

## Research Note

***Syphacia muris* infection delays the onset of hyperglycemia in WBN/Kob-*Lep<sup>fa</sup>* rats, a new type 2 diabetes mellitus model**K. TAIRA<sup>1</sup>, R. YAZAWA<sup>2</sup>, A. WATANABE<sup>2</sup>, Y. ISHIKAWA<sup>2</sup>, M. OKAMOTO<sup>3</sup>, A. TAKAHASHI<sup>1</sup>, F. ASAI<sup>2\*</sup>

<sup>1</sup>Laboratory of Parasitology, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagami-hara, Kanagawa 252-5201, Japan; <sup>2</sup>Laboratory of Pharmacology, School of Veterinary Medicine, Azabu University, \*E-mail: [asai@azabu-u.ac.jp](mailto:asai@azabu-u.ac.jp); <sup>3</sup>Laboratory of Veterinary Immunology, School of Veterinary Medicine, Azabu University

## Article info

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## Summary

Diabetes mellitus is one of the most common endocrine disorders and its continuous global increase is due to factors as population growth, urbanization, aging, and increasing prevalence of obesity and physical inactivity. The effect of pinworm infection on the development of hyperglycemia was examined in WBN/K-*Lep<sup>fa</sup>* (*fa/fa*) rats, a new model of the obese type 2 diabetes mellitus (T2DM) with pancreatitis. The rats were orally administered *Syphacia muris* eggs (infected group) and distilled water (control group). Hyperglycemia onset in the infected group was significantly delayed compared to the control group. Neither body weight nor intake of food and water were affected by *S. muris* infection. This study demonstrated that *S. muris* infection delayed the onset of T2DM in *fa/fa* rats and suggested that elucidation of the underlying mechanism and relevant pathways in the helminth-mediated protection may lead to the development of a new strategy to prevent diabetes mellitus.

**Keywords:** WBN/Kob-*Lep<sup>fa</sup>* rats; type 2 diabetes mellitus; *Syphacia muris*

## Introduction

Globally it has been estimated that 382 million people worldwide with a prevalence of 8.3 % in the age group of 20-79 suffer from diabetes mellitus (DM) in 2013, and this number is expected to rise markedly in the next 20 years (International Diabetes Federation, 2013). Type 2 diabetes mellitus (T2DM) characterized by insulin resistance and pancreatic  $\beta$ -cell dysfunction constitutes about 85 to 95 % of all DM cases in high-income countries and accounts for even higher percentage in low- and middle-income countries (Shaw *et al.*, 2010).

Helminths in general seem to protect onset of type 1 DM, an autoimmune disease. Several reports have described relations between helminth infection and prevention the onset of type 1 DM (Cooke *et al.*, 1999; Saunders *et al.*, 2007; Liu *et al.*, 2009; Hubner *et al.* 2012). There are few reports concerning relations between helminth infection and T2DM. While, recent studies have demonstrated that a low-grade chronic inflammation characterized by alterations in circulating immune-modulatory factors is associated with the pathogenesis of T2DM (Donath and Shoelson, 2011; Daniele *et al.*, 2014), and Wu *et al.* (2011) reported that helminth infections ameliorate diet-induced insulin resistance by an immune-modulation.

The WBN/Kob-*Lep<sup>fa</sup>* (*fa/fa*) rat is a new congenic strain developed by introduction of the *fa* gene of the Zucker fatty (ZF) rat into the parental WBN/Kob (lean) rat genome (Akimoto *et al.*, 2008). The WBN/Kob rats are a relevant animal model used in the study of non-obese diabetes. These rats typically show symptoms that include chronic pancreatitis and pancreatic endocrine disorders (Nakama *et al.*, 1985; Tsuchitani *et al.*, 1985). Conversely, Zucker fatty rats are obese and have hyperinsulinemia over their lifetime (Shiota and Printz, 2012). Previous reports showed that *fa/fa* rats have severe insulin resistance and develop hyperglycemia at 7-9 weeks of age (Akimoto *et al.*, 2008; Kaji *et al.*, 2012; Okuno *et al.*, 2013; Nagakubo *et al.* 2014) and that pancreatitis with inflammatory infiltration is observed prior to the onset of hyperglycemia (Kaji *et al.* 2012; Okuno *et al.*, 2013; Nagakubo *et al.*, 2014). These features make the *fa/fa* rat a useful novel model for evaluating the course of the obese T2DM. We recently observed that *fa/fa* rats accidentally infected with rat pinworms *Syphacia muris* tend to show delayed onset of hyperglycemia. To our knowledge, there are few reports concerning possible links between nematode infections and delayed onset of T2DM. The objective of this study was therefore to determine whether experimental infection with *S. muris* delays the onset of hyperglycemia in *fa/fa* rats.

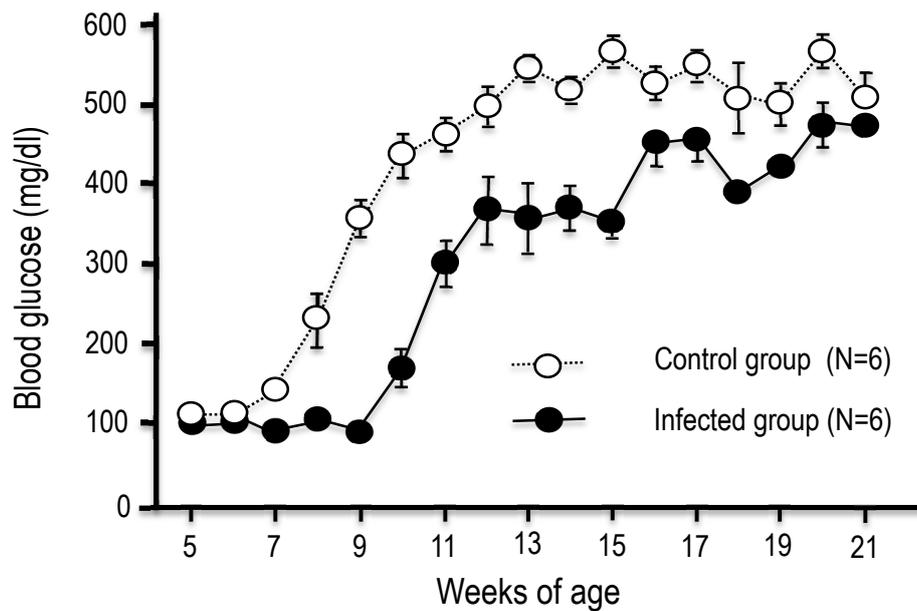


Fig. 1. The effect of inoculation of *Syphacia muris* eggs on the development of hyperglycemia with aging in WBN/Kob-*Lep<sup>fa</sup>* (*fa/fa*) rats. At 5 weeks of age, the infected group (n = 6) was inoculated with 100 eggs/rat, and the control group (n = 6) was inoculated with distilled water. Data are the means  $\pm$  SEM of 6 animals in each group. The infected group showed significantly lower blood glucose level than the control group ( $P < 0.0001$ , repeated-measures one-way ANOVA).

## Materials and Methods

For an experimental study, 12 male *fa/fa* rats had been obtained at 4 weeks of age from Japan SLC, Inc. (Shizuoka, Japan). Rats were housed in plastic cages (2 rats/cage) under the following conditions: temperature,  $21 \pm 2$  °C; humidity,  $55 \pm 5$  %; lights on for 12 h per day from 8:00 to 20:00. Rats were allowed free access to food (CRF-1; Charles River Laboratories Japan Inc., Kanagawa, Japan) and tap water from a plastic water bottle. All rats were handled according to the experimental animal guidelines outlined by Azabu University.

*S. muris* eggs were harvested from the uterus of female worms obtained from the colon and rectum of experimentally infected male Wistar rats (Stahl, 1961). Collected eggs were incubated at 25 °C for 1 – 2 days in a solution of 0.5 % (v/v) formalin. The incubated eggs were then stored at 10 °C for 2 weeks. Prior to inoculation, the egg suspension was washed with distilled water to remove the formalin.

Twelve *fa/fa* rats were divided into 2 groups. At 5 weeks of age, *fa/fa* rats were inoculated using stomach tubes with approximately 100 *S. muris* eggs/ml/rat (n = 6, infected group), and other rats were inoculated with 1 ml/rat of distilled water (n = 6, control group). Infection with *S. muris* was confirmed using the cellophane tape method to detect pinworm eggs in the perianal region of rats.

Blood glucose and body weight were measured weekly. Food and water intake was also monitored for each cage. Blood samples were collected from the tail vein, and the blood glucose level was measured using an Accu-Chek Aviva self-monitoring device (Roche Diagnostics, Ltd., Tokyo, Japan).

At 17 weeks post-inoculation (21 weeks of age), all rats were euthanized using exsanguination under pentobarbital anesthesia (Kyoritsu Seiyaku Corporation, Tokyo, Japan). The pancreas,

adipose tissues surrounding the testis and mesenteric adipose tissues were removed and weighed. The caecum to the rectum was dissected, and the number of parasitized worms was counted under a light microscope. Worms in which the uterus was observed were regarded as females, those with mamelons were regarded as males, and other small worms were regarded as larvae.

Pancreatic tissues were isolated from *S. muris*-infected 12-week-old *fa/fa* rats and from non-infected control *fa/fa* rats. Tissues were fixed in a 10 % neutral buffered formalin solution, and processed using standard histological techniques as described previously (Nagakubo et al., 2014). Following paraffin-embedding, 4- $\mu$ m-thick sections were cut and stained with hematoxylin and eosin for histopathological evaluation. Further, the tissue sections were stained with an antibody against insulin.

Data are reported as average values  $\pm$  standard error of the mean (SEM). Statistical analysis was conducted using repeated-measures one-way ANOVA for comparison of blood glucose, body weight and food/water intake. The analysis was performed using two-tailed tests with a significance level of 5 % ( $P < 0.05$ ) using IBM SPSS statistics software (Version 20; SPSS Inc., Chicago, IL, USA).

## Results

In the infected group,  $429.2 \pm 107.9$  (mean  $\pm$  SEM, n = 6) female worms,  $309.4 \pm 152.0$  male worms and  $194.8 \pm 52.8$  larvae of *S. muris* were isolated from the large intestines during necropsy performed at 17 weeks post-inoculation. No worms were found in the rats in the control group (n = 6).

No significant difference was recorded in blood glucose levels between the control and the infected groups before inoculation ( $106 \pm 10$  mg/dl for the control group,  $104 \pm 2$  mg/dl for infected

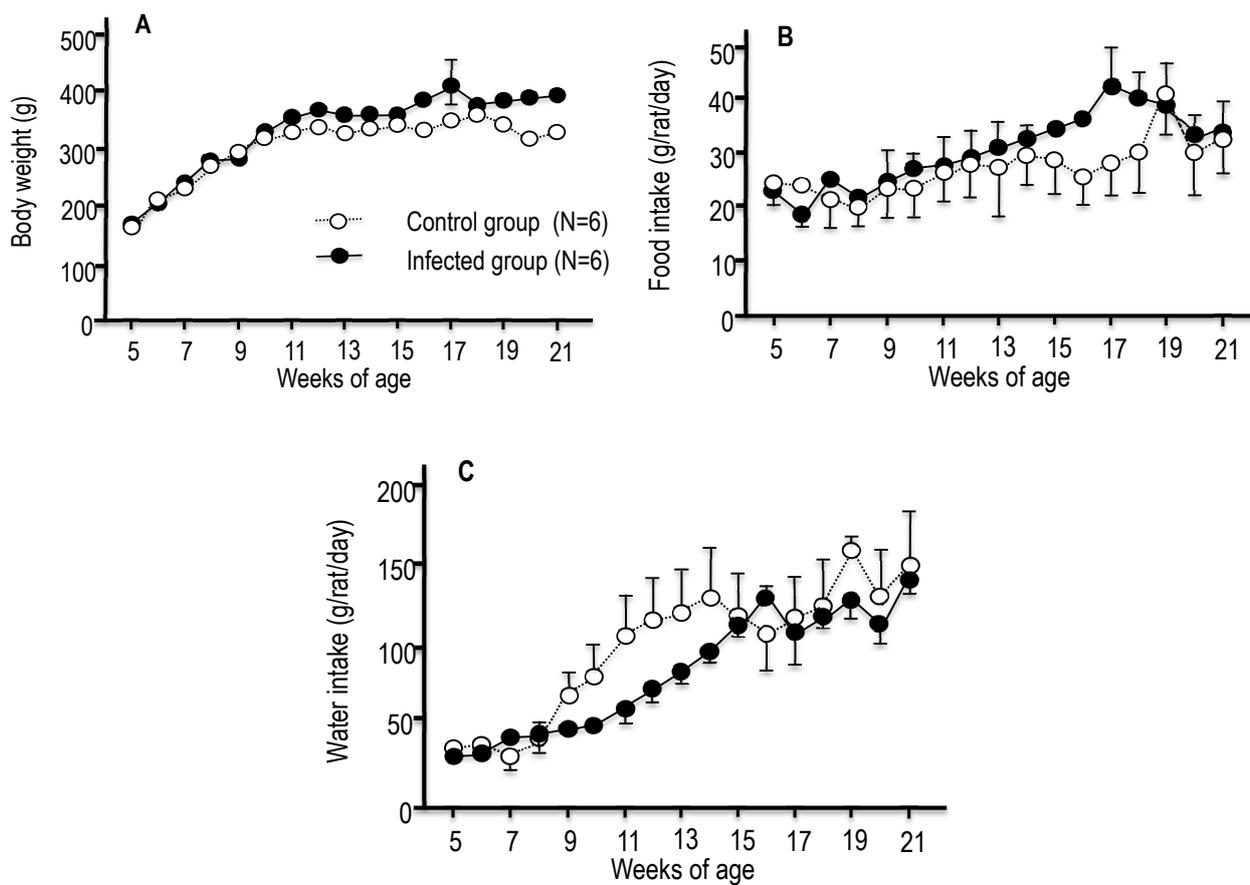


Fig. 2. The effect of inoculation of *Syphacia muris* eggs on the body weight (A) and intake of food (B) and water (C) in WBN/Kob-*Lep<sup>fa</sup>* rats. At 5 weeks of age, the infected group was inoculated with 100 eggs/rat and the control group was inoculated with distilled water. Data are the means  $\pm$  SEM of 6 animals in each group. No significant differences were observed between the infected group and the control group ( $P > 0.09$ , repeated-measures one-way ANOVA).

group). In the control group, the blood glucose level increased at 8 weeks of age ( $233 \pm 39$  mg/dl), and the levels plateaued at 13 weeks of age ( $547 \pm 14$  mg/dl). Unlike this, the blood glucose level in the infected group increased at 10 weeks of age ( $168 \pm 26$  mg/dl) (Fig. 1). Overall, the infected group showed significantly ( $P < 0.0001$ ) lower blood glucose level than the control group (Fig. 1).

There were no significant differences in body weight and food and water intake between the infected group and the control group during the experiment (Fig. 2. A – C). No apparent differences in the weight of the pancreas ( $0.85 \pm 0.07$  g for the control group,  $0.60 \pm 0.08$  g for infected group), adipose tissues surrounding the testis ( $10.46 \pm 0.87$  g for the control group,  $10.83 \pm 1.19$  g for infected group) and mesenteric adipose tissues ( $8.07 \pm 0.92$  g for the control group,  $8.89 \pm 0.37$  g for infected group) were seen between the groups.

Histopathological and immunohistochemical stained sections of the pancreas of *S. muris*-infected *fa/fa* rat (12-week-old) and of non-infected *fa/fa* rat are shown in Fig. 3. A region of absence of exocrine glands was seen in the non-infected rat, in where islet-like cells, undifferentiated cells and hemosiderosis were observed (Fig. 3. B). Similar regions were also seen in the infected rat (Fig. 3. A). However, more number of insulin positive cells were seen in the infected rat (Fig. 3. C) than those in the non-infected rat (Fig. 3. D).

## Discussion

The present study demonstrated for the first time that experimental infection with *S. muris* delays the onset of hyperglycemia in *fa/fa* rats, a model for T2DM associated with pancreatitis and obesity. This finding was corroborated by the significantly lower blood glucose observed in the infected group compared to the control group.

The T2DM is now a common and serious global health problem, which, in most countries, has evolved in association with rapid cultural and social changes, ageing populations, increasing urbanization, dietary changes, decreased physical activity and other unhealthy lifestyle and behavioural patterns (Fonseca and John-Kalarickal, 2010). To investigate connection with this disease, infections with pinworm *S. muris*, a ubiquitous nematode that parasitizes mainly in the large intestines of wild and experimental rats (*Rattus norvegicus*), was employed. The lifecycle of *S. muris* is direct, and host animals are infected with the nematode via an oral intake of their eggs; the prepatent period of this nematode is 7–8 days (Stahl, 1961; Taffs, 1976; Torre *et al.*, 2013). In the present study, 100 *S. muris* eggs were orally administered to 5-week-old *fa/fa* rats at the pre-diabetic stage, and eggs were detected 2 weeks after inoculation in the perianal region of all *fa/fa* rats in the infection group. Subsequently, a considerable number of adult worms and larvae were detected in

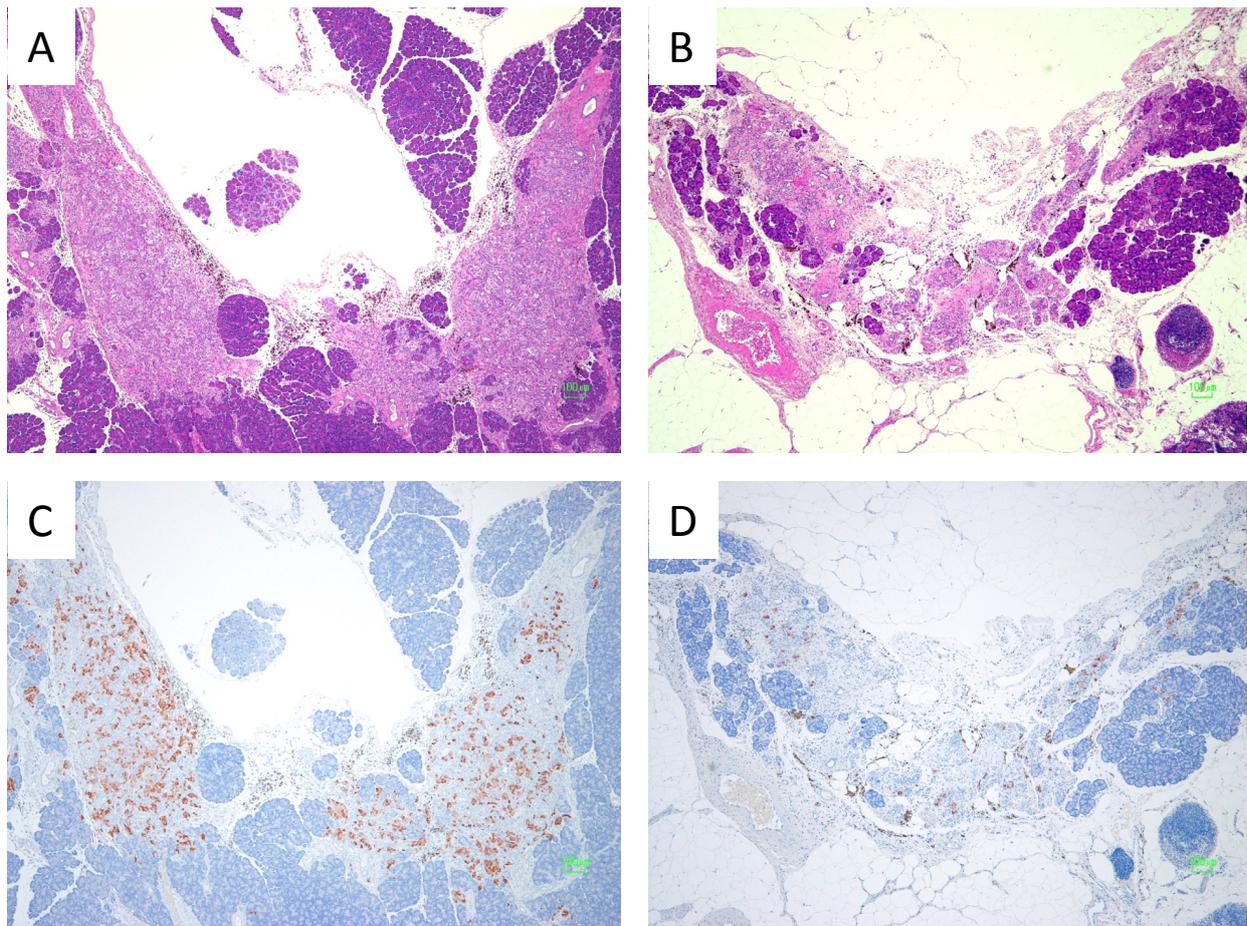


Fig. 3. Histopathological and immunohistochemical stained sections of the pancreas of *Syphacia muris*-infected WBN/Kob-*Lep<sup>fa</sup>* (*fa/fa*) rats. Hematoxylin and eosin stained sections of *S. muris*-infected rat (A) and of non-infected rat (B). Sections immunostained for insulin from *S. muris*-infected rat (C) and from non-infected rat (D).

the large intestine during necropsy conducted 17 weeks after inoculation. These results indicate that repeated infection with *S. muris* was established in the *fa/fa* rats.

A typical phenotype encountered in *fa/fa* rats is characterized by obesity resulting from hyperphagia. Younger *fa/fa* rats exhibit euglycemia accompanied by marked hyperinsulinemia, indicating that severe insulin resistance is compensated by increased insulin secretion in these animals (Okuno *et al.*, 2013). These authors also reported that hyperglycemia thereafter develops in association with a marked decline in plasma insulin levels. Excessive caloric intake and consequent obesity cause insulin resistance and impaired pancreatic function (Di Marzo, 2008). Previous reports showed that dietary restriction inhibits the development of diabetes in *fa/fa* rats by reducing pancreatitis (Akimoto *et al.*, 2010). In our study, however, *S. muris* infection has not affected the body weight, food and water intake, or the weights of examined organs. These results are consistent with the fact that infection with pinworms is generally asymptomatic (Taffs, 1976).

Since *fa/fa* rats develop pancreatitis with inflammatory infiltration prior to the onset of hyperglycemia (Akimoto *et al.*, 2012), inflammatory cytokine production may trigger the onset of hyperglycemia in this model. Histopathological and immunohisto-

chemical evaluations of the pancreas in the present study suggested that traces of inflammation, where exocrine components of the pancreas were not seen, were observed both in the *S. muris*-infected and the non-infected *fa/fa* rats. However, more number of the insulin positive cells in these post inflammatory region was noted in the infected rat. The observations may support that *S. muris* infection relates to suppress pancreatitis of *fa/fa* rats.

Elucidating the mechanism of delayed onset of T2DM may lead to the development of a new type of prophylaxis that can be used in the treatment of diabetes mellitus. Further studies, which include immunological approaches, are needed to elucidate the mechanism of the delayed onset of T2DM in *S. muris*-infected *fa/fa* rats. Other parasite infections should also be studied to determine whether the onset of hyperglycemia in *fa/fa* rats can be delayed.

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