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REGULAR ARTICLE

# Cotton rat (*Sigmodon hispidus*) develops metabolic disorders associated with visceral adipose inflammation and fatty pancreas without obesity

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## Abstract

Obesity induces metabolic disorders such as type 2 diabetes, hypertension, and cardiovascular diseases and has become a global health concern. Recent studies imply that fat accumulation in nonadipose tissue correlates with metabolic disorders. However, there are no suitable animal models to evaluate this phenomenon. This study investigated the characteristics of metabolic disorders found in cotton rat (*Sigmodon hispidus*). Blood biochemical examinations revealed that cotton rats, predominantly males, developed hyperinsulinemia, hyperglycemia, and dyslipidemia when fed a normal diet. The islets increased in size through β-cell hyperplasia, which was associated with serum insulin level in both sexes, strongly indicating insulin resistance. In male cotton rats, oxidative stress was observed in β cells, and macrophage infiltration into the visceral white adipose tissue was reported, both of which were associated with serum insulin level without visceral obesity. In contrast, female cotton rats developed hyperinsulinemia without histopathological changes that were reported in males. Adipocytes were found to be accumulated in the pancreas but not in the liver of both sexes during aging. Pancreatic fat accumulation was associated with the serum insulin level only in females. Taken together, cotton rats developed metabolic disorders associated with visceral fat inflammation in the absence of obesity. In addition, pancreatic ectopic fat may also be related to the early stages of these conditions. Thus, the cotton rat may serve as a novel and useful model for metabolic disorders characterized by visceral adipose inflammation and ectopic fat accumulation in the pancreas without obesity.

**Keywords** Cotton rat · Ectopic fat · Metabolic disorder · Obesity · Visceral fat inflammation

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## Introduction

Obesity, characterized by excessive energy storage in the form of triglycerides in the adipose tissue, has become a global health concern worldwide. Obesity, particularly visceral fat obesity, induces adipocyte hypertrophy and hyperplasia as well as aberrant production of adipokines in the visceral white adipose tissue (Yao et al. 2014). Adipokines trigger local chronic inflammation by recruiting inflammatory cells, which further exacerbate obesity (Yao et al. 2014). In addition, adipokines are known to modulate systemic metabolic homeostasis by altering glucose absorption and insulin sensitivity in the liver, skeletal muscle, and adipose tissue itself, thereby inducing compensatory hyperinsulinemia in response to an increase in insulin demand, a condition termed as insulin resistance (Yao et al. 2014). Thus, the obesity-inflammation-insulin resistance axis has been recognized as the basis for several metabolic diseases,

including type 2 diabetes, hypertension, dyslipidemia, and cardiovascular diseases (Yao et al. 2014). However, the involvement of visceral fat obesity in metabolic disorders remains controversial. STAMP2-deficient mice exhibit inflammation restricted to the visceral adipose tissues without visceral fat obesity and develop spontaneous metabolic disorders, which manifest as insulin resistance, glucose intolerance, hyperglycemia, dyslipidemia, and fatty liver disease (Wellen et al. 2007). These results indicate that factors other than obesity may trigger the progression of metabolic disorders. Recent studies have highlighted the association between fat accumulation in nonadipose tissues and metabolic disorders in mice and humans (Wang et al. 2008; Catanzaro et al. 2016). Leptin receptor-deficient *db/db* mice that overexpress adipocyte-specific leptin receptor showed a decrease in body weight and fat weight, but had exacerbated diabetes owing to fat accumulation in nonadipose tissues such as the liver, heart, skeletal muscle, and pancreas (Wang et al. 2008). However, little is known about their pathogenesis and molecular mechanisms, given the lack of an appropriate animal model because ectopic fat accumulation is accompanied by visceral obesity when feeding animals a high-fat diet (Fraulob et al. 2010; Matsuda et al. 2014).

The cotton rat (*Sigmodon hispidus*), also known as the hispid cotton rat, is an experimental rodent originating from the southern USA. Cotton rats are moderate-sized rodents and have a weight between 80 and 300 g (Faith et al. 1997) or 70 to 200 g (Niewiesk and Prince 2002). In cotton rats, the lifespan is about 18 months in the laboratory colonies (Tajima and Horiuchi 1989), and the life expectancy of 50% is about 14 months in the inbred strain (Niewiesk and Prince 2002). Although the laboratory cotton rats tend to have shorter lifespan than the laboratory mice and rats (Tajima and Horiuchi 1989), their cause of death has been poorly understood. Many studies have reported the association of this rodent with an increased susceptibility to pathogenic human viruses, protozoans, metazoans, respiratory disease viruses, and bacteria such as *Leishmania* and *Echinococcus* (Kroeze and Tanner 1985; Azazy et al. 1997; Blanco et al. 2014). In addition, unique disease phenotypes were identified in cotton rats, including fragile tails, stomach cancers, and cardiomyopathy (Faith et al. 1997). In our previous studies, we identified additional unique phenotypes such as pharyngeal pouch remnants, female-dominant chronic kidney disease, and pyometra (Ichii et al. 2016, 2018; Nakamura et al. 2018). These reports indicate that cotton rat may serve as a useful model for studying various human diseases, other than just human infectious diseases. In the present study, we demonstrate that cotton rats develop metabolic disorders associated with chronic inflammation in visceral white adipose tissue and ectopic fat accumulation, specifically in the pancreas, in the absence of obesity.

## Materials and methods

### Animals

Animal experimentation was performed according to the instruction of Hokkaido Institute of Public Health (approval No. K27-03). Male and female cotton rats were originally provided by Tanabe Seiyaku Co. Ltd. (Toda, Japan) in 1971 and were inbred by brother-sister mating since 1982 at the Hokkaido Institute of Public Health (Sapporo, Japan) under conventional conditions (Waldum et al. 1999). The animals were allowed free access to tap water and commercial diet (CMF, 20% of calories from fat, Oriental Yeast Co., Ltd., Tokyo, Japan). The animals were divided into three life stages: juvenile (approximately 1–3 months of age), adult (approximately 6–8 months of age), and old (9–12 months of age) (Faith et al. 1997).

### Blood examination and urinalysis

Blood was collected from the vena cava under deep anesthesia with isoflurane at 2 to 5 p.m., and serum was prepared by centrifugation. Levels of glucose, triglyceride, total cholesterol (T-Cho), high-density lipoprotein cholesterol (HDL-Cho), uric acid, aspirate aminotransferase (AST), alanine aminotransferase (ALT), and lipase were measured using a Fuji Dri-Chem 7000v instrument (Fujifilm, Tokyo, Japan). Non-HDL-Cho was calculated by subtracting HDL-Cho from T-Cho, and the atherogenic index was calculated by dividing non-HDL-Cho from HDL-Cho (Kayamori and Igarashi 1994). Free fatty acid (FFA) level was measured using the NEFA C-test kit (Wako, Osaka, Japan). Insulin concentration was analyzed using an enzyme-linked immunosorbent assay (ELISA, ultra-sensitive rat insulin ELISA kit, Morinaga, Kanagawa, Japan). Voided urine was collected and urine glucose levels were measured using urine test strips (Siemens Healthineers, Forchheim, Germany).

### Histopathological analysis

Cotton rats were euthanized by cutting the abdominal aorta. Their pancreas, visceral fat, and liver were fixed using 10% neutral-buffered formalin, followed by paraffin embedding. The sections were stained with hematoxylin and eosin (HE). Immunostaining was performed to detect glucagon (for α cell), insulin (for β cell), proliferating cell nuclear antigen (PCNA, for proliferating cell), 4-hydroxy-2-nonenal-modified proteins (4-HNE, for oxidative stress-related lipid peroxidation products), and Iba1 (for macrophages). The details of the procedures are listed in Table 1. In brief, the deparaffinized sections were heated for antigen retrieval and incubated with primary and secondary antibodies, as previously reported (Ichii et al. 2016, 2018; Nakamura et al. 2018). For immunohistochemistry, the color was developed

**Table 1** List of antibodies used for immunohistochemistry and immunofluorescence

| Antibody | Source | Dilution | Antigen retrieval | Application |
|---|---|---|---|---|
| Mouse anti-insulin antibody | Nichirei (Tokyo, Japan) | Prediluted | CB, 90 °C, 30 min | Primary Ab for IHC and IF |
| Rabbit anti-glucagon antibody | Nichirei | Prediluted | CB, 90 °C, 30 min | Primary Ab for IF |
| Goat anti-proliferating cell nuclear antigen | Santa Cruz Biochemistry (Santa Cruz, USA) | 1:2000 | CB, 90 °C, 30 min | Primary Ab for IF |
| Rabbit anti-4-hydroxy-2-nonenal-modified protein | Abcam (Cambridge, UK) | 1:200 | Not done | Primary Ab for IHC |
| Rabbit anti-Iba1 | Wako | 1:2000 | CB, 90 °C, 30 min | Primary Ab for IHC |
| Goat anti-rabbit IgG | Nichirei | Prediluted | – | Secondary Ab for IHC |
| Rabbit anti-mouse IgG | Nichirei | Prediluted | – | Secondary Ab for IHC |
| Rabbit anti-goat IgG | Nichirei | Prediluted | – | Secondary Ab for IHC |
| Alexa Fluor 488-labeled donkey anti-mouse IgG | Life Technologies (Carlsbad, USA) | 1:1000 | – | Secondary Ab for IF |
| Alexa Fluor 594-labeled donkey anti-rabbit IgG | Life Technologies | 1:1000 | – | Secondary Ab for IF |
| Alexa Fluor 488-labeled donkey anti-goat IgG | Life Technologies | 1:1000 | – | Secondary Ab for IF |
| Alexa Fluor 594-labeled donkey anti-mouse IgG | Life Technologies | 1:1000 | – | Secondary Ab for IF |

*CB*, 0.01 M citrate buffer (pH 6.0); *Ab*, antibody; *IHC*, immunohistochemistry; *IF*, immunofluorescence

by using the streptavidin-biotin method and 3,3′-diaminobenzidine tetrahydrochloride-$H_2O_2$ solution. The sections were counterstained with hematoxylin. Fluorescent signals were detected using BZ-X710 (Keyence, Osaka, Japan), and histometric analysis of images was performed using the BZ-H3A application software and hybrid Cell Count software (BZ-H3C, Keyence). 4-HNE scores in the islets were graded as follows: negative (score 0), weakly positive (lightly stained but clearly differentiated from negative background, score 1), moderately positive (densely stained areas at two regions, score 2), and strongly positive (densely stained areas at three or more regions, score 3) (Kanda et al. 2010).

### Statistical analysis

The results are expressed as mean ± standard error. Mann-Whitney *U* test was used to compare data between males and females of the same age. Kruskal-Wallis test was used for comparing the data among different age groups of the same sex, and multiple comparisons were performed using Scheffé's method when a significant difference was noted. Spearman's correlation test was used to analyze the correlation between two values.

## Results

### Association between biochemical alterations and metabolic disorders in cotton rats

First, we examined the metabolic parameters in cotton rats. Body weight of the cotton rats increased in adult and old age than in juvenile age of both sexes, and their values were within the reference range (Faith et al. 1997; Niewiesk and Prince 2002) (Fig. 1a). At adult and old ages, insulin levels were elevated in both sexes, although males showed remarkably higher values than females (Fig. 1b). Glucose levels increased in adult and old males but were unchanged with aging in females (Fig. 1c). On the other hand, urine glucose was negative (< 100 mg/dl) in all adult and old cotton rats in both sexes. Lipid parameters such as triglyceride, T-Cho, and non-HDL-Cho levels were higher in adult males than in juvenile males and age-matched females (Fig. 1d–f). Atherogenic index was higher in adult and old female cotton rats than that observed in juveniles (Fig. 1g). FFA and uric acid levels increased in an age-dependent manner in both sexes (Fig. 1h, i). We examined the correlation between insulin level, an indicator for insulin resistance, and other biochemical parameters (Table 2). In males, the level of serum insulin correlated with that of glucose as well as lipid parameters such as triglycerides, T-Cho, non-HDL-Cho, and atherogenic index. However, in females, serum insulin level correlated only with the levels of the aforementioned lipid parameters, but not glucose. Among the lipid parameters, triglyceride level showed the highest correlation with insulin level in both sexes.

### Histological changes in the islets of cotton rats

Based on the results of blood biochemistry, we histologically examined the islets containing insulin-producing β cells. In cotton rats, the glucagon$^+$ α cells localized at the periphery and the insulin$^+$ β cells localized in the central region of the islets were observed, as seen in mice and rats (Dolenšek et al. 2015; Tsuchitani et al. 2016) (Fig. 2(a)). The islet area, specifically the β-cell area, tended to increase with aging (Fig. 2(a, a′)). A significant increase in the islet area was observed with aging in

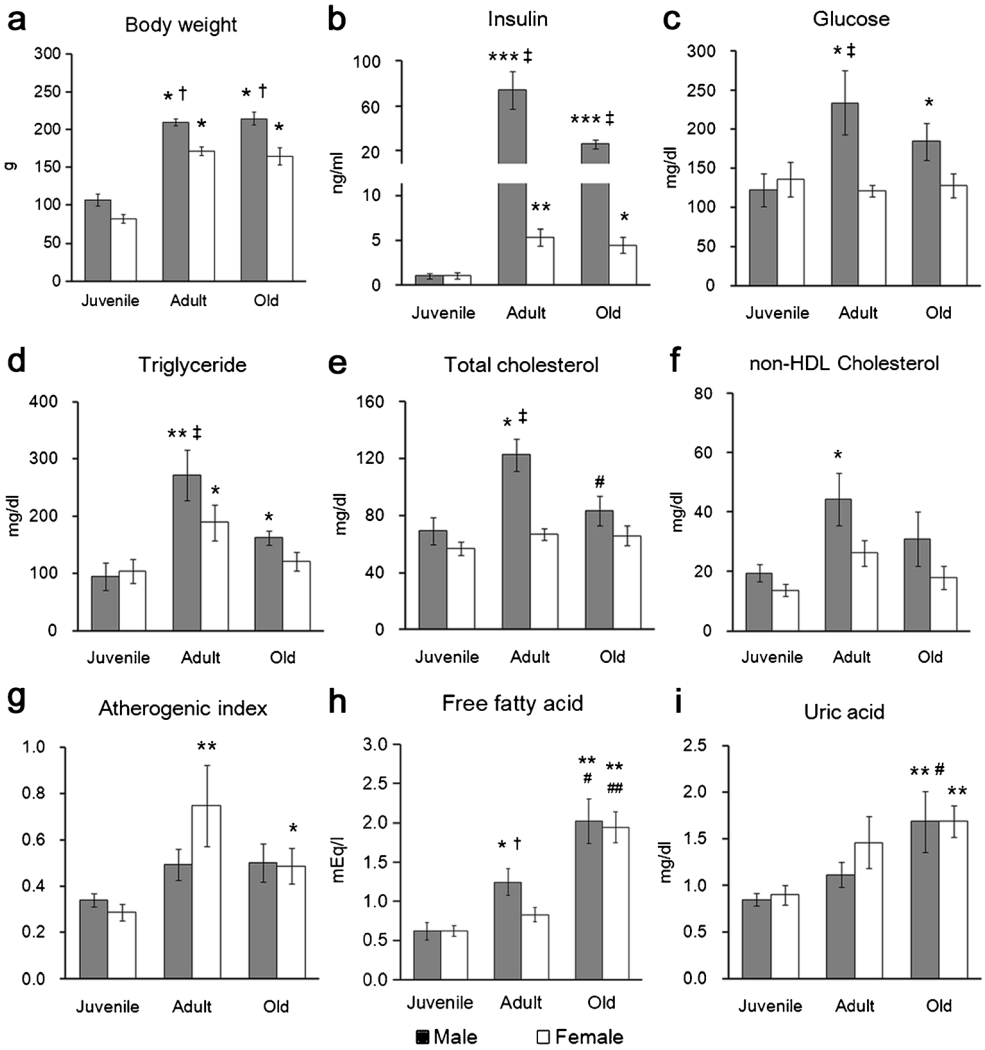


**Fig. 1** Blood biochemistry associated with metabolic parameters in cotton rats. **a** Body weight. **b** Insulin. **c** Glucose. **d** Triglyceride. **e** Total cholesterol. **f** Nonhigh-density lipoprotein cholesterol (non-HDL cholesterol). **g** Atherogenic index. **h** Free fatty acid. **i** Uric acid. Values = mean ± standard error. The number of animals is 5 or more in each group. Significant difference from the juveniles of the same sex is indicated by $^{*}P < 0.05$, $^{**}P < 0.01$, or $^{***}P < 0.001$. Significant difference between adults and old cotton rats of the same sex is indicated by $^{\#}P < 0.05$ or $^{\#\#}P < 0.01$. Significant sex-related difference in the same age is indicated by $^{\dagger}P < 0.05$ or $^{\ddagger}P < 0.01$

both sexes, and aged males had a larger islet area than age-matched females (Fig. 2(b)). Consistent with the increase in islet area, several β cells showed a positive reaction to PCNA (Fig. 2(c)). PCNA$^{+}$ β cells in the islets significantly increased with aging in males, but not in females (Fig. 2(d)). The insulin$^{+}$ β cells were also localized in the pancreatic duct (Fig. 2(e)).

In cotton rats with hyperglycemia, vacuolated cells were observed in the islets that were positive for insulin, suggesting these to be β cells (Fig. 2(f, f')). The islets of hyperglycemic animals showed stronger signals of 4-HNE, a marker for oxidative stress-related lipid peroxidation products. In particular, the cytoplasm

**Table 2** Correlation between serum insulin level and other biochemical parameters

| Biochemical parameters | Sex | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Glucose | Triglyceride | T-Cho | Non-HDL-Cho | Atherogenic index | Free fatty acid | Uric acid |
| Insulin | All | 0.544*** | 0.700*** | 0.598*** | 0.628*** | 0.317* | 0.488** | 0.250 |
| | Male | 0.477* | 0.795*** | 0.781*** | 0.808*** | 0.569** | 0.331 | 0.183 |
| | Female | 0.192 | 0.651** | 0.394 | 0.507* | 0.467* | 0.376 | 0.262 |

$n = 19$–$20$ (male cotton rats). $n = 21$–$22$ (female cotton rats). All examined ages (1–12 months) are included in these data. Spearman's rank correlation coefficient ($^{*}P > 0.05$, $^{**}P > 0.01$, $^{***}P > 0.001$)

*T-Cho*, total cholesterol; *HDL-Cho*, high-density lipoprotein cholesterol

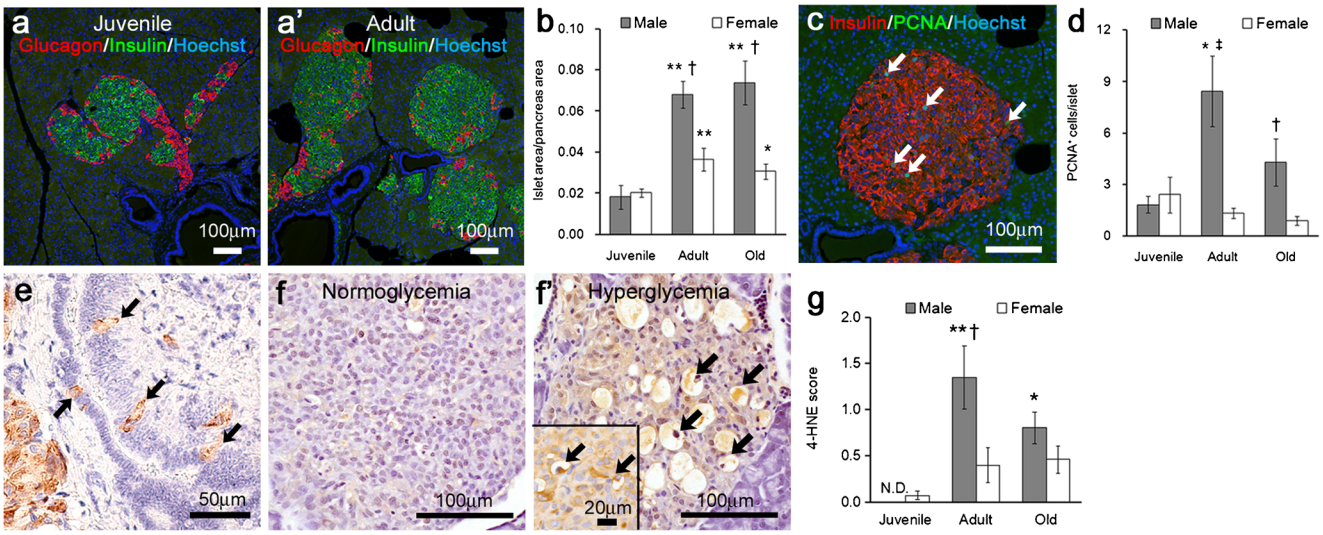


**Fig. 2** Histological changes in the islets of cotton rats. (a, a′) Immunofluorescence of glucagon and insulin in male cotton rats. (b) Age-related changes in the islet area. (c) Immunofluorescence of insulin and proliferating cell nuclear antigen (PCNA) in adult male cotton rats. Arrows indicate proliferating β cells. (d) Number of $PCNA^+$ β cells per islet. (e) Immunohistochemistry of insulin in the pancreatic duct of an adult male. Arrows indicate β cells localized in the pancreatic duct. (f, f′) Immunohistochemistry for 4-hydroxy-2-nonenal-modified proteins (4-HNE) in adult males. Inset indicates immunohistochemistry for insulin. Arrows indicate vacuolated cells. (g) 4-HNE scores in the islets. The number of animals is 5 or more in each group. Significant difference from the juveniles of the same sex is indicated by $^*P < 0.05$ or $^{**}P < 0.01$. Significant sex-related difference in the same age is indicated by $^{\dagger}P < 0.05$ or $^{\ddagger}P < 0.01$

of vacuolated cells was intensely stained for 4-HNE (Fig. 2(f, f′)). 4-HNE scores significantly increased in adult and old males but remained unchanged with aging in females (Fig. 2(g)).

## Cellular infiltration into visceral white adipose tissue without obesity

Accumulation of lipids and chronic inflammation in the visceral adipose tissue trigger insulin resistance (Boutens and Stienstra 2016). Therefore, we examined the relationship between visceral white adipose tissue and metabolic disorders in cotton rats. Macroscopic observation revealed that the visceral fat was obvious in both sexes and seemed to increase with aging. However, the visceral fat failed to surround the kidneys completely, even at an old age (Fig. 3(a–a‴)). Histological analysis revealed that adipocytes tended to increase in size in both sexes at adult and old ages (Fig. 3(b–b‴)). In adult and old males, inflammatory cells infiltrated into the visceral white adipose tissue and surrounded the adipocytes to form a crown-like structure (Fig. 3(b′, b″)). Fewer crown-like structures were found in females than in males of adult and old ages (Fig. 3(b‴, b⁗)). These crown-like structures comprised abundant $Iba1^+$ macrophages (Fig. 3(c)) and several neutrophils (Fig. 3(d)). The adipocytes increased in size in an age-dependent manner in both sexes and were reported to be larger in males than in females at adult and old ages (Fig. 3(e)). In males, the number of crown-like structures increased at adult and old ages, whereas in females, their numbers were unchanged with aging (Fig. 3(f)).

## Ectopic fat accumulation specifically in the pancreas

In addition to the chronic inflammation of the visceral white adipose tissue, ectopic fat accumulation is also associated with insulin resistance (Catanzaro et al. 2016). In the liver, fat accumulation was not observed in both sexes, although slight vacuolation was apparent in male cotton rats (Fig. 4(a, a′)). In addition, neither AST nor ALT levels were elevated with aging (Fig. 4(b, b′)).

On the other hand, the adipocytes accumulated in both intralobular and interlobular spaces of the pancreas in both sexes, and this accumulation tended to increase with age (Fig. 5(a–a⁗)). However, adipocytes showed no infiltration into the islets (Fig. 5(a–a⁗)). The adipose tissue area in the pancreas significantly increased with age in both sexes and was higher in males than in females at adult and old ages (Fig. 5(b)). In contrast, the exocrine area decreased with age in both sexes and the decrease was more prominent in males than in females at adult and old ages (Fig. 5(c)). Even at a juvenile age, acinar cells were compressed by the adjacent fat tissues and showed vacuolated features (Fig. 5(d)). Consistent with these findings, the level of serum lipase, a marker of damaged pancreas, decreased with age in both sexes (Fig. 5(e)).

## Correlation between biochemical and histopathological parameters

We examined the correlation between the biochemical parameters associated with metabolic disorders and histopathological parameters (Table 3). Triglyceride level was selected as the

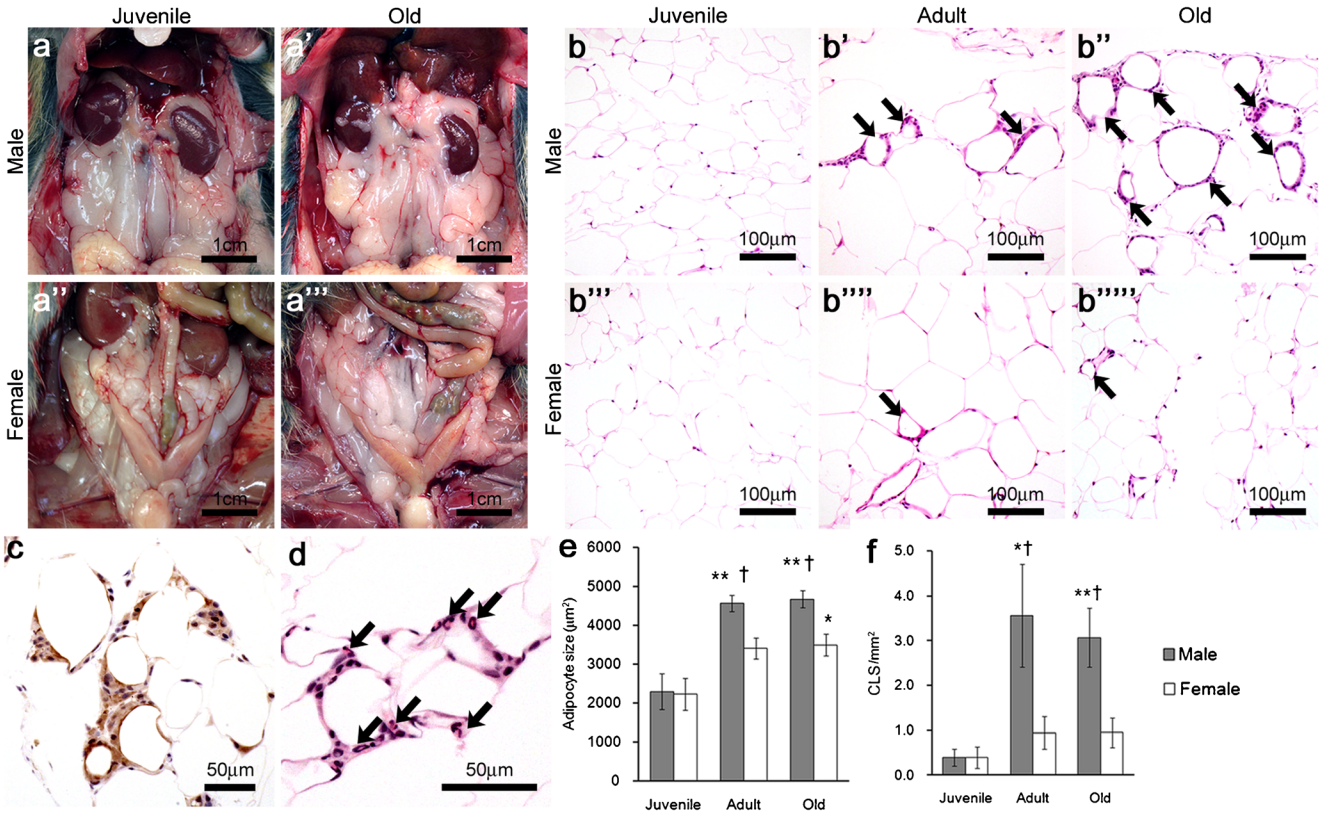


**Fig. 3** Cellular infiltration into the visceral white adipose tissue in cotton rats. (a–a‴) Gross anatomical features of the visceral adipose tissue of cotton rats. (b–b⁗) Histology of the visceral white adipose tissue of cotton rats. Arrows indicate cellular infiltration referred to as crown-like structures (CLS). HE staining. (c, d) Component cells of CLS. CLS mainly composed of Iba1$^{+}$ macrophages (c) and several neutrophils (d, arrows, HE staining). (e) Adipocyte size of the visceral white adipose tissue. (f) Number of CLS in the visceral white adipose tissue. Values = mean ± standard error. The number of animals is 5 or more in each group. Significant difference from the juveniles of the same sex is indicated by $^{*}P < 0.05$ or $^{**}P < 0.01$. Significant sex-related difference in the same age is indicated by $^{\dagger}P < 0.05$

lipid parameter, owing to its highest correlation with serum insulin level. In both sexes, serum insulin level was associated with the visceral adipocyte size and islet area. In contrast, several indices showed sex-associated differences. In males, serum insulin level was associated with crown-like structures and 4-HNE scores. However, in females, serum insulin level was associated with fat area and exocrine area in the pancreas. Glucose level was associated with visceral adipocyte size, 4-HNE score, fat area, and exocrine area in the pancreas only in males. Triglyceride level was associated with the islet area and 4-HNE scores in males and visceral adipocyte size in females.

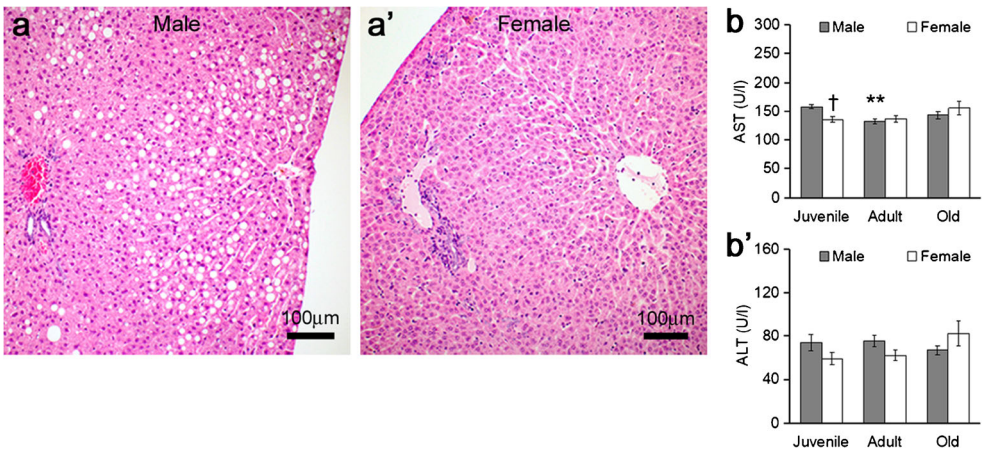


**Fig. 4** Hepatic histology in cotton rats. (a, a′) Histology of the liver in old cotton rats. (b, b′) Hepatic biochemical parameters in cotton rats. Values = mean ± standard error. The number of animals is 5 or more in each group. Significant difference from the juveniles of the same sex is indicated by $^{**}P < 0.01$. Significant sex-related difference in the same age is indicated by $^{\dagger}P < 0.05$

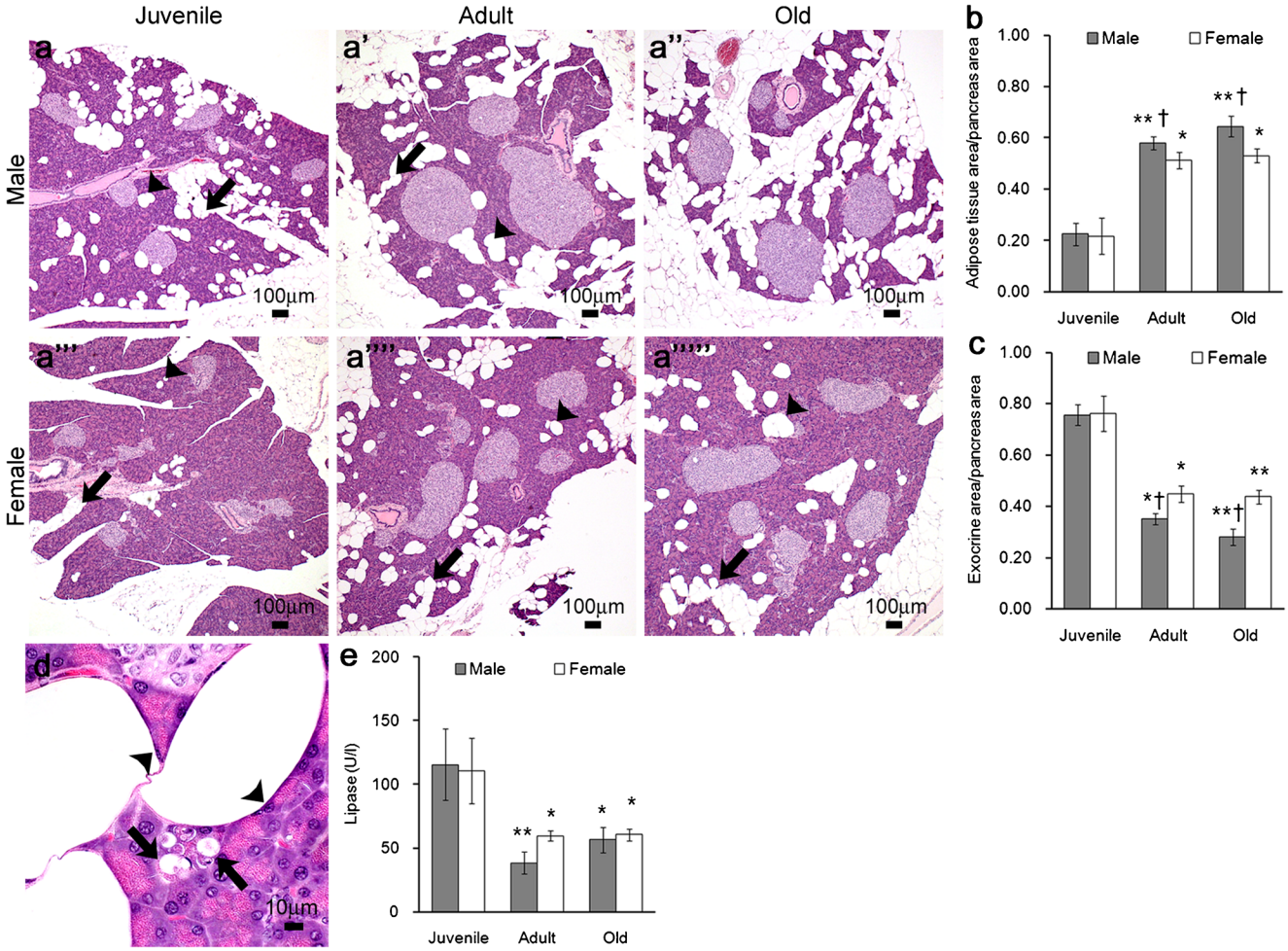


**Fig. 5** Pancreatic fat infiltration in cotton rats. (a–a'''') Histology of the pancreas in cotton rats. Arrow and arrowheads indicate adipocyte accumulation in interlobular and intralobular spaces, respectively. (b, c) Age-related changes in the area of adipose tissue (b) and exocrine part (c) in the pancreas. (d) Acinar cells of juvenile male cotton rats. Arrows and arrowheads indicate vacuolated and compressed acinar cells, respectively. (e) Serum lipase value in cotton rats. The number of animals is 5 or more in each group. Significant difference from the juveniles of the same sex is indicated by $^{*}P < 0.05$ or $^{**}P < 0.01$. Significant sex-related difference in the same age is indicated by $^{\dagger}P < 0.05$

## Discussion

Here, we demonstrate that cotton rats, especially males, developed metabolic disorders that manifested as hyperinsulinemia, mild hyperglycemia, and dyslipidemia. Absence of urinary glucose indicated that the cotton rats did not develop severe diabetes. The serum insulin level showed a positive association with lipid parameters in both sexes and with glucose levels in males, suggestive of the relationship between hyperinsulinemia and disruption of both glucose and lipid metabolism. Although hyperinsulinemia is also related to gout (Miao et al. 2009), serum insulin level showed no correlation with uric acid levels, suggesting a poor association between hyperinsulinemia and gouty in cotton rats.

Insulin resistance is the basis of metabolic disorders (Riant et al. 2009; Yao et al. 2014). In response to conditions characterized by an increase in insulin demand, such as insulin resistance and normal early postnatal growth, the islets increase in size and number through the proliferation of pre-existing β cells as well as neogenesis of β cells by the progenitor cells in the pancreatic duct (Gu and Sarvetnick 1993; Bock et al. 2003; Inada et al. 2008). In aged cotton rats, both proliferation and neogenesis of β cells were observed, strongly indicating compensatory hyperplasia of β cells against insulin resistance. In addition, aged male cotton rats predominantly developed hyperglycemia, and their β cells showed degeneration and elevated oxidative stress, which was associated with glucose levels. Oxidative stress plays a central role in glucose toxicity and dysfunction of β cells (Kooptiwut et al. 2017). Thus, these results indicate that male cotton rats exhibited progressed metabolic disorders.

In mice, rats, and humans, obesity and subsequent fat accumulation in the visceral adipose tissue induce local chronic inflammation characterized by the formation of crown-like structures and result in insulin resistance (Boutens and Stienstra 2016). In obese mice and rats, the exaggerated

**Table 3** Correlation between biochemical and histological parameters

| Biochemical parameters | Sex | Histological parameters | | | | | |
|---|---|---|---|---|---|---|---|
| | | Visceral white adipose tissue | | Islet | | Pancreas | |
| | | Adipocyte size | CLS | Islet area | 4-HNE score | Fat area | Exocrine area |
| Insulin | All | 0.809*** | 0.608*** | 0.762*** | 0.673*** | 0.738*** | − 0.813*** |
| | Male | 0.480* | 0.519* | 0.547* | 0.652** | 0.405 | − 0.379 |
| | Female | 0.710*** | − 0.144 | 0.520* | 0.275 | 0.696*** | − 0.740*** |
| Glucose | All | 0.496** | 0.410** | 0.270 | 0.552*** | 0.484** | − 0.487** |
| | Male | 0.503* | 0.076 | 0.121 | 0.828*** | 0.577** | − 0.525* |
| | Female | 0.233 | 0.273 | − 0.038 | 0.037 | 0.327 | − 0.252 |
| Triglyceride | All | 0.545*** | 0.317* | 0.567*** | 0.369* | 0.376* | − 0.431** |
| | Male | 0.397 | 0.402 | 0.560** | 0.610** | 0.156 | − 0.164 |
| | Female | 0.545** | − 0.024 | 0.381 | − 0.043 | 0.373 | − 0.364 |

$n = 19–20$ (male cotton rats). $n = 21–22$ (female cotton rats). All examined ages (1–12 months) are included in these data. Spearman's rank correlation coefficient (*$P > 0.05$, **$P > 0.01$, ***$P > 0.001$)

*CLS*, crown-like structure; *4-HNE*, 4-hydroxy-2-nonenal-modified protein

visceral fat surrounds the kidneys (Fraulob et al. 2010; Matsuda et al. 2014). In our study, cotton rats failed to develop severe obesity when fed a normal diet, because excess storage of the visceral fat was not observed and the body weight was within the reference range (Faith et al. 1997; Niewiesk and Prince 2002), and their kidneys were not surrounded by visceral fat. However, in males, visceral adipocytes were surrounded by macrophages and neutrophils, and the formation of crown-like structures was associated with hyperinsulinemia. These results indicate that histopathological alterations of visceral white adipose tissue induced hyperinsulinemia without obesity in aged male cotton rats.

In contrast to male cotton rats, females exhibited milder metabolic disorders and histopathological changes in both the islets and the visceral adipose tissue. Clinical and experimental studies have revealed that estrogen attenuates oxidative stress of β cells, prevents fat accumulation in visceral adipose tissue, improves insulin sensitivity, and exerts protective effect against metabolic disorders (Kooptiwut et al. 2017; Riant et al. 2009). In female cotton rats, milder metabolic disorders may be similarly attributed to estrogen, although further studies must address the contribution of estrogen to these phenotypes. Nevertheless, aged female cotton rats showed higher serum insulin levels without both obesity and histopathological changes in the visceral white adipose tissue, suggesting that other factors may affect insulin resistance in cotton rats.

Apart from the fat accumulation in the white adipose tissue, ectopic fat in nonadipose tissues such as the liver, skeletal muscle, heart, and pancreas is related to insulin resistance (Sattar and Gill 2014). In cotton rats, we failed to observe a remarkable level of lipids in the liver or elevated levels of biochemical markers for hepatic injury, suggesting that the liver had poor contribution to metabolic disorders. Fat accumulation in the pancreas has been referred as various synonyms such as pancreatic steatosis, pancreatic lipomatosis, fatty pancreas, lipomatous pseudohypertrophy, fatty replacement, fatty infiltration, and nonalcoholic fatty pancreas disease (NAFPD) (Catanzaro et al. 2016; Nolte et al. 2016). For cotton rats, we thought that NAFPD, defined as fat accumulation associated with obesity and metabolic disorders, was the most appropriate terminology, as the phenotype was associated with hyperglycemia in males and hyperinsulinemia in females. Obesity has been recognized as the most important risk factor for developing NAFPD (Catanzaro et al. 2016). However, patients with lipodystrophy also develop ectopic fat stores (Lanktree et al. 2010). NAFPD is related to insulin resistance in prediabetes, but not progressed type 2 diabetes in humans. However, these observations are still controversial (Tushuizen et al. 2007; Lingvay et al. 2009; Catanzaro et al. 2016). Fat accumulation in the pancreas promotes acute/chronic pancreatitis and pancreatic cancer (Catanzaro et al. 2016). Thus, the pathogenesis and clinical significance of NAFPD is yet to be fully elucidated in humans, and appropriate animal models are needed for the elucidation of mechanisms underlying NAFPD. In female cotton rats that developed mild hyperinsulinemia without obesity and chronic inflammation in the visceral white adipose tissue, NAFPD showed a significant positive association with serum insulin, suggesting that it is related to insulin resistance in cotton rats without obesity. In male cotton rats presenting progressive metabolic diseases, NAFPD was associated with blood glucose but not with insulin levels. These data may reflect that NAFPD had no correlation with β-cell function in progressed diabetes in humans (Tushuizen et al. 2007; Lingvay et al. 2009). Acinar cells located in the vicinity of adipocytes

showed degenerated features and serum lipase level decreased with age, suggestive of impaired endocrine functions of the pancreas. Taken together, cotton rats may be useful for the elucidation of the pathogenesis and mechanisms of NAFPD in humans.

In conclusion, we found that cotton rats, especially males, develop metabolic disorders without obesity. Although it is unclear whether wild cotton rats develop these diseases, the candidate molecules derived from genetic characteristics would be indicators of these phenotypes in laboratory cotton rats. Whole-genome sequencing and other hormone levels in serum may be useful tools for cotton rats because mRNAs and amino acid sequences are insufficient. In conclusion, cotton rats may serve as a novel and useful model for metabolic disorders with ectopic fat accumulation, specifically in the pancreas, in the absence of obesity.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving animals were in accordance with the ethical standards of Hokkaido Institute of Public Health (approval No. K27-03). This article does not contain any studies with human participants performed by any of the authors.

## References

Azazy AA, Chance ML, Devaney E (1997) A time-course study of circulating antigen and parasite-specific antibody in cotton rats infected with Leishmania donovani. Ann Trop Med Parasitol 91:153–162

Blanco JC, Boukhvalova MS, Perez DR, Vogel SN, Kajon A (2014) Modeling human respiratory viral infections in the cotton rat (*Sigmodon hispidus*). J Antivir Antiretrovir 6:40–42

Bock T, Pakkenberg B, Buschard K (2003) Increased islet volume but unchanged islet number in ob/ob mice. Diabetes 52:1716–1722

Boutens L, Stienstra R (2016) Adipose tissue macrophages: going off track during obesity. Diabetologia 59:879–894

Catanzaro R, Cuffari B, Italia A, Marotta F (2016) Exploring the metabolic syndrome: nonalcoholic fatty pancreas disease. World J Gastroenterol 22:7660–7675

Dolenšek J, Rupnik MS, Stožer A (2015) Structural similarities and differences between the human and the mouse pancreas. Islets 7: e1024405

Faith RE, Montgomery CA, Durfee WJ, Aguilar-Cordova E, Wyde PR (1997) The cotton rat in biomedical research. Lab Anim Sci 47:337–345

Fraulob JC, Ogg-Diamantino R, Fernandes-Santos C, Aguila MB, Mandarim-de-Lacerda CA (2010) A mouse model of metabolic syndrome: insulin resistance, fatty liver and non-alcoholic fatty pancreas disease (NAFPD) in C57BL/6 mice fed a high fat diet. J Clin Biochem Nutr 46:212–223

Gu D, Sarvetnick N (1993) Epithelial cell proliferation and islet neogenesis in IFN-gamma transgenic mice. Development 118: 33–46

Ichii O, Nakamura T, Irie T, Kouguchi H, Nakamura D, Nakamura S, Sato S, Yokoyama K, Horino T, Sunden Y, Elewa YH, Kon Y (2016) Female cotton rats (*Sigmodon hispidus*) develop chronic anemia with renal inflammation and cystic changes. Histochem Cell Biol 146:351–362

Ichii O, Nakamura T, Irie T, Kouguchi H, Sotozaki K, Horino T, Sunden Y, Elewa YHA, Kon Y (2018) Close pathological correlations between chronic kidney disease and reproductive organ-associated abnormalities in female cotton rats. Exp Biol Med 243:418–427

Inada A, Nienaber C, Katsuta H, Fujitani Y, Levine J, Morita R, Sharma A, Bonner-Weir S (2008) Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. Proc Natl Acad Sci U S A 105:19915–19919

Kanda Y, Shimoda M, Hamamoto S, Tawaramoto K, Kawasaki F, Hashiramoto M, Nakashima K, Matsuki M, Kaku K (2010) Molecular mechanism by which pioglitazone preserves pancreatic β-cells in obese diabetic mice: evidence for acute and chronic actions as a PPARγ agonist. Am J Physiol Endocrinol Metab 298: E278–E286

Kayamori F, Igarashi K (1994) Effects of dietary nasunin on the serum cholesterol level in rats. Biosci Biotechnol Biochem 58:570–571

Kooptiwut S, Wanchai K, Semprasert N, Srisawat C, Yenchitsomanus PT (2017) Estrogen attenuates AGTR1 expression to reduce pancreatic β-cell death from high glucose. Sci Rep 7:16639

Kroeze WK, Tanner CE (1985) *Echinococcus multilocularis*: responses to infection in cotton rats (*Sigmodon hispidus*). Int J Parasitol 15: 233–238

Lanktree MB, Johansen CT, Joy TR, Hegele RA (2010) A translational view of the genetics of lipodystrophy and ectopic fat deposition. Prog Mol Biol Transl Sci 94:159–196

Lingvay I, Esser V, Legendre JL, Price AL, Wertz KM, Adams-Huet B, Zhang S, Unger RH, Szczepaniak LS (2009) Noninvasive quantification of pancreatic fat in humans. J Clin Endocrinol Metab 94: 4070–4076

Matsuda A, Makino N, Tozawa T, Shirahata N, Honda T, Ikeda Y, Sato H, Ito M, Kakizaki Y, Akamatsu M, Ueno Y, Kawata S (2014) Pancreatic fat accumulation, fibrosis, and acinar cell injury in the Zucker diabetic fatty rat fed a chronic high-fat diet. Pancreas 43: 735–743

Miao Z, Yan S, Wang J, Wang B, Li Y, Xing X, Yuan Y, Meng D, Wang L, Gu J, Zhang S, Li C, Wang CY (2009) Insulin resistance acts as an independent risk factor exacerbating high-purine diet induced renal injury and knee joint gouty lesions. Inflamm Res 58:659–668

Nakamura T, Ichii O, Irie T, Mizoguchi T, Shinohara A, Kouguchi H, Sunden Y, Otsuka-Kanazawa S, Elewa YHA, Koshimoto C, Nagasaki KI, Kon Y (2018) Cotton rats (*Sigmodon hispidus*) possess pharyngeal pouch remnants originating from different primordia. Histol Histopathol 33:555–565

Niewiesk S, Prince G (2002) Diversifying animal models: the use of hispid cotton rats (*Sigmodon hispidus*) in infectious diseases. Lab Anim 36:357–372

Nolte T, Brander-Weber P, Dangler C, Deschl U, Elwell MR, Greaves P, Hailey R, Leach MW, Pandiri AR, Rogers A, Shackelford CC, Spencer A, Tanaka T, Ward JM (2016) Nonproliferative and proliferative lesions of the gastrointestinal tract, pancreas and salivary glands of the rat and mouse. J Toxicol Pathol 29:1S–125S

Riant E, Waget A, Cogo H, Arnal JF, Burcelin R, Gourdy P (2009) Estrogens protect against high-fat diet-induced insulin resistance and glucose intolerance in mice. Endocrinology 150:2109–2117

Sattar N, Gill JM (2014) Type 2 diabetes as a disease of ectopic fat? BMC Med 12:123

Tajima S, Horiuchi S (1989) Biological reference data book on experimental animals, 1st ed (Japanese). Soft Science, Tokyo

Tsuchitani M, Sato J, Kokoshima H (2016) A comparison of the anatomical structure of the pancreas in experimental animals. J Toxicol Pathol 29:147–154

Tushuizen ME, Bunck MC, Pouwels PJ, Bontemps S, Van Waesberghe JH, Schindhelm RK, Mari A, Heine RJ, Diamant M (2007) Pancreatic fat content and β-cell function in men with and without type 2 diabetes. Diabetes Care 30:2916–2921

Waldum HL, Rørvik H, Falkmer S, Kawase S (1999) Neuroendocrine (ECL cell) differentiation of spontaneous gastric carcinomas of cotton rats (*Sigmodon hispidus*). Lab Anim Sci 49:241–247

Wang MY, Grayburn P, Chen S, Ravazzola M, Orci L, Unger RH (2008) Adipogenic capacity and the susceptibility to type 2 diabetes and metabolic syndrome. Proc Natl Acad Sci U S A 105:6139–6144

Wellen KE, Fucho R, Gregor MF, Furuhashi M, Morgan C, Lindstad T, Vaillancourt E, Gorgun CZ, Saatcioglu F, Hotamisligil GS (2007) Coordinated regulation of nutrient and inflammatory responses by STAMP2 is essential for metabolic homeostasis. Cell 129:537–548

Yao L, Herlea-Pana O, Heuser-Baker J, Chen Y, Barlic-Dicen J (2014) Roles of the chemokine system in development of obesity, insulin resistance, and cardiovascular disease. J Immunol Res 181450