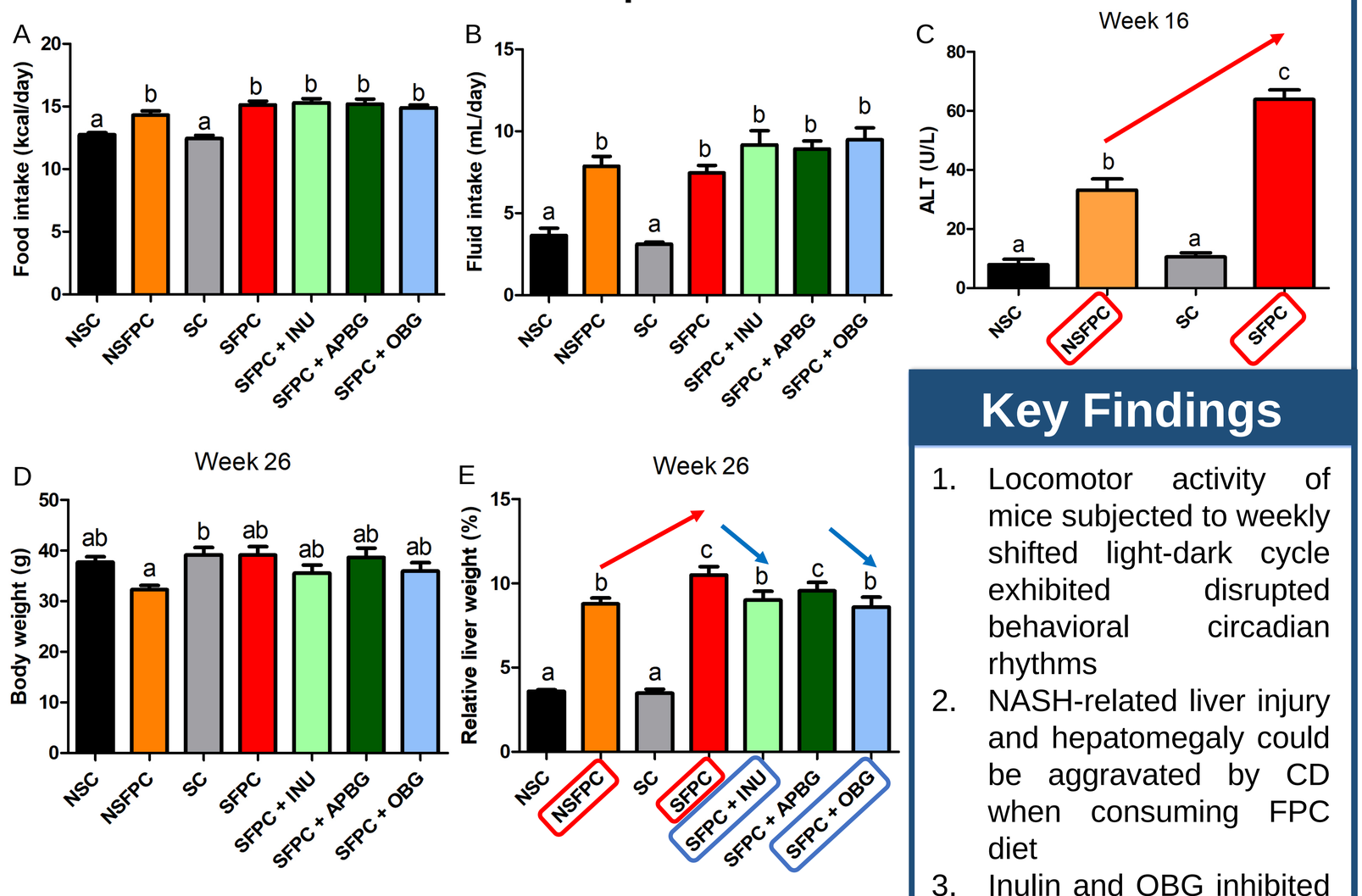


Fig. 2. (A) Average fluid intake; (B) Average food intake; (C) Week 16 serum alanine aminotransferase (ALT); (D) Week 26 body weight; (E) Week 26 relative liver weight. Data is expressed as mean ± SEM. Different letters represent statistical differences, $p < 0.05$.

Key Findings

1. Locomotor activity of mice subjected to weekly shifted light-dark cycle exhibited disrupted behavioral circadian rhythms
2. NASH-related liver injury and hepatomegaly could be aggravated by CD when consuming FPC diet
3. Inulin and OBG inhibited the increase in relative liver weight induced by CD and FPC diet

Conclusion

Our study revealed that inulin and BG isolated from oat but not *A. pullulans* reversed the increase in relative liver weight at the dose level of 500mg/kg, suggesting that β-1,3/1,4-glucan could be more effective to attenuate NASH-associated hepatomegaly than β-1,3/1,6-glucan. Serum biochemical analyses, histological examinations of the liver, and assessment of cecal short-chain fatty acids content and gut microbiota will be performed in the future to delineate the protective mechanism of BG on NASH.