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Usefulness of Obese Animal Models in Antiobesity Drug Development

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Abstract

Obese animal models have played key roles to elucidate the etiology of obesity and develop antiobesity drugs. In the first half of the chapter, we introduce the characteristics of obese animal models. In the second half of the chapter, we show the results of pharmacological studies using obese animal models for new antiobesity drugs.

Keywords: animal model, diabetes, obesity

1. Introduction

The number of obese patients is rapidly increasing, due to the change of lifestyle, such as eating habits of high calorie-diet and sedentary life. Obesity and the obesity-related diseases, such as diabetes mellitus, dyslipidemia, and hypertension, are risk factors for several severe diseases, including cardiovascular disease and cancer, and deteriorate the quality of life (QOL) of patients and result in high medical expenses [1, 2]. Moreover, nonalcoholic fatty liver disease (NAFLD) is recently well recognized as the most common chronic liver disease, and the NAFLD is strongly associated with obesity and the related diseases [3, 4]. In Western countries, 4–22% of NAFLD patients lead to hepatocellular carcinoma [5]. Metabolic abnormalities based on obesity, such as hyperinsulinemia, dyslipidemia, and ectopic lipid accumulation, induce the various complications including microangiopathy and nonalcoholic steatohepatitis (NASH).

Obesity is considered to be caused by an imbalance in individual energy, and energy homeostasis in body is maintained by a balance between energy intake and energy expenditure. When the former exceeds the latter, overt energy is accumulated in adipose tissue and resulting in
The basic therapies for obesity are appropriate dietary restriction for the purpose of decreasing energy intake and effective exercise for the purpose of promoting energy expenditure. The lifestyle modifications, such as diet therapy and exercise, mainly occupy the treatments for obesity; however, medical therapy is performed on patients who do not show weight loss effect by the lifestyle modifications.

Medical therapy is a fundamental step in reducing the accumulation of excess fat. To reduce excess fat accumulation and excess body weight, antiobesity drugs that reduce lipid absorption in the intestine or appetite have been developed. In past years, centrally acting drugs, such as phentermine, mazindol, and fenfluramine, had been approved as antiobesity drugs, but the drugs have since been withdrawn in the USA and Europe [7, 8]. Mazindol is now available only in Japan [9]. In the 1990s, another type of antiobesity drug, orlistat, which inhibits lipid absorption in the intestine, was approved in the USA and Europe and is now also available [10]. Thereafter, sibutramine and rimonabant were developed; however, both drugs were withdrawn because of adverse effects [11]. Development of drug combinations, such as qsymia and contrave, has been recently promoted [12], and serotonin (5HT2c)-R agonist lorcaserin was accepted by the FDA in 2012 [13]. In addition, a variety of drugs with various mechanisms, such as protein tyrosine phosphatase (PTP) IB inhibitors, microsomal triglyceride transfer protein (MTP) inhibitors, diacylglycerol acyltransferase (DGAT) 1 inhibitors, and monoacylglycerol acyltransferase (MGAT) inhibitors, have been investigated in clinical and basic stages [14–19].

Animal models have played important roles in the development of these antiobesity drugs. Obese animal models are essential to elucidate the etiology for the drug development. In the first half of this chapter, we introduce the characteristics of obese animal models. Obese animal models are divided into two types: genetic and nongenetic models. An overview of the pathophysiological features, such as body weight, blood chemical parameters, and histopathology of microangiopathy, is presented for both types. Moreover, an obese model is expected to be used as a NASH model. An overview of the development of NASH-like hepatic lesions in each model is also presented. In the second half of this chapter, results of pharmacological studies using the obese animal models for new antiobesity drugs are shown. The pharmacological effects were investigated using both genetic and nongenetic animal models.

2. Obese animal model

2.1. Genetic mouse model

2.1.1. ob/ob mouse

Lepr<sup>ob</sup> mutation on chromosome 6 was discovered at the Jackson laboratory in a multiple recessive stock in 1949 [20], and the Lepr<sup>ob</sup> mutation was subsequently transferred to B6 inbred strain background. Lepr<sup>ob</sup> mutation on the B6 background (ob/ob) mice shows obesity, hyperinsulinemia, and relatively mild hyperglycemia.
Body weights in ob/ob mice significantly increased as compared with those in lean mice at 7 weeks of age (mean values; ob/ob mice, 44.0 g vs. lean mice, 23.1 g). The body weights periodically increased, reaching a maximum level of approximately 55 g at 11 weeks of age. With overt obesity, blood insulin levels in ob/ob mice also significantly increased as compared with those in lean mice. The blood insulin levels in ob/ob mice showed a remarkable increase at 7 weeks of age (mean values; ob/ob mice, 23.7 ng/ml vs. lean mice, 4.2 ng/ml). Blood glucose levels in ob/ob mice increased as compared with those in lean mice from 7 to 11 weeks of age, but the levels decreased with aging and normalized after 12 weeks of age. Since the pancreatic islets in ob/ob mice have a proliferative activity with an increase of blood insulin levels, the hyperglycemia is improved with aging. Moreover, ob/ob mice show the overt fat accumulation with hyperphagia. In ob/ob mice, the de novo lipogenesis and the hepatic fatty acid synthesis are significantly elevated [21].

The ob/ob mice fed a standard diet show fatty liver, but do not represent NASH-like lesion. NASH-like lesion is induced in ob/ob mice by methionine-choline deficient (MCD) and high-fat (HF) diets [22, 23].

2.1.2. db/db mouse

In 1966, a recessive Lepr<sup>db</sup> mutation (db/db) was found on chromosome 4 in C57BL KS/J inbred strain [24]. The db/db mouse was produced by backcrossing among the C57BL KS/J inbred strains.

db/db mice show a development of obesity after weaning, but the metabolic abnormalities, including hyperglycemia, are more severe as compared with those in ob/ob mice. Body weights in db/db mice significantly increased as compared with those in lean mice at 7 weeks of age (mean values; db/db mice, 42.4 g vs. lean mice, 28.4 g). The body weights periodically increased, reaching a maximum level of approximately 50 g at 11 weeks of age. The degree of weight gain in db/db mice was mild as compared with that in ob/ob mice. With obesity, the blood insulin levels in db/db mice increased as compared with those in lean mice at 7 weeks of age (mean values; db/db mice, 10.8 ng/ml vs. lean mice, 3.2 ng/ml). However, the insulin levels decreased gradually with aging, and the level in db/db mice at 11 weeks of age was comparable with that in lean mice. Blood glucose level at 7 weeks of age in db/db mice was about 700 mg/dl, and the hyperglycemia is sustained over the life span. The fluctuation in blood insulin and glucose levels is associated with the pancreatic β cell mass in db/db mice.

In examination of renal lesions in db/db mice, the creatinine clearance decreases after 20 weeks of age, and the substantial glomerular changes, such as albuminuria, mesangial area enlargement, and basement membrane thickening, are observed [25]. There are some reports of neuropathy and retinopathy in db/db mice [26, 27]. Impaired motor nerve conduction velocities (MNCV) are observed during the early phase of the diabetic syndrome. In morphological studies, db/db mice show loss or shrinkage of myelinated fibers in sural nerve and ventral root, and axonal atrophy after 25 weeks of age [28]. In the retina, pathological changes, such as loss of pericytes, acellular capillaries, and blood-retinal barrier breakdown, are observed.
The db/db mice fed a standard diet show fatty liver, but do not represent NASH-like lesion. Like ob/ob mice, NASH-like lesion is induced in ob/ob mice by methionine-choline deficient (MCD) and high-fat (HF) diets [29, 30].

2.1.3. KKA\(^y\) mouse

KK mouse, which is a spontaneously diabetic model, was established by Kondo et al. [31]. Furthermore, Nakamura et al. established KKA\(^y\) mouse, which is an obese diabetic model, by introducing the yellow obese gene (A\(^y\)) into the KK mice [32, 33].

In KKA\(^y\) mice, metabolic abnormalities, such as obesity, hyperinsulinemia, and hyperglycemia, are observed from 6 weeks of age, but the abnormalities are improved with aging [34].

Glomerular lesions, such as glomerulosclerosis, glomerular basement membrane (GBM) thickening, and nodular-like changes, are observed after 16 weeks of age [35]. Moreover, in retina of KKA\(^y\) mice, the apoptosis cell number for retinal neural cells in the ganglion cell layer increased with aging [36].

It is reported that NASH-like lesions are observed in KKA\(^y\) mice fed a MCD diet [37].

2.1.4. Tsumura Suzuki obese diabetics (TSOD) mouse

In 1992, two inbred strains: Tsumura Suzuki obese diabetics and Tsumura Suzuki nonobese (TSNO) mice were established by selective breeding of obese mice in ddy strain [38, 39].

In the male TSOD mice, metabolic abnormalities, such as hyperinsulinemia, hyperglycemia, and dyslipidemia, are developed with the increase of body weight. In the examination of pancreatic islets, the hypertrophy is observed with the increase in number of \(\beta\) cells and the degranulation of \(\beta\) cells [38].

In histopathological analyses in kidney, glomerular lesions, such as GBM thickening and mesangial area enlargement, are observed after 18 weeks of age [40]. The sensory neuropathy is observed after 12 months of age, and the motor neuropathy is also shown after 14 months of age. In histological analyses in sciatic nerves, a decrease in the density of nerve fibers is observed after 18 months of age. Moreover, the degenerative changes of myelinated fibers and the separation of myelin sheaths are observed with intralamellar edema and remyelination. Retinal lesions in TSOD mice are not reported.

2.2. Genetic rat model

2.2.1. Zucker fatty (ZF) rat

Zucker rats were originally bred to be a genetic model for research on obesity and hypertension. Two types of Zucker rat: a lean Zucker rat, denoted as the dominant trait (Fa/Fa) or (Fa/fa); and the characteristically obese Zucker rat (ZF) rat, which is actually a recessive trait (fa/fa) of the leptin receptor [41, 42]. ZF rats show overt obesity with hyperphagia (mean ± standard deviation in body weights at 12 weeks of age: ZF rats, 476.4 ± 39.4 g vs. lean rats, 306.9
± 21.9 g; mean ± standard deviation in body weights at 18 weeks of age; ZF rats, 634.8 ± 50.3 g vs. lean rats, 409.9 ± 33.5 g), and hyperinsulinemia (mean ± standard deviation in body weights at 6 weeks of age: ZF rats, 8.9 ± 1.9 ng/ml vs. lean rats, 1.1 ± 0.3 ng/ml; mean ± standard deviation in body weights at 18 weeks of age: ZF rats, 29.8 ± 12.4 ng/ml vs. lean rats, 2.7 ± 1.2 ng/ml). Blood glucose levels in ZF rats were comparable to those in lean mice from 8 to 12 weeks of age, but the glucose levels in ZF rats slightly increased as compared with those in lean rats after 12 weeks of age.

There are some reports of microangiopathy in ZF rats. Renal lesions, such as glomerular area expansion and tubular cast accumulation are observed at 24 weeks of age in ZF rats [43]. Decreased hind limb pressure pain threshold is an early indicator of insulinopenia and neuropathy, and the decreased pressure pain threshold is observed at 10 weeks of age in ZF rats [44]. Histological changes in retina of ZF rats are not reported, but some markers of inflammation, including nuclear factor (NF)-κβ, increased in the retina [45].

There are few reports of NASH-like lesions in ZF rats. Obese and hypertensive SHRSP-ZF rats treated with a high fat diet and carbon tetrachloride show the pathophysiological and histopathological characteristics of NASH [46].

2.2.2. Zucker diabetic fatty (ZDF) rat

Zucker diabetic fatty rats derived from the ZF strain exhibit obesity with diabetes. It is reported that characteristics of the male ZDF rat maintained on Purina 5008 diet include obesity, hyperinsulinemia, and hyperglycemia beginning at 6–7 weeks of age [47]. Also, in our study, obesity and hyperinsulinemia were observed at 6 weeks of age (mean ± standard deviation in body weights: ZDF rats, 221.8 ± 9.3 g vs. lean rats, 157.6 ± 5.0 g; mean ± standard deviation in insulin levels; ZDF rats, 23.1 ± 4.3 ng/ml vs. lean rats, 1.1 ± 0.3 ng/ml). By 14 weeks of age, blood glucose levels steadily increase, reaching an average of approximately 800 mg/dl. Since the ZDF rats develop diabetes, the degree of obesity in ZDF rats is mild as compared with that in ZF rats (body weights at 9 weeks of age: ZDF rats, 314.3 ± 10.7 g vs. ZF rats, 414.6 ± 19.2 g vs. lean rats, 277.6 ± 11.9 g; body weights at 13 weeks of age: ZDF rats, 388.3 ± 17.7 g vs. ZF rats, 572.5 ± 33.2 g vs. lean rats, 352.0 ± 15.8 g).

In examination of renal lesion, pathological changes, such as glomerulosclerosis and tubulointerstitial scarring/inflammation are observed. ZDF rats at 8 weeks of age show neither glomerulosclerosis nor evidence of tubulointerstitial lesions. Renal hypertrophy is slightly observed at 12 weeks of age, and the renal hypertrophy is more prominent by 16 weeks of age [48]. Glomerulosclerosis commences after 20 weeks of age, and is associated with glomerular hypertrophy and mild mesangial expansion with podocyte injury [49]. Furthermore, tubulointerstitial scarring and inflammation are observed in ZDF rats at 22 weeks of age [50]. In ZDF rats, the retinal capillaries demonstrated hypercellularity, and the retinal capillary basement membrane thickness revealed thicker membrane as compared with lean rats [51, 52]. The blood-retinal barrier is broken at 26 weeks of age in ZDF rats, and the inflammatory state and cell death by apoptosis in retina are observed [53]. In examination of sciatic nerve functions in ZDF rats, motor nerve conduction velocity (MNCV) decreases after 12 weeks of age, and vascular relaxation of sciatic nerve is impaired after 8 weeks of age [54, 55]. In the
histological analyses from 24 to 28 weeks of age after the onset of diabetes, ZDF rats do not represent sympathetic neuroaxonal dystrophy [56].

ZDF rats show fatty liver with insulin resistance, but the NASH-like lesions are not reported.

2.2.3. Otsuka Long-Evans Tokusima fatty (OLETF) rat

Otsuka Long-Evans Tokushima fatty rats show an impaired glucose tolerance from 8 weeks of age, and the plasma glucose level becomes higher from 18 weeks of age [57]. In kidney of OLETF rats, pathological changes such as diffuse glomerulosclerosis and nodular lesion are observed [58]. The proliferation in mesangial cells is observed at 25 weeks of age, and the mesangial area enlargement is observed with extracellular matrix accumulation and GBM thickening after 40 weeks of age. Nodular-like lesions are observed after 65 weeks of age, and the lesions expand to the proliferated mesangial area. Tubular interstitial lesions, such as mononuclear cell filtration and fibrosis, are also observed. In the retinal capillaries after 56 weeks of age, the basement membranes are thicker, and the ratio of pericyte area decreases [59]. Regarding cataract, slight lens fiber swelling is observed in the anterior and/or posterior subcapsular regions at 40 weeks of age in OLETF rats [60]. In examination of peripheral nerve functions in OLETF rats, MNCV tends to decrease after about 40 weeks of age as compared with lean rats [61].

In NASH-like lesions of OLETF rats, there are some reports of MCD diet-induced steatohepatitis [62, 63]. The steatohepatitis is accelerated in OLETF rats after 8 weeks fed MCD diet. Furthermore, the MCD + HF diet leads to rapid development of precirrhosis in OLETF rats.

2.2.4. Wistar fatty rat

In Wistar fatty rats, glucose intolerance accompanied by exaggerated insulin secretion and an increase of basal plasma glucose level are observed at 8 weeks of age, and an increase of basal plasma insulin level also increased at 14 weeks of age [64].

Kidney enlargement and glomerular hypertrophy are observed at 20 and 42 weeks of age in Wistar fatty rats [65]. In histopathological analyses, glomerular lesions, including mesangial area enlargement and tubular lesions, are observed. Intercellular adhesion molecular (ICAM)-1 expression on the glomeruli is significantly observed at 15 weeks of age, and progresses further at 29 weeks of age [66]. The other complications, such as retinopathy, neuropathy, and NASH, are not reported in Wistar fatty rats.

2.2.5. Spontaneously Diabetic Torii (SDT) fatty rat

Spontaneously Diabetic Torii fatty rats of both sexes show a significant hyperphagia and obesity after weaning, and especially, the increase of body weight in female rats is remarkable. In the male SDT fatty rats, the blood insulin levels increase after weaning, but the insulin levels decrease after 16 weeks of age. The female SDT fatty rats show hyperinsulinemia from 4 to 8 weeks of age, and the insulin levels decrease with aging. Serum glucose levels in SDT fatty rats of both sexes are elevated from 6 weeks, and the hyperglycemia is sustained for a long time afterwards.
With early incidence of diabetes mellitus, diabetic complications, such as nephropathy, retinopathy, and neuropathy, are observed at younger ages than the SDT rats [67, 68]. In histopathological analyses of the male rats, tubular lesions are observed after 8 weeks of age, and glomerular lesions are also observed after 16 weeks of age [67]. The glomerulosclerosis are observed from 16 weeks of age, and the nodular-like lesions are observed at 40 weeks of age. The renal tubular lesions, such as Armanni-Ebstein lesions and tubular dilation, are observed from 8 weeks of age. In histopathological analyses of the female rats, tubular lesions are observed from 16 weeks of age, and glomerular lesions are also observed from 32 weeks of age [68]. In lens of the male rats, histopathological changes, such as hyperplasia of epithelium and vacuolation of fiber, are observed after 8 weeks of age. Similar changes are observed after 16 weeks of age in the female SDT fatty rats. The male and female SDT fatty rats show the retinal lesions, such as folding and thickening, after 40 weeks of age [67, 68]. The decrease in caudal MNCV is observed at 24 weeks of age in the male SDT fatty rats [67]. In histopathological analyses, the male rats show the decrease in fiber number and the atrophy in myelinated nerve at 40 weeks of age.

It is reported that female SDT fatty rats fed a standard diet develop HASH-like hepatic lesions [4]. Hepatic lipid content significantly increases in female SDT fatty rats from 8 to 32 weeks of age. Histopathologically, severe hepatosteatosis accompanied by inflammation was observed from 8 weeks of age, and fibrosis started to occur at 32 weeks of age (Figure 1). Female SDT fatty rats have the potential to become an important animal model of NASH with diabetes and obesity.

**Figure 1.** Histological analysis of liver in female Spontaneously Diabetic Torii fatty and Sprague-Dawley (SD) rats [4]. Liver sections are from SDT fatty at 32 weeks of age (A, B) and SD at 40 weeks of age (C, D). Hematoxylin and eosin (HE) stain (A, C) and Sirius Red stain (B, D). Bar = 200 µm.

### 2.2.6. cp/cp rat

The LA/N-corpulent (LA/N-cp) rat is a normotensive strain derived from Koltesky’s original mutant strain of the spontaneously hypertensive rat (SHR). When homozygous for the cp gene (cp/cp), the rats are hyperphagous, obesity, hyperinsulinemia, hyperglycemia, and dyslipide-
mia [69]. The levels of body weight, systolic blood pressure, serum TG and blood glucose in cp/cp rats being 1.43, 1.65, 25.4, and 1.25 times, respectively, compared with those in control rats, Wistar Kyoto rats at 19 or 20 weeks of age [70].

Renal lesions, including glomerular and tubular changes, are observed in cp/cp rats, from 24 to 36 weeks of age [71]. In light microscopy, cp/cp rats develop glomerular lesions, characterized by glomerular hypertrophy, mesangial expansion, and focal and segmental glomerular sclerosis. Also, interstitial lesions, such as tubular hypertrophy and atrophy, inflammation cell infiltration, and thickening of tubular basal membrane, are prominent. In electron microscopy, thickening of glomerular basal membrane (GBM) and glomerular epithelial injuries, such as pseudocyst formation, vacuolization, detachment from the GBM, podocyte depletion, and foot process effacement are observed. Retinal lesions in cp/cp rats are also reported [72]. In cp/cp rats at 24 weeks of age, partial capillary obstruction and acellular, tortuous, irregular capillaries are observed by light microscope. In electron microscopy, thickening and irregularity of the basement membrane along with remnants of pericytes or so-called ghost pericytes are observed. Neuropathy in cp/cp rats is not reported.

It is reported that cp/cp rats fed a diet of AIN-93G show NASH-like lesions after 23 weeks [73].

2.2.7. WBN/Kob fatty rat

WBN/Kob fatty rat is a new congenic strain for the fa allele of the leptin receptor gene, and the homozygous rat provides a model of type 2 diabetes with obesity [74]. Male and female WBN/Kob fatty rats show inflammatory cell infiltration of the pancreas and impaired glucose tolerance at 7 weeks of age. Furthermore, the rats developed diabetes with pancreatitis at 3 months of age. From 7 to 12 weeks of age, the body weight and body mass index (BMI) of male WBN/Kob fatty rats are significantly greater than those of lean rats. Female WBN/Kob fatty rats have a significantly greater body weight and BMI than lean rats from 5 to 32 weeks of age. Male WBN/Kob fatty rats show hyperinsulinemia until 8 weeks of age, but after 8 weeks their insulin levels decrease with the increase of blood glucose levels. There has been no report regarding microangiopathy and NASH.

2.3. Nongenetic rodent model

2.3.1. Diet-induced obese models

Diet-induced obesity (DIO) animal model is a created model to study obesity and its comorbidities, such as insulin resistance, type 2 diabetes, dyslipidemia, hypertension, and atherosclerosis. In this model, an animal is fed a HF diet or HF/high sucrose or fructose diet for long term. As a result, it becomes obese with several glucose and lipid metabolic abnormalities, such as impaired glucose tolerance, increased fasting glucose level, hyperlipidemia, and hyperinsulinemia. The DIO models have become one of the most important tools for understanding the relationship of high-calorie Western diets and the development of obesity [75]. In recent years, Western diet-loaded genetic animal models have investigated to elucidate the
pathophysiology of obese related diseases including NAFLD/NASH and pancreatic lesion with diabetes and develop the new therapies of the diseases [76].

HF diet-induced obesity models are commonly used to gain a greater understanding of pathophysiology in obesity and develop antiobesity drugs. When choosing the HF diet, the fat level in diet should be taken into consideration. The low-fat diet has about 10% of the calories coming from fats, while the HF diet has about 30–50% of the calories coming from fats, and the very HF diet contains greater than 50 kcal% fats. When those diets are used to induce obesity, there is a dose response for body weight [77]. The source of dietary fat is also important. The rodents fed diets with fish oil do not gain so much weight and are more insulin sensitive as compared with those fed saturated fats [78]. Moreover, there are variable responses in physiological parameters, such as glucose tolerance, insulin resistance, and blood lipid levels, on strain and gender [79]. HF diet promotes the incidence of diabetes, and induces NASH-like lesions in genetic obese models. It is reported that HF diet-fed db/db mouse shows NASH-like lesions [29].

In rodent models, high-fructose or/sucrose diets elevates triglyceride and glucose production in liver, and this increased availability of nutrients leads to insulin resistance and hypertriglyceridemia [80]. Unless fed for a prolonged period of time, these high-fructose or/sucrose diets do not appear to lead to excessive weight gain [81]. Since high-fructose or/sucrose diets induce the elevation of lipid production in liver, these diets may be more effective to produce NASH-like hepatic lesions.

3. Antiobesity drugs

3.1. Protein tyrosine phosphatase 1B inhibitor

PTP1B is a 50-KD cytosolic tyrosine dephosphorylase consisting of 435 amino acids that are ubiquitously expressed in organs throughout the body. Originally, PTP1B was known to dephosphorylate phosphorylated insulin receptor (IR) β subunit and IR substrate in order to negatively regulate insulin signal transmission [82]. PTP1B is also reportedly related to the negative regulation of leptin signal transmission and to dephosphorylate phosphorylated signal transducer and activator of transcription 3 (STAT3) [83]. Therefore, PTP1B inhibitors are expected to be developed as antiobesity drugs as well as antidiabetes drugs. PTP1B KO mice are protected from diet-induced obesity, and neuronal PTP1B KO mice also show increased leptin signaling in the hypothalamus, reductions in feeding, body weight and adiposity, and increases in energy expenditure [84].

Ito et al. reported the antiobesity effects of JTT-551, which was developed as a novel PTP1B inhibitor [85, 19]. The single administration of JTT-551 and leptin enhanced STAT3 phosphorylation in the hypothalamus of DIO mice, and the food intake resulted in a significant reduction as compared with that in the control group. The food intake in JTT-551 administration without leptin treatment did not result in the reduction. DIO mice at 8 weeks of age were given 10 or 100 mg/kg of JTT-551 contained in food for 6 weeks and the chronic effects were investigated.
In the JTT-551 100 m/kg group, the cumulative calorie intake tended to decrease from 2 weeks after treatment and significantly decreased from 6 weeks after treatment. Body weight in JTT-551 treatment tended to decrease dose-dependently and the decreases in the JTT-551 100 mg/kg group were significant from 5 to 6 weeks after treatment. PTP1B inhibitor is a unique target that shows not only an improvement of glucose metabolism but also an antiobesity effect possibly by enhancement of leptin signaling.

### 3.2. Microsome triglyceride transfer protein inhibitor

MTP is localized in the endoplasmic reticulum in hepatocytes and enterocytes, and MTP leads the transfer of triglyceride (TG) and cholesteryl ester between membranes [86]. The protein participates in the assembly of TG-rich lipoproteins, such as chylomicron particles in the small intestine and very low-density lipoprotein (VLDL) particles in the liver, thereby also participating in the mobilization and secretion of TG-rich lipoproteins from enterocytes and hepatocytes [87]. Since enteric MTP has been shown to play a critical role in the absorption of fat or cholesterol, the inhibition of MTP in small intestine is expected to induce the potential of weight loss as an antiobesity drug.

Since the in vivo effects of MTP inhibitors were reported, it has been pointed out that inhibition of hepatic MTP could lead to the potent blockade of VLDL release, resulting in reduced plasma lipids but inducing fatty liver and hepatic dysfunction [88]. In fact, while the potential benefits of MTP inhibition, such as lowering chylomicron-TG and VLDL-TG levels, are demonstrated in animal experiments and in clinical studies, several major toxicity issues affect the clinical development of MTP inhibitors [89]. In clinical studies of BAY 13-9952 and BMS-201038, for example, hepatotoxicity indicated by the elevation of transaminase level halted their developments. Therefore, the compounds designed to show a high selectively inhibition for intestine-MTP have been developed and lipid-absorption inhibitors are expected to show pharmacological effects, including weight loss, without any hepatotoxicity.

Mera et al. designed the compound, JTT-130, that would be rapidly metabolized during the absorption process to avoid inhibition of hepatic MTP after oral administration [90, 91]. JTT-130 was designed to be rapidly hydrolyzed to its inactive metabolite (M1) by cleavage of ester group in the structures. The IC\textsubscript{50} values of JTT-130 on MTP inhibitory activities were 0.83 nM for TG transfer and 0.74 nM for cholesteryl ester (CE) transfer, respectively. No inhibitory effect of M1 on MTP was observed at concentrations of M1 increasing up to 30,000 nM. Antiobesity effects were investigated in a DIO model, Sprague-Dawley rat fed a 35% fat diet [15]. JTT-130 treatment decreased body weights with suppression of food intake (Figure 2A and B). Interestingly, the pharmacological effects were not observed in rats fed with the 3.1% fat diet (Figure 2C and D), and JTT-130 showed antiobesity effects in a dietary fat-dependent manner. The elevation of plasma levels of gut hormones, such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), was observed in DIO rats, and the elevation of gut peptides may be related with body weight loss with JTT-130 treatment. The antiobesity effect of JTT-130 was also investigated using a genetic model, ZDF rat [92]. Male ZDF rats at 7 weeks of age were fed a regular diet with JTT-130 as a food admixture for 6 weeks. JTT-130 treatment decreased the food intake in the ZDF rats throughout the treatment period, resulting in reduction in the body
weight in the first 4 weeks of the treatment period. However, the body weights of the JTT-130-treated ZDF rats were comparable to those of the control ZDF rats after 5 weeks of treatment. The body weight change is considered to be induced by the improvement of metabolic abnormalities in whole body with JTT-551 treatment.

Furthermore, JTT-130 treatment has been reported as ameliorating impaired glucose and lipid metabolism in ZDF rats [92], and attenuates dyslipidemia in hyperlipidemic hamsters and rabbits [93]. It is expected that intestine-specific MTP inhibitors will be useful in treatment of diabetes and atherosclerosis as well as obesity.

### 3.3. Acyl-CoA: diacylglycerol acyltransferase 1 inhibitor

DGAT1 is an enzyme that catalyzes the final step of TG synthesis, i.e., synthesis of TG from diacylglycerol and fatty acyl-CoA. DGAT1 is expressed in various organs, and is especially highly expressed in the small intestine, fat tissue, and testes [94]. The enzyme is involved in TG absorption from the small intestine and fat accumulation in adipose tissues [95]. Indeed, DGAT1-knockout (−/−) mice show resistance to the antiobesity effects of a HF diet; wherein body weight gain is suppressed, fat weight and TG contents decrease, and energy consumption in the liver and skeletal muscles accelerates, as well as observing improvements in insulin and leptin resistance, in comparison with wild-type mice [96]. Since the inhibition of DGAT1 is expected to result in two kinds of pharmacological effects: (1) inhibition of fat absorption in the small intestine and (2) inhibition of fat synthesis in adipose tissues, DGAT1 inhibitors are likely to become a good therapeutic option for obesity.
Tomimoto et al. reported antiobesity effects with JTT-553, which was discovered as a novel DGAT1 inhibitor [97]. A single administration of JTT-553 inhibited the increase of plasma TG levels after olive oil loading in Sprague-Dawley (SD) rats, suggesting that JTT-553 inhibited fat absorption in the small intestine. Furthermore, JTT-553 suppressed TG synthesis in adipose tissues [98]. The antiobesity effects of JTT-553 were investigated in DIO rats, SD rats fed a 35% fat diet, and a genetic model, the KKAY mouse. In DIO rats, body weight and visceral fat in the JTT-553 administration group decreased dose-dependently; however, the suppressive effects of JTT-553 on body weight were not observed with the 3.1% fat diet. Interestingly, the antifeeding effects of JTT-553 were observed in DIO rats, which was not observed in DGAT1-knockout (−/−) mice. A single administration of JTT-553 decreased food consumption depending on dietary fat content. The difference in appetite between DGAT1 inhibitor-treated and knockout mice remains unknown. In KKAY mice, JTT-553 decreased the food intake and body weight (Figure 3). Repeated administration of JTT-553 showed decreases of the liver and fat weights, and the liver TG content. The DGAT1 inhibitor was considered to suppress food consumption via the elevation of levels of gut hormones, such as GLP-1, in plasma [99]. Furthermore, JTT-553 was administrated to DIO mice and antiobesity effects and antidiabetic effects were investigated at the same time [98]. JTT-553 decreased body weight and food consumption, and treatment resulted in improvements in hyperinsulinemia and hyperlipidemia. In the glucose tolerance test, JTT-553 treatment resulted in ameliorations of insulin resistance. In addition, JTT-553 treatment resulted in significant reductions in fat mass, and increased glucose utilization of epididymal adipose tissues in the presence of insulin.

Figure 3. Effects of Acyl-CoA: diacylglycerol acyltransferase1 inhibitor, JTT-553 on body weights gain in KKAY mice on a 35% fat diet [98]. JTT-553 was dosed as food admixture to KKAY mice for 5 weeks. Data represent mean ± standard deviation (n = 7–8). \(* p < 0.05, ** p < 0.01: significantly different from 35% control group, \#p < 0.05, \##p < 0.01: significantly different from 3.1% control group.

3.4. Acyl-CoA: monoacylglycerol acyltransferase 2 inhibitor

MGAT2 is an enzyme that catalyzes the esterification of monoacylglycerol (MG), i.e., synthesis of diglycerides from MG and fatty acyl-CoA [100, 101]. The genes encoding three MGATs,
MGAT1, MGAT2, and MGAT3 have been identified \[102–104\]. MGAT1 is mainly expressed in the heart, lung, skeletal muscle, and pancreas, but not in the small intestine. Both MGAT2 and MGAT3 are mainly expressed in human small intestine, whereas only MGAT2 is expressed in mouse small intestine \[102\].

MGAT2 is involved in the resynthesis of TG in the intestine, and plays an important role in the assembly and secretion of chylomicrons. In fact, MGAT2 KO mice demonstrate reduced fat uptake in the small intestine and delay in the absorption of fat into circulation \[105\]. In addition, the elevation of postprandial GLP-1 and not PYY levels are observed in MGAT2 KO mice fed a HF diet \[106\]. The chronic function of MGAT2 on metabolic disorders is investigated using MGAT2 KO mice. MGAT2 deficient mice are protected from HF diet-induced obesity and glucose intolerance \[106\]. Moreover, MGAT2 deficiency results in increased metabolic rates, decreased food consumption, and protection from obesity in genetically obese Agouti mice, suggesting that MGAT2 regulates energy balance \[106, 107\]. The intestinal function of MGAT2 and the effect of this function on obesity are also investigated using intestine-specific MGAT2 KO mice \[108\]. Intestinal-specific deletion of MGAT2 alters TG metabolism in the small intestine and delays fat absorption. These mice are protected from obesity and impair glucose metabolism when feed a HF diet. Thus, there is considerable interest that inhibition of MGAT2 is a feasible target for obesity and other metabolic disorders caused by excess dietary calories. Although, the physiological role of MGAT2 has been mainly investigated using genetically modified mice, the detailed pharmacological characteristics of MGAT2 inhibitors have not been reported.

Okuma et al. reported the pharmacological profile of JTP-103237, which was discovered as a novel MGAT2 inhibitor. A single administration of JTT-103237 reduced plasma TG after lipid loading. In addition, JTT-103237 increased MG and fatty acid content, which are MGAT2 substrates, in the small intestine. A single administration of JTT-103237 tended to elevate plasma levels of GLP-1 and PYY after olive oil loading, and the antifeedding effect of JTT-103237 was observed independent of dietary fat content. After repeated dosing, JTT-103237 reduced food consumption and body weight, and increased energy expenditure in DIO mice. Furthermore, JTT-103237 reduced hepatic steatosis in high sucrose and very low fat (HSVLF)-fed mice, through the suppression of TG synthesis related genes, such as sterol regulatory element-binding protein (SREBP)-1c, fatty acid synthesis, and stearoyl-CoA desaturase (SCD)-1. The inhibition of hepatic MGAT2 activity is considered to directly reduce hepatic TG synthesis. 2-MG content in the small intestine is considered to increase by administration of MGAT2 inhibitor. The effects of 2-MG on food intake and diarrhea were evaluated and compared with the long-chain fatty acid (LCFA) in rats by intrajejunal infusion \[109\]. 2-MG did not induce diarrhea under the condition in which it comparably reduced food intake as compared with LCFA, suggesting that 2-MG stimulates satiety without inducing diarrhea, different from LCFA. From these findings, MGAT2 inhibition may prove to be a useful strategy target for treating obesity and related metabolic disorders.
4. Conclusion

Obesity is the consequence of an imbalance between energy intake and energy expenditure, and basic therapies for obesity are appropriate dietary restriction to decrease energy intake and effective exercise to increase energy consumption. However, maintaining these lifestyle modifications, such as diet therapy and exercise, are difficult and therapeutic effects are limited. Medical therapy then becomes a pivotal step.

It is important to elucidate the complex mechanisms of obesity in developing new antiobesity therapies, including the discovery of novel drugs. In particular, investigations using obese animal models are essential to clarify the pathophysiology and develop new antiobesity drugs. Several drug types that target various mechanisms, such as increased satiety with anorexia, inhibition of nutritional absorption, and acceleration of energy consumption, have been developed using various obese animal models including genetic models and nongenetic models. To help develop new antiobesity therapies, including the understanding of pathophysiology of obesity, the importance of the obese animal models will be a constant in the future.

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[97] Tomimoto D, Okuma C, Ishii Y, Akiyama Y, Ohta T, Kakutani M, et al. Pharmacological characterization of [trans-5’-(4-amino-7,7-dimethyl-2-trifluoromethyl-7H-pyrimido[4, 5-b][1,4]oxazin-6-yl)-2’,3’-dihydrospiro(cyclohexane-1,1’-inden)-4-yl]acetic acid mono-


