Abnormalities in the metabolism of fatty acids and triacylglycerols in the liver of the Goto-Kakizaki rat, a model for non-obese type 2 diabetes

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Abstract The Goto-Kakizaki (GK) rat is widely used as animal model for spontaneous-onset

type 2 diabetes without obesity; nevertheless, little information is available on the metabolism of fatty acids and triacylglycerols (TAG) in their livers. We investigated the mechanisms underlying the alterations in the metabolism of fatty acids and TAG in their livers, in comparison with Zucker (*fa*/*fa*) rats, which are obese and insulin resistance. Lipid profiles, the expression of genes for enzymes and proteins related to the metabolism of fatty acid and TAG, *de novo* synthesis of fatty acids and TAG *in vivo*, fatty acid synthase activity *in vitro*, fatty acid oxidation in liver slices, and very-low-density-lipoprotein (VLDL)-TAG secretion *in vivo* were estimated. Our results revealed that (i) the TAG accumulation was moderate, (ii) the *de novo* fatty acid synthesis was increased by up-regulation of fatty acid synthase in a post-transcriptional manner, (iii) fatty acid oxidation was also augmented through the induction of carnitine palmitoyltransferase 1a, and (iv) the secretion rate of VLDL-TAG remained unchanged, in the livers of GK rats. These results suggest that, despite the fact that GK rats exhibit non-obese type 2 diabetes, the up-regulation of *de novo* lipogenesis is largely compensated by the up-regulation of fatty acid oxidation, resulting in only moderate increase in TAG accumulation in the liver.

Keywords Fatty acid synthesis · Triacylglycerol metabolism · Fatty acid β-oxidation · Liver · Non-obese diabetes · Goto-Kakizaki rat

Abbreviations

ACC	Acetyl-CoA carboxylase
ACLY	ATP-citrate lyase
ACOT1	Acyl-CoA thioesterase 1
ACOX1	Peroxisomal acyl-CoA oxidase 1
ACSL	Long-chain acyl-CoA synthetase
APOC3	Apolipoprotein CIII
CE	Cholesteryl ester(s)
CPT1a	Carnitine palmitoyltransferase 1a
CYP	Cytochrome P450
DAG	Diacylglycerol(s)
DGAT	Diglyceride acyltransferase
ELOVL	Fatty acid elongase
FABP1	Fatty acid-binding protein 1
FABPpm	Plasma membrane-associated fatty acid-binding protein
FAS	Fatty acid synthase
FAT/CD36	Fatty acid translocase
FATP	Fatty acid transport protein
FFA	Unesterified fatty acid(s)
GCK	Glucokinase
GK rat	Goto-Kakizaki rat
GPAT	Glycerol-3-phosphate acyltransferase
G6Pase	Glucose-6-phosphatase
G6PD	Glucose-6-phosphate dehydrogenase
HNF4α	Hepatic nuclear factor 4α
LCAD	Long-chain acyl-CoA dehydrogenase
LPK	L-type pyruvate kinase

LXRα	Liver X receptor α
MCAD	Medium-chain acyl-CoA dehydrogenase
MCD	Malonyl-CoA decarboxylase
ME1	Malic enzyme 1
MUFA	Monounsaturated fatty acid(s)
P-ACC	Phospho-acetyl-CoA carboxylase
PEPCK	Phosphoenolpyruvate carboxykinase
PGC1α	Peroxisome proliferator-activated receptor gamma coactivator 1α
PPAR	Peroxisome proliferator-activated receptor
SCD	Stearoyl-CoA desaturase
SREBP-1c	Sterol regulatory element-binding protein-1c
TAG	Triacylglycerol(s)
T2D	Type 2 diabetes
TLC	Thin-layer chromatography
UCP2	Uncoupling protein 2
VLCAD	Very long-chain acyl-CoA dehydrogenase
VLDL	Very low-density lipoprotein
WI rat	Wistar rat, a control corresponding to the GK rat
ZF rat	Obese Zucker (*fa*/*fa*) rat
ZL rat	Lean Zucker (?/+) rat

Introduction

Diabetes is a heterogeneous group of disorders characterized by high blood glucose levels, with type 2 diabetes (T2D) being more common than type 1. The impaired life expectancy of patients with T2D has been linked not only to vascular complications and renal disease, but also to progressive liver disease [1]. Well-characterized and suitable animal models are indispensable for elucidating the molecular mechanisms underlying the pathogenesis of T2D and developing novel therapeutics against T2D. Several animal models of spontaneous T2D have been provided, most of which have hyperglycemia, hypertriglyceridemia, insulin resistance, fatty liver, and, in particular, obesity [2, 3]. Obesity is common in patients with T2D; however, not all diabetic patients are obese. In contrast to Western populations, the prevalence of T2D has increased in spite of the low prevalence of obesity in some populations, including Japanese [4, 5]. Therefore, animal models of non-obese T2D are required in order to investigate this type of diabetes. Unlike most animal models developed for T2D, the Goto-Kakizaki (GK) rat is an animal model of spontaneous-onset T2D without obesity. The GK rat exhibits mild hyperglycemia, impaired glucose tolerance, impaired insulin secretion, progressive reductions in the β-cell mass, and the development of long-term diabetic complications without obesity [6, 7]. Impaired insulin sensitivity has also been reported not only in the skeletal muscles and adipose tissues of GK rats, but also in their livers [8, 9].

T2D with obesity is generally associated with fatty liver [1, 10], a disorder characterized by the abnormal accumulation of lipids due to their overproduction and/or a defect in their disposal. A recent meta-analytical study revealed a two-fold higher risk of T2D with non-alcoholic fatty liver disease [11], and T2D has been shown to have an impact on the development and progression of liver disease, which may lead to steatohepatitis, cirrhosis, and eventual liver failure [1]. Previous studies demonstrated the dysregulation of lipid metabolism in the livers of rodents with defects in leptin or its receptor [2, 3]. We previously showed the aberrant regulation of fatty acid modifications (desaturation and elongation) in GK rats [12]; nevertheless, metabolic defect(s) in lipids have been poorly characterized in GK rats. It is conceivable that the liver plays a key role in regulating glucose and lipid

metabolism and that fatty liver is one of the major phenotypes of metabolic disorders closely associated with hepatic insulin resistance [1, 10, 13]. Therefore, information on disorders in lipid metabolism in the livers of GK rats is indispensable to understand molecular basis underlying pathogenesis of non-obese T2D and to develop novel therapeutics against non-obese T2D because GK rats is widely utilized as an animal model for T2D without obesity. In this context, the present study aimed (i) to determine whether lipid accumulation occurs and, if this is the case, (ii) to reveal the metabolic mechanisms underlying lipid accumulation in the livers of GK rats, in comparison with those in obese Zucker (*fa*/*fa*) (ZF) rats, an animal model that is a commonly studied as a model of fatty liver, hepatic insulin resistance, and obesity. Our findings show for the first time that *de novo* lipogenesis is increased while fatty acid degradation is also elevated, such that triacylglycerols (TAG) moderately accumulate in the livers of GK rats.

Materials and Methods

Materials

The following materials were obtained from the indicated commercial sources: [1-^{14}C] acetic acid (55 Ci/mol) and [1-^{14}C]palmitic acid (16:0) (56.0 Ci/mol), (American Radiolabeled Chemicals, Inc., St. Louis, MO. USA); acetyl-CoA and malonyl-CoA (Sigma-Aldrich, St. Louis, MO, USA); anti-acetyl-CoA carboxylase (ACC) rabbit monoclonal antibody, anti-carnitine palmitoyltransferase 1a (CPT1a) mouse monoclonal antibody, anti-fatty acid synthase (FAS) rabbit monoclonal antibody, anti-1TBP18 mouse monoclonal antibody, and anti-β-actin mouse monoclonal antibody (Abcam, Cambridge, UK); anti-phospho-acetyl-CoA carboxylase $^{\text{Ser78 and 80}}$ (P-ACC) mouse monoclonal antibody, anti-sterol regulatory element binding protein-1c (SREBP-1c) mouse monoclonal antibody, goat anti-mouse IgG horseradish peroxidase-conjugated antibody, and goat anti-rabbit IgG horseradish

peroxidase-conjugated antibody (Santa Cruz Biotechnologies Inc., Santa Cruz, CA, USA).

Animals

All animal procedures were approved by the Institutional Animal Care Committee of Josai University in accordance with the Guidelines for the Proper Conduct of Animal Experiments (Science Council of Japan). Five-week-old male GK rats and their corresponding control Wistar (WI) rats were obtained from Clea Japan Inc. (Tokyo, Japan). Five-week-old male lean Zucker (?/+) (ZL) and ZF rats were obtained from Charles River Japan (Tokyo, Japan). Animals were fed a standard diet (CE-2, Clea Japan Inc.) *ad libitum*, allowed free access to water, and were killed in the fed state at the age of 10 weeks. Animals were anesthetized with diethyl ether, and blood was withdrawn from the inferior vena cava. The liver was rapidly removed, washed with saline, and weighed. Livers were used for histopathological analyses, lipid analyses, and in the preparation tissue lysates, nuclear extracts, cytosol, and liver slices. One part of the liver was frozen in liquid nitrogen and then stored at –80 °C for the later determination of mRNA. The liver portions used to prepare the cytosol were perfused with ice-cold saline.

Histopathological Analysis

Isolated livers were fixed in 10 % neutral-buffered formalin, embedded in paraffin wax, sectioned (3–4-μm thick), and stained with hematoxylin and eosin. In order to visualize fat deposition in the liver, frozen sections (10–12-μm thick) were cut on a cryostat and stained with Oil Red O and hematoxylin. Sections were evaluated by scanning the entire tissue specimen under low-power magnification (× 40) and then confirmed under higher power magnification (× 100, × 200, and × 400). The severity of histopathological findings was scored as (0) normal, (1) minimal, (2) mild, (3) moderate, and (4) marked lipid deposition in hepatocytes. All histopathological scoring and evaluations were carried out

in a blind evaluation without knowledge of the treatment. Images were obtained under a light microscope (Olympus BX53; Olympus, Tokyo, Japan) equipped with a DP72 digital camera (Olympus).

Biochemical Analysis of Serum Parameters

Serum glucose, TAG, unesterified fatty acids (FFA), and total cholesterol were measured using colorimetric enzymatic assay kits from Wako Pure Chemicals (Osaka, Japan). Serum insulin was measured using a rat RIA kit from Millipore Corp. (Billerica, MA, USA).

Lipid Analysis

Total lipids were extracted from a piece of the liver by the reported method [14]. Cholesterol and phospholipid phosphorus were measured using previously reported methods [15, 16]. In order to analyze fatty acid profiles, cholesteryl ester (CE), TAG, diacylglycerol (DAG), FFA, and phospholipid were separated by thin-layer chromatography (TLC) on silica gel G plates, which were developed with *n*-hexane/diethyl ether/acetic acid (80:30:1, v/v), as described previously [17]. Fatty acid methyl esters were prepared using sodium methoxide/methanol for TAG and phospholipids, and HCl/methanol for DAG and FFA. CE, which was extracted from silica gel, was saponified once, and the fatty acids released were then extracted separately from cholesterol and converted into methyl esters using HCl/methanol. The composition of fatty acid methyl esters was determined by gas–liquid chromatography as described previously [12].

Real-Time Quantitative PCR

mRNA expression was analyzed by real-time PCR as described previously [12, 18]. The sequences of

primers used in this study are listed in Table 1.

Western Blot Analysis

Western bolt analyses of FAS, ACC1, P-ACC1, and CPT1a were performed using the tissue lysates prepared as described previously [18]. Proteins (15 μg each) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 10 % (for CPT1a) or 7.5 % (for ACC1, P-ACC1 and FAS) gels. Proteins were transferred to polyvinylidene difluoride membranes, incubated with the primary antibody, incubated with the secondary antibody, visualized using the ECL Prime Western Blotting Detection Reagent (GE Healthcare Japan, Tokyo, Japan), and then detected using a luminoimage analyzer. A Western bolt analysis of SREBP-1c was performed using nuclear extracts (20 μg of protein) of livers as reported previously [19] with some modifications as described previously [3].

Measurement of the *In Vivo* Synthesis of Fatty Acids and TAG in the Liver

The hepatic synthesis of fatty acids and TAG was estimated by measuring the incorporation *in vivo* of [1-^{14}C]acetic acid into fatty acid and TAG in the liver as reported previously [20] with some modifications. In brief, [^{14}C]acetic acid was dissolved in 0.9 % NaCl (40 μCi/mL). Under light anesthesia with diethyl ether, [^{14}C]acetic acid at a dose of 160 μCi/kg of body weight was intraperitoneally injected. Five minutes after the injection, livers were immediately isolated and frozen in liquid nitrogen. Acetyl-CoA was extracted from one portion of the liver and determined using the reported method [21]. Lipids were extracted from another portion of the liver [14]. One portion of the extracted lipids was saponified with 10 % methanolic KOH at 80 °C for 60 min under a nitrogen atmosphere. After being diluted with water, unsaponifiable matter was extracted with *n*-hexane three times, samples were acidified with 6 M HCl, and fatty acids were extracted with *n*-hexane three times.

The unsaponifiable matter and fatty acids were mixed with scintillation fluid, and radioactivities were measured using a liquid scintillation counter (Aloka LSC 6100; Hitachi–Aloka, Tokyo, Japan). Lipids were separated into lipid classes by TLC, and the separated lipids were extracted from silica gel [17]. The extract was mixed with scintillation fluid, and radioactivity was measured.

Assay for FAS

One portion of the perfused liver was homogenized in 1.5 volumes of a phosphate-bicarbonate buffer (70 mM $KHCO_3$, 85 mM K_2HPO_4, 9 mM KH_2PO_4, and 1 mM dithiothreitol) (pH 8.0) in a Potter glass–Teflon homogenizer. The homogenates were centrifuged at 20,000 × *g* for 10 min. The supernatant obtained was centrifuged at 105,000 × *g* for 60 min, and the resulting supernatant was stored at –80 °C until used. Protein concentrations were determined by the reported method [22] using bovine serum albumin (BSA) as a standard. FAS activity was determined spectrophotometrically as reported previously [23]. The assay mixture contained 33 μM acetyl-CoA, 100 μM malonyl-CoA, 100μM NADPH, 1 mM EDTA, 1 mM 2-mercaptoethanol, 50–100 μg cytosolic protein, and 100 mM phosphate buffer (pH 7.0) in a total volume of 1 mL. NADPH oxidation was followed at 340 nm at 30 °C. A correction was made for the rate of NADPH oxidation in the absence of malonyl-CoA.

***Ex Vivo* Fatty Acid Oxidation in Liver Slices**

Fatty acid oxidation was measured utilizing liver slices as reported previously [24]. Rats were killed and their livers were quickly removed. The left lobe was separated, and precision-cut liver slices (600-μm thick; 75–85 mg) were prepared with a Krumdieck tissue slicer (Alabama Research Development, Munford, AL, USA). [1-^{14}C] 16:0 was purified just before use by TLC on silica gel G plates, which were developed with *n*-hexane/diethyl ether/acetic acid (80:30:1, v/v). Liver slices were incubated in glass vials that contained 2 mL of Krebs–Henseleit buffer (pH 7.4) containing 5 mM

glucose, 0.25 mM [1-^{14}C]16:0 (0.3 μCi), and 0.6 % BSA (essentially fatty acid-free) at 37 ^{o}C for 30 and 90 min under an O_2–CO_2 atmosphere (95:5, by vol.) with shaking (90 oscillations/min). The vials were capped with rubber stoppers, from which plastic center-wells were suspended. The incubation was terminated by an injection of 1 mL of 0.6 M $HClO_4$ into the vial, and 0.2 mL of 1 M benzethonium hydroxide in methanol was injected into the center well. The vials were shaken (60 oscillation/min) at room temperature for 45 min in order to trap radio-labeled CO_2 in benzethonium hydroxide. The contents of the center well were transferred to a counting vial and mixed with scintillation fluid, and radioactivity was then measured using a liquid scintillation counter. Liver slices were homogenized with the incubation mixture that was acidified with $HClO_4$. After centrifugation at 1,500 × g for 10 min, the supernatant was neutralized with 5 M KOH, and its pH was adjusted to 4 using 3 M acetate buffer (pH 4.0); the aqueous phase obtained was extracted five times with petroleum ether to remove traces of [^{14}C]16:0. An aliquot of the aqueous phase was mixed with scintillation fluid, and radioactivity was determined as acid-soluble oxidation products. The slope between 30 and 90 min of the incubation was used to calculate the rate of 16:0 oxidation.

Very Low-Density Lipoprotein (VLDL)–TAG Secretion

Rats that had been starved for 12 h were intravenously injected with 20 % (w/v) Triton WR-1339 (Sigma-Aldrich) in saline at a dose of 300 mg/kg body weight. Blood was collected from the retro-orbital plexus under diethyl ether anesthesia 1, 2, 3, 4 and 6 h after this administration. Serum was obtained from the blood by centrifugation (at 1,200 × *g* for 15 min). The VLDL secretion rate was determined by measuring changes in serum TAG levels [25]. The amount of total blood was calculated as one-twelfth of the body weight.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). The significance of differences between two groups was analyzed using the Student's *t*-test.

Results

Comparison with ZF Rats

In order to gain a better understanding of pathophysiological characteristics of GK rats, general features were compared between GK and ZF rats.

The pathophysiological parameters of GK and ZF rats were compared (Table 2). The body weights of GK rats were 86 % those of WI rats, whereas no significant difference was found in relative liver weights between WI and GK rats. On the other hand, the body weights of ZF rats were 161 % those of ZL rats, and the relative liver weights of ZF rats were 1.24-fold higher than those of ZL rats. Regarding white adipose tissues, the relative weight of mesenteric fat was1.12-fold greater in GK rats than in WI rats, whereas the relative weight of epididymal fat did not differ between these rats. The relative weights of epididymal and mesenteric fat were 3.16-fold and 2.55-fold greater, respectively, in ZF rats than in ZL rats. Serum glucose and insulin levels in the fed state were 1.92-fold and 1.79-fold higher, respectively, in GK rats than in WI rats. No significant differences were observed in serum glucose levels between ZL and ZF rats, whereas serum insulin levels were 7.16-fold higher in ZF rats than in ZL rats. Regarding serum lipid parameters, TAG concentrations in GK and ZF rats were 59 % and 610 %, respectively, those in their respective controls. Serum levels of cholesterol and FFA were 1.66-fold and 1.88-fold higher, respectively, in GK rats than in WI rats, while cholesterol and FFA levels in ZF rats were 183 % and 207 %, respectively, those in ZL rats.

The hepatic lipid profiles of GK rats were shown in Table 2. TAG and DAG contents were 1.48-fold and 1.17-fold higher, respectively, in GK rats than in WI rats. TAG and DAG contents were

5.72-fold and 1.19-fold greater, respectively, in ZF rats than in ZL rats. Hepatic FFA concentrations did not significantly differ between GK and WI rats, or between ZF and ZL rats. Although no significant differences were observed in the hepatic contents of phospholipids and cholesterol between GK and WI rats, their contents in ZF rats were 91 % and 79 %, respectively, those in ZL rats. Hepatic CE contents did not significantly differ between WI and GK rats, or between ZL and ZF rats. The histopathological features of Oil Red O-stained sections of livers from WI, GK, ZL, and ZF rats were shown in Figure 1. In WI and ZL rats, neither vacuolation nor lipid accumulation was observed in hepatocytes, whereas several sinusoidal cells such as stellate cells were stained by Oil Red O (Fig. 1a, c). Mild to moderate lipid accumulation in peripheral hepatocytes was detected in GK rats (Fig. 1b). In contrast, ZF rats revealed moderate to severe diffuse fatty deposition in hepatocytes (Fig. 1d). The intensity of lipid deposition in the hepatocytes of each animal was scored (Fig. 1e). The fatty acid profiles of hepatic TAG in GK and ZF rats were compared with those in the respective controls (Table 3). The proportions of palmitoleic acid (16:1n-7) (4.10-fold), oleic acid (18:1n-9) (1.67-fold), and *cis*-vaccenic acid (18:1n-7) (1.80-fold) were markedly higher in ZF rats than those in ZL rats. In contrast, the proportion of linoleic acid (18:2n-6) in ZF rats was 34.9 % that in ZL rats. The proportions of 16:1n-7 (1.50-fold) and 18:1n-7 (1.33-fold) were moderately higher in GK rats than in WI rats, whereas no significant differences were observed in the proportion of 18:1n-9 between GK and WI rats. The proportion of 18:2n-6 was slightly reduced in GK rats (84.0 % that in WI rats). Changes in the proportions of monounsaturated fatty acids (MUFA) in phospholipids, DAG, FFA and CE of GK and ZF rats were similar to those in TAG, whereas the changes observed in these lipid classes were less than those in TAG (Table 3). It is noteworthy that the proportion of arachidonic acid (20:4n-6) increased in phospholipids in GK rats, and the proportion of 8, 11, 14-eicosatrienoic acid (20:3n-6) proportion increased, whereas that of 20:4n-6 decreased in phospholipids in ZF rats. These alterations may have been due to the enhanced expression of genes encoding Δ6 fatty acid desaturase and Δ5 fatty acid desaturase in the livers of GK rats, but not ZF rats [12].

In order to gain an insight into the molecular mechanisms underlying the accumulation of TAG

in the livers of GK rats, the mRNA levels of key enzymes and proteins involved in the metabolism of fatty acids and TAG were measured and compared with those in the livers of ZF rats (Table 4). The levels of mRNAs encoding enzymes related to *de novo* fatty acid synthesis [FAS, ACC1, glucose-6-phsphate dehydrogenase (G6PD), ATP-citrate lyase (ACLY), and malic enzyme 1 (ME1)] and glycerolipid synthesis [glycerol-3-phosphate acyltransferase (GPAT) 1 and diacylglycerol acyltransferase (DGAT) 2] were significantly up-regulated in the livers of ZF rats, whereas mRNAs for GPAT4, lipin 2, lipin 3, and DGAT1 remained unchanged. Among the enzymes related to *de novo* fatty acid synthesis, the expression of mRNAs for FAS and ACC1 was unchanged in the livers of GK rats, whereas the mRNA levels of *G6pd, Acly*, and *Me1* were significantly up-regulated. Regarding glycerolipid synthesis, the mRNA level of *Lipin2* was up-regulated, the expression of mRNAs for GPAT4, lipin 3, DGAT1, and DGAT2 was unchanged, and the mRNA level of *Gpat1* was considerably down-regulated. It is noteworthy that the levels of mRNAs encoding the enzymes involved in fatty acid modifications [stearoyl-CoA desaturase 1 (SCD1) and fatty acid elongase (ELOVL6)] were markedly up-regulated in the livers of ZF rats, and also that the mRNA level of SCD1 was up-regulated (1.93-fold) while that of ELOVL6 was unchanged in the livers of GK rats. The expression of *Elovl5* was up-regulated in GK rats, but not in ZF rats. Regarding the levels of proteins participating in the trafficking of fatty acids, fatty acid translocase (FAT/CD36) and long-chain acyl-CoA synthetase (ACSL) 5, were significantly higher in ZF rats than in ZL rats, whereas no significant differences were observed in the levels of mRNAs encoding fatty acid transport protein (FATP) 2, FATP4, FATP5, plasma membrane-associated fatty acid-binding protein (FABPpm), fatty acid-binding protein 1 (FABP1), ACSL1, or ACSL3 between ZL and ZF rats. The expression of the genes for FAT/CD36 and FATP5 was down-regulated in GK rats, whereas no significant difference was observed in the expression of other genes involved in the trafficking of fatty acids between WI and GK rats. Regarding fatty acid degradation, the level of mRNAs encoding CPT1a and uncoupling protein 2 (UCP2) were significantly higher in GK rats than in WI rats, whereas the expression of these genes was slightly down-regulated or unchanged in ZF rats. On the other hand, no significant

differences were detected in the levels of mRNAs for medium-chain acyl-CoA dehydrogenase (MCAD), very long-chain acyl-CoA dehydrogenase (VLCAD), or peroxisomal acyl-CoA oxidase 1 (Acox1) between WI and GK rats, or between ZL and ZF rats, except for the mRNA levels of long-chain acyl-CoA dehydrogenase (LCAD) being slightly higher in ZF rats, but not in GK rats than in their respective controls. The expression of genes for ACC2 and malonyl-CoA decarboxylase (MCD) was unchanged in the livers of GK and ZF rats. As for lipoprotein metabolism, apolipoprotein CIII (APOC3) mRNA levels were lower in GK rats than in WI rats, and higher in ZF rats than in ZL rats. The levels of mRNA encoding microsomal triglyceride transfer protein (MTP) did not differ between WI and GK rats, or between ZL and ZF rats. Concerning glucose metabolism, the level of mRNA for L-type pyruvate kinase (LPK) was markedly up-regulated in the livers of ZF rats, whereas that for phosphoenolpyruvate carboxykinase (PEPCK) was significantly down-regulated; no significant differences were observed in the levels of mRNAs for glucokinase (GCK), glucose-6-phosphatase (G6Pase), or glucose transporter type 2 (GLUT2) between ZL and ZF rats. The expression of *Pepck* and *G6pase* was up-regulated in the livers of GK rats; no significant differences were observed in the expression of *Lpk*, G*ck*, or *Glut2* between GK and WI rats. The expression of genes for IRS-1 and IRS-2 in the livers in ZF rats were 79 % and 34 %, respectively, those in ZL rats. The levels of mRNA for IRS-1 in the liver were 1.27-fold higher in GK rats than in WI rats and no significant difference was found in the expression of *irs-2* between WI and GK rats.

The expression of the genes for peroxisome proliferator-activated receptor α (PPARα), peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α), and lipin 1 were significantly higher in GK rats than in WI rats (Fig. 2a). Moreover, the expression of the gene for acyl-CoA thioesterase 1 (ACOT1), a typical PPARα target gene, was up-regulated in GK rats (Table 4). The level of mRNA for sterol regulatory element-binding protein-1c (SREBP-1c) in the liver was significantly higher in ZF rats than in ZL rats (Fig. 2b), whereas this level in GK rats was 60 % that in WI rats (Fig. 2a). The nuclear content of the mature 68-kDa form of SREBP-1c in the liver was 2.45-fold higher in ZF rats than in ZL rats, whereas no significant difference was observed between

WI and GK rats (Fig. 2c). No significant differences were noted in the expression of genes for carbohydrate response element-binding protein (ChREBP), liver X receptor α (LXRα), or hepatic nuclear factor 4α (HNF4α) between WI and GK rats, or between ZL and ZF rats (Fig. 2a, b). The expression of *Lpk*, a typical target gene of ChREBP, was elevated in ZF rats, while that of *lipin1*, a direct target gene of HNF4α, was up-regulated in GK and ZF rats; however, the expression of the gene for cytochrome (CYP) 7A1, a typical LXRα target gene, was unchanged in GK and ZF rats (Table 4).

De Novo Fatty Acid Synthesis in GK Rat Livers

In order to estimate *de novo* fatty acid synthesis, [1-^{14}C]acetate was intraperitoneally injected into WI and GK rats, and its incorporation into fatty acids in the liver was compared. The radioactivity found in fatty acids was 1.48-fold higher in GK rats than in WI rats (Fig. 3a). Since the hepatic content of acetyl-CoA was 1.46-fold greater in GK rats than in WI rats (Fig. 3b), the amount of fatty acids synthesized *de novo* in the liver was calculated to be 1.72-fold higher in GK rats than in WI rats (Fig. 3c). FAS activity in the hepatic cytosol was 2.10-fold higher in GK rats than in WI rats; this activity was 44 % that in ZF rats (Fig. 3d). FAS and ACC1 protein levels in the liver were 2.29-fold and 1.77-fold, respectively, higher in GK rats than in WI rats (Fig. 3e, f). FAS protein levels in GK rats were 21 % those in ZF rats, and ACC protein levels in GK rats were 30 % those in ZF rats.

TAG Synthesis in GK Rat Livers

The distribution of radioactivity from [^{14}C]acetate, which was administered *in vivo* to rats, among lipid classes in the liver was compared between WI and GK rats (Fig. 4a). The proportion of radioactivity found in TAG in the liver was 1.72-fold greater in GK rats than in WI rats, while that residing in cholesterol in GK rats was 26 % that in WI rats (Fig. 4a). The amount of [^{14}C]acetate incorporated into TAG in the liver was 2.77-fold higher in GK rats than in WI rats, while that incorporated into

cholesterol in the livers of GK rats was 42 % that in the livers of WI rats (Fig. 4b). No significant differences were observed in the amounts of [^{14}C]acetate incorporated into DAG, phospholipids, FFA, or CE between WI and GK rats (Fig. 4b). Since the hepatic content of acetyl-CoA was 1.46-fold greater in GK rats than in WI rats (Fig. 3b), the amounts of acetyl-CoA incorporated into TAG, DAG, phospholipids, and FFA in the liver were calculated to be 2.74-fold, 1.75-fold, 1.36-fold, and 1.63-fold higher, respectively, in GK rats higher than in WI rats; the amount of acetyl-CoA incorporated into cholesterol was 41 % that in WI rats (Fig. 4c).

Fatty Acid Oxidation in GK Rat Livers

In order to confirm the functional significance of changes in the gene expression and protein contents of the enzymes involved in fatty acid degradation, [1-^{14}C]16:0 oxidation rates in the livers of WI and GK rats were compared using liver slices (Fig. 5). The rates of formation of CO_2 and acid-soluble oxidation products in liver slices were 2.22-fold and 2.52-fold higher, respectively, in GK rats than in WI rats (Fig. 5a, b); total β-oxidation products formed in liver slices were 2.48-fold greater in GK rats than in WI rats (Fig. 5c). The results of the Western bolt analysis revealed that the content of the CPT1a protein in the liver was 1.39-fold greater in GK rats than in WI rats (Fig. 5d); moreover, the ratio of protein content of P-ACC to that of ACC was 1.91-fold higher in GK rats than in WI rats (Fig. 5e).

VLDL–TAG Secretion in GK Rats

The rates of hepatic VLDL–TAG secretion were compared between WI and GK rats utilizing Triton WR-1339, which prevents TAG from lipolysis by lipoprotein lipase. No significant difference was observed in this rate between WI and GK rats (Fig. 5f).

Discussion

Hepatic lipid profiling revealed that the hepatic content of TAG was moderately higher in GK rats (1.48-fold), and markedly greater (5.72-fold) in ZF rats than in their respective controls. Although the close relationship between hepatic lipid accumulation and insulin resistance has already been established, recent studies have indicated that DAG rather than TAG itself is the direct cause for insulin resistance [26, 27]. The result of the present study showed that, despite the marked difference in TAG accumulation, the hepatic content of DAG was elevated to largely the same extent in GK and ZF rats. Previous studies demonstrated that GK and ZF rats both exhibited hepatic insulin resistance [9, 28]. In addition to increases in DAG levels in the livers of ZF rats, the expression of *Irs-2* was markedly lower, whereas that of *Irs-1* was slightly reduced. Moreover, the nuclear content of the mature form of SREBP-1c was significantly increased in their livers. These results coincide with previous findings showing that high SREBP-1c activity resulting from hyperinsulinemia negatively correlated with IRS-2 expression in *ob*/*ob* mice [29]. Under such conditions, an insulin signal may fail to suppress the transcription of gluconeogenic enzymes through IRS-2; however, an insulin signal through IRS-1 may continually stimulate the cleavage of SREBP-1c, thereby activating the expression of lipogenic genes. The expression of *Pepck* was reduced while that of lipogenic enzymes was entirely up-regulated in ZF rats. On one hand, the expression of *Irs-1* was slightly high while that of *Irs-2* was unchanged in the livers of GK rats. Therefore, the expression of genes for the gluconeogenic enzymes, PEPCK and G6Pase, was increased, likely because of moderately high insulin levels in GK rats. However, the nuclear content of the mature form of SREBP-1c was unchanged, and the expression of *Fas* and *Acc1* was not altered in the liver. Taken together, the present results imply that the mechanism underlying hepatic TAG accumulation in GK rats is evidently distinct from that operating in the livers of ZF rats.

Since the liver does not serve as a storage depot for TAG, the steady-state concentration of TAG

in the liver is kept low under physiological conditions. Therefore, TAG accumulation in the liver arises from an imbalance between lipid acquisition (*de novo* lipogenesis and fatty acid uptake from the circulation) and disposal (fatty acid oxidation and the export of TAG as a component of VLDL) [30]. The present study showed the up-regulated expression of genes for key enzymes involved in *de novo* fatty acid synthesis in the livers of ZF rats. It is widely known that most lipogenic enzymes are regulated by SREBP-1c and ChREBP. Serum insulin levels in ZF rats were markedly high, and the gene expression of *Srebp-1c* and nuclear level of the mature form of SREBP-1c were elevated in their livers. Therefore, SREBP-1c appears to play a central role in the development of TAG accumulation in ZF rats because insulin markedly increases the expression of *Srebp-1c* and nuclear content of the mature form of SREBP-1c in the liver [31]. Since the expression of *Lpk* is under the regulation of ChREBP [32] and was found to be up-regulated in the livers of ZF rats, ChREBP may also induce genes for *de novo* fatty acid synthesis in the livers of ZF rats. In addition to the genes for enzymes related to *de novo* fatty acid synthesis, the expression of *Dgat2*, *Scd1*, *Elovl6*, *Gpat1*, and *Acsl5*, which were up-regulated in the livers of ZF rats, are also known to be elevated by insulin and SREBP-1c [33, 34]. DGAT2 co-localizes with SCD, suggesting that it may be linked with the esterification of endogenously formed MUFA in order to produce TAG [35]. This appears to be causal for the markedly increased proportion of 18:1n-9 in TAG in ZF rats. Importantly, apoptosis was enhanced in hepatocytes loaded with saturated fatty acids, but not unsaturated fatty acids, and this was found to be mediated by endoplasmic reticulum stress [36]. Therefore, TAG, which contains 18:1n-9 at high proportions, may serve as a protective reservoir in the pathogenesis of fatty liver [37, 38]. GPAT1 resides on the outer mitochondrial membrane and plays a role in diverting fatty acids towards the formation of TAG for storage and away from β-oxidation [39]. ACSL5 also acts as a branch-point for directing fatty acids into the pathways of complex lipid synthesis and away from β-oxidation [34]. Hyperinsulinemia has been shown to increase serum levels of FFA, which are taken up by the liver through FAT/CD36, FATP2, and FATP5, thereby driving the production of TAG [30, 40]. Therefore, increase in the expression of the *Fat*/*Cd36* gene in ZF rats may contribute to the hepatic accumulation

of TAG [41]. In contrast to increases in lipid acquisition, a marked elevation was not observed in the expression of the genes encoding enzymes involved in the degradation of fatty acids in the livers of ZF rats; the expression of the *Cpt1a* genes, the products of which paly crucial roles in degrading fatty acids [42, 43], was slightly reduced. Previous studies demonstrated that fatty acid oxidation was down-regulated in the livers of ZF rats [44, 45]. Since ZF rats are in leptin-related hyperphagia [2], the liver may convert surplus nutrients into TAG, which are increasingly secreted into the circulation as VLDL [30, 46]. Consequently, the changes occurring in fatty acid oxidation, but not VLDL secretion, appear to be causal for the accumulation of TAG in the livers of ZF rats. In contrast to ZF rats, the livers of GK rats exhibited no considerable up-regulation in the expression of genes encoding enzymes and proteins related to lipogenesis and fatty acid trafficking, except for significant increases in the expression of *G6pd*, *Acly*, and *Me1* and significant decreases in that of *Gpat1*, *Fat*/*Cd36*, and *Fatp5*. In contrast, the expression of genes for key enzymes and transcription factors participating in fatty acid degradation, CPT1a, UCP2, PPARα, PGC1α, and lipin 1, was significantly up-regulated in the livers of GK rats. PGC1α is known to increase the expression of lipin 1, which physically associates with PGC1α and PPARα in the nucleus in order to stimulate the transcription of PPARα target genes [47]. CPT1a, and UCP2 are considered to be under the control of PPARα [48, 49]. In the livers of GK rats, the expression of the *Srebp-1c* gene was markedly reduced and nuclear content of the mature form of SREBP-1c was unchanged. In GK rats, the expression of *Scd1* was up-regulated because this gene is under the control of not only SREBP-1c, but also PPARα; on one hand, the expression of *Elovl6* was unchanged. As a result, the proportion of 18:1n-9, which is synthesized by the concerted actions of SCD1 and ELOVL6, in hepatic TAG was markedly less than in ZF rats. We previously demonstrated that the activity of SCD was elevated, whereas that of ELOVL6 was not changed in the livers of GK rats, and that the activities of SCD and ELOVL6 were significantly augmented in the livers of ZF rats [12]. The present results, taken together, suggest that lipid acquisition (*de novo* lipogenesis and fatty acid uptake from the circulation) in the livers of GK rats is not as active as that in ZF rats and also that lipid degradation, one of lipid disposal processes, actively operates.

Since the changes that occurred in the expression of genes related to lipid acquisition and disposal did not clearly explain the moderate increase observed in TAG accumulation in the livers of GK rats, functional analyses were conducted on FAS activity *in vitro*, *de novo* synthesis of fatty acids and TAG *in vivo*, fatty acid oxidation in liver slices, and VLDL-TAG secretion *in vivo* in GK rats. Regarding lipid acquisition, the activity and protein level of FAS were significantly higher in GK rats than in control rats. Since the expression of the *Fas* gene was unchanged in the livers of GK rats, this induction of FAS is most likely to be responsible for a post-transcriptional regulation, such as an increase in translation or a decrease in degradation [50]. The levels of ACC protein in the liver were also significantly increased in the livers of GK rats, although the ratio of P-ACC to ACC in the livers of GK rats was higher than that of control rats. Moreover, serum glucose levels were higher, and the expression of *Acly*, *Me1*, and *G6pd* was elevated in their livers, implying increased supply of acetyl-CoA and NADPH from glucose through the glycolysis and pentose phosphate pathways. In fact, hepatic concentrations of acetyl-CoA were significantly higher in GK rats than in control rats. Collectively, these findings strongly suggest that *de novo* fatty acid synthesis is up-regulated *in vivo* in the livers of GK rats. As expected, the *in vivo* incorporation of [^{14}C]acetate into fatty acids increased and the formation of TAG, DAG, phospholipids and FFA from [^{14}C] acetate *in vivo* was confirmed to be significantly elevated in the livers of GK rats. Serum levels of FFA in GK rats were high and almost the same as those in ZF rats, indicating that the chronically increased FFA flux from the circulation may result in the storage of excess TAG within the liver irrespective of the reduced expression of *Fat*/*Cd36* in GK rats. Thus, lipid acquisition appears to be enhanced in the livers of GK rats. Regarding lipid disposal, [^{14}C]16:0 oxidation, which was measured using liver slices of GK rats, was markedly higher than that of control rats. In accordance with the results of gene expression, the hepatic level of the CPT1a protein was higher in GK rats than in control rats. The increase observed in the ratio of P-ACC to ACC may reduce the conversion of acetyl-CoA to malonyl-CoA and, moreover, the elevation of FAS activity may increase the utilization of malonyl-CoA for the synthesis of 16:0 in the livers of GK rats. These states may reinforce the activation of CPT1a by decreasing malonyl-CoA

concentrations [51]. Moreover, decreases in the expression of *Gpat1* may lead to increases in fatty acid oxidation because GPAT1 and CPT1 compete for the same long-chain acyl-CoA substrates, particularly newly synthesized fatty acyl-CoAs, and channel them toward either glycerolipid synthesis or β-oxidation [39]. The export of TAG as a VLDL component is the only means by which to reduce hepatic TAG concentrations other than fatty acid oxidation. Since serum concentrations of TAG were lower in GK rats than in control rats, impairments in TAG secretion by VLDL may worsen TAG accumulation. However, no significant reduction was observed in the rate of VLDL−TAG secretion in GK rats. The low level of serum TAG may be due to a decrease in the gene expression of apolipoprotein CIII, which inhibits lipoprotein lipase [52]. These findings, taken together, suggest that an increase in TAG accumulation due to elevated *de novo* lipogenesis and, possibly, a flux of FFA from the circulation is predominant and that the TAG accumulation is partially offset by increased fatty acid oxidation in the livers of GK rats.

Previous studies demonstrated that FAS was required in order to generate an endogenous ligand for PPARα in the liver [50, 53, 54], and this transcription factor promotes fatty acid oxidation and gluconeogenesis in the liver [55]. Moreover, insulin resistance in the liver increases glucose production [56]. These findings coincide with our present results; the expression of genes for fatty acid oxidation, *Cpt1a*, and gluconeogenesis, *Pepck* and *G6pase*, was up-regulated in the livers of GK rats. Although the increase observed in *Cpt1a* expression in the livers of GK rats was moderate (1.67-fold), it is important to note that the stimulation of fatty acid oxidation achieved by a moderate increase in the expression of *Cpt1a* gene is sufficient to markedly reduce hepatic TAG accumulation [57, 58].

In conclusion, the present study revealed that, in the livers of GK rats, (i) the accumulation of TAG was moderate, (ii) the *de novo* synthesis of fatty acids was increased by elevating the protein levels of FAS, apparently in a post-transcriptional manner, (iii) the promotion of the gene expression of PPARα concomitant with PGC1α and lipin 1contributed to suppressing the further accumulation of TAG by enhancing fatty acid oxidation through CPT1a induction, and (iv) the VLDL−TAG secretion rate remained unchanged. The present study demonstrated for the first time that *de novo* lipogenesis

was up-regulated, while fatty acid degradation was also elevated, such that TAG moderately accumulated in the livers of GK rats. The detailed molecular mechanisms underlying these aberrant metabolic alterations, particularly the non-transcriptional up-regulation of FAS, in the livers of GK rats still remain to be investigated.

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Conflict of Interest The authors declare no conflicts of interest.

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Figure legends

Fig. 1 Representative images showing the histology of liver sections. Liver sections from the WI rat (**a**), GK rat (**b**), ZL rat (**c**) and ZF rat (**d**) were stained with Oil Red O. Scale bars indicate 200 μm. Inserts show a higher magnification of the respective figure; scale bars in the inserts indicate 20 μm. (**e**) The intensity of fat accumulation in hepatocytes. The severity of histopathological findings was scored as (0) normal, (1) minimal, (2) mild, (3) moderate, and (4) marked lipid deposition in hepatocytes.

WI, Wistar rat; GK, Goto-Kakizaki rat, ZL, Lean Zucker (?/+) rat; ZF, Obese Zucker (*fa*/*fa*) rat.

Fig. 2 Gene and protein expression of transcription factors related to the synthesis and degradation of lipids in the liver. (**a**) The levels of mRNA in the livers of GK rats relative to those in the livers of WI rats. (**b**) The levels of mRNA in the livers of ZF rats relative to those in the livers of ZL rats. (**c**) The protein levels of the mature form (68 kDa) of SREBP-1c in the nucleus. Immunoblots were carried out on nuclear extracts from livers. Values represent means ± SD ($n = 4–14$). $^{*, **, ***}$ Significantly different from WI rats ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). $^{\#, \#\#}$ Significantly different from ZL rats ($^{\#}P < 0.05$; $^{\#\#}P < 0.01$).

WI, Wistar rat; GK, Goto-Kakizaki rat, ZL, Lean Zucker (?/+) rat; ZF, Obese Zucker (*fa*/*fa*) rat.

Fig. 3 *De novo* fatty acid synthesis in GK rat livers. GK and WI rats were intraperitoneally injected with [^{14}C]acetic acid, and the hepatic synthesis of fatty acids was estimated by measuring the incorporation of [^{14}C]acetic acid into fatty acids *in vivo*. Five minutes after the injection, rats were killed and livers were immediately isolated (**a**, **b**, **c**). (**a**) The incorporation of [^{14}C]acetic acid into fatty acids. Lipids were extracted from one portion of the liver; saponified once, and fatty acids were obtained by removing unsaponifiable matter. (**b**) Acetyl-CoA concentrations in the liver. Acetyl-CoA was extracted from one portion of the liver and determined. (**c**) The amount of acetyl-CoA

incorporated into fatty acids. This value was calculated from the data in Figures **a** and **b**. (**d**) FAS activity. The cytosol was prepared from the liver and FAS activity in the cytosol was assayed. Immunoblots were performed using extracts from the livers (**e**, **f**). (**e**) The FAS protein in the liver; the visible bands represent FAS and β-actin as indicated. (**f**) The ACC protein in the liver; visible bands represent ACC and β-actin as indicated. Values represent means ± SD ($n = 4$). $^{*, ****}$ Significantly different from WI rats ($^{*}P < 0.05$; $^{***}P < 0.001$). $^{\#, \#\#\#}$ Significantly different from ZL rats ($^{\#}P < 0.05$; $^{\#\#\#}P < 0.001$).

WI, Wistar rat; GK, Goto-Kakizaki rat, ZL, Lean Zucker (?/+) rat; ZF, Obese Zucker (*fa*/*fa*) rat.

Fig. 4 *De novo* lipogenesis in GK rat livers. Rats were intraperitoneally injected with [^{14}C]acetic acid, and lipogenesis was estimated by measuring the incorporation of [^{14}C]acetic acid into hepatic lipids *in vivo*. Five minutes after the injection, livers were immediately isolated; lipids were extracted and separated into TAG, DAG, PL, FFA, CE, and C by TLC. (**a**) The proportion (%) of radioactivity distributed among lipid classes. (**b**) Radioactivity incorporated into lipid classes. (**c**) The amount of acetyl-CoA incorporated into lipid classes. This value was calculated from the data in Figures 4b and 3b. Values represent means ± SD ($n = 4$). $^{*, **, ***}$ Significantly different from WI rats ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

WI, Wistar rat; GK, Goto-Kakizaki rat; TAG, triacylglycerols; DAG, diacylglycerols; PL, phospholipids; FFA, free fatty acids; CE, cholesteryl esters; C, cholesterol.

Fig. 5 Fatty acid disposal processes, fatty acid oxidation and VLDL–TAG secretion, in GK rat livers. Liver slices were incubated with [^{14}C] 16:0; $^{14}CO_2$ produced was trapped and [^{14}C]-labeled acid-soluble products were extracted (**a**, **b**, **c**). (**a**) $^{14}CO_2$ production. (**b**) [^{14}C]-labeled acid-soluble products. (**c**) The sum of $^{14}CO_2$ and [^{14}C]-labeled acid-soluble products. Immunoblots for CPT1a, ACC, and P-ACC were performed using liver extracts (**d**, **e**). (**d**) The protein levels of CPT1a; (**e**) The protein level ratio of P-ACC/ACC. (**f**) The VLDL–TAG secretion rate. Rats that had been starved for

12 h were intravenously injected with Triton WR1339. Blood was collected 1, 2, 3, 4, and 6 h after this administration. The VLDL secretion rate was determined by measuring changes in serum TAG levels. Values represent means ± SD (n = 4−6). $^{*, **, ***}$ Significantly different from WI rats ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

WI, Wistar rat; GK, Goto-Kakizaki rat.

Table 1 Sequences of primers used for real-time PCR.

Gene	Forward primer (5'−3')	Reverse primer (5'−3')	Accession No.
Fas	CGCCGACCAGTATAAACCCA	GTTGTAATCGGCACCCAAGTC	M76767
Acc1	AACGCCTTCACACCACCTTG	AGTCGCAGAAGCAGCCCAT	J03808
G6pd	CTTTGGACCCATCTGGAATCG	TCAAAATAGCCCCCACGACC	NM_017006
Acly	AAACTGTATCGCCCAGGCAGT	GTAACGCAGCACGTGATCCAT	J05210
Me1	ACAATACAGTTTGGCATTCCG	AGGATTCGCTCTCCATCAGTCA	NM_012600
Gpat1	AGACACAGGCAGGGAATCCAC	AATTCCCGGAGAAGCCCAG	AF021348
Gpat4	TTGGAGTCCTGGAATTTGCTGA	GGCTAATCCCTGTGAATGCCA	NM_001047849
Lipin2	ACCCTGTTCCCAGCCCATCAG	GGTGCTGGCTTCTTTTGTGA	NM_001108236
Lipin3	ATCCTGAGTTCTCGTTGGTC	GATCTCAAAGTGTCCACGCC	NM_001014184
Dgat1	CCGTGGTATCCTGAATTGGT	GGCGCTTCTCAATCTGAAAT	NM_053437
Dgat2	ATCTTCTCTGTCACCTGGCT	ACCTTTCTTGGGCGTGTTCC	NM_001012345
Scd1	TCACCTTGAGAGAAGAATTAGCA	TTCCCATTCCCTTCACTCTGA	J02585
Elovl5	ACCACCATGCCACTATGCTCA	GGACGTGGATGAAGCTGTTG	AB071985
Elovl6	AGAACACGTAGCGACTCCGAA	CAAACGCGTAAGCCCAGAAT	AB071986
Fat/Cd36	CGAAGGCTTGAATCCTACCG	TGTTGACCTGCAGTCGTTT	NM_031561
Fatp2	TTCAACAGTGGCGATCTCCTG	ACCGGAAGGTGTCTCCAACT	NM_031736
Fatp4	CCTGGTGTACTATGGATTCCGC	GCTGAAAACTTCTTCCGGATCA	NM_001100706
Fatp5	TTGCGAACGTACGGCAAGTAG	AAGGCGGTCTCGGAAGTAGAAG	NM_024143
Fabppm	TCTGCCAATCCTATGCCAA	CACCCTTTTGGCTTCTTC	NM_013177
Fabp1	CGGCAAGTACCAAGTGCAGAG	CTGACACCCCCTTGATGTCCT	BC086947
Acsl1	TCAGAGCAGTTCATCGGCATC	GTCGGTTCCAAGCGTGTCATA	NM_012820
Acsl3	GGTGGCCAAAATGTGACAATG	AAACTCTCCAATATCGCCAGT	NM_057107
Acsl5	CAAACATGGCTGCTTTCCTCA	ACCCTGGACAAGCCTCTCAAA	NM_053607
Cpt1a	AAGGCAGCGTTCTTCGTGA	GTCAAAGCATCTTCCATGC	NM_031559
Mcad	CTTTGCCTCTATTGCGAAGGC	TCCGAAAATCTGCACAGCATC	J02791
Lcad	TGTATTGGTGCCATAGCCATGA	CCCAGACCTTTTGGCATTTGT	L11276
Vlcad	ACAGCTTTCGTAGTGGAACGGA	CTGGCACCTTGACTCCATCAA	D30647
Acox1	ACTACGACGACCTCCCCAAGA	TGGCCACGCAGGTAGTTCA	NM_031315
UCP2	CAAGACCATTGCACGAGAGGA	CAGTTGACAATGGCATTTCGG	NM_019354.2
Acc2	AGAGCGGCCGGGTGAACTA	CGCGTGCACTTTTGAAGGG	NM_053922.1
Mcd	CGGGAAATGAACGGAGTGCTAA	CAGCCTCACAATCGCTGATCTT	NM_053447.1
Apoc3	GACAATCGCTTCAAATCCCT	CGGCTCAAGAGTTGGTGTTG	NM_012501
Mtp	ACGTGGTATTCCCGCCTCA	CGTCAAAGCATTTCGTTCTCG	BC012686
Gck	TGTCACCGACTGCGACATTG	GCATGCGATTTATGACCCCA	M25807
G6pase	CAGCCTCTTCAAAAACCTGG	GAGCGACTTGCGGAGTTCTC	L37333
Pepck	TGGCTGGATGAAGTTTGATG	GCCCGGAGCAACTCCAAAAA	NM_198780
Lpk	TGTGTACCACCGCCAGTTGTT	AGCACTTGAAGGAAGCCTCCA	M17685
Glut2	CACACCAGCACATACGACACC	ACTGCAAAGCTGGACACAGA	NM_012879
Pparα	AATGCCCTCGAACTGGATGAC	CACAATCCCCTCCTGCAACTT	NM_013196
Pgc1α	CGATGACCCTCCTCACAC	TTGGCTTGAGCATGTTGCG	NM_031347
Lipin1	AGGGAGGGAGATGGTGGTTT	CTCTCCGGTATTGTGGCCCTT	NM_001012111

Srebp1c	GGAGCCATGGATTGCACATT	AGGAAGGCTTCCAGAGAGGA	AF286469
Chrebp	AATAGAGGAGCTCAATGCT	CCCAGAACTTCCAGTTGTGC	AB074517
Lxrα	CCACAGCTCAGCCCAGAA	GGCGTGACTCGAAGTCGGT	NM_031627
Hnf4α	CATCTTCTTTGACCCAGATGCC	CATACTGCCGGTCGTTGATGT	NM_022180.1
Irs-1	TACATCCCAGGTGCTACCAT	CCAGCCGAGTGAGTTCTCTT	NM_012969.1
Irs-2	TGCTACAGCACATTGCCCCG	TTCCAAAATCCGACCCACAG	NM_0101168633.1
Acot1	ACTACGACGACCTCCCCAAGA	TGGCCACGCAGGTAGTTCA	NM_031315.1
Cyp7a1	GAATTGCCGTGTTGGTGAG	AGGTACGGAATCAACCCGTTC	NM_012942.2
β-Actin	TGCAGAAGGAGATTACTGCC	CGCAGCTCAGTAACAGTCC	V01217

Table 2 Physiological, serum and hepatic measurements

	WI	GK	ZL	ZF
Body weight (g)	311 ± 3	266 ± 19**	281 ± 8	452 ± 42$^{\#\#\#}$
Organ weights (g/100 g body weight)				
Liver	4.21 ± 0.23	4.43 ± 0.11	3.80 ± 0.07	4.72 ± 0.30$^{\#\#\#}$
Epididymal fat	0.99 ± 0.11	0.95 ± 0.06	0.86 ± 0.10	2.72 ± 0.30$^{\#\#\#}$
Mesenteric fat	0.67 ± 0.04	0.75 ± 0.05*	0.62 ± 0.07	1.58 ± 0.10$^{\#\#}$
Serum parameters				
Glucose (mmol/L)	9.99 ± 1.17	19.15 ± 2.39***	10.66 ± 0.72	12.21 ± 2.11
TAG (μmol/L)	1.94 ± 0.62	1.14 ± 0.36*	1.46 ± 0.38	8.91 ± 1.37$^{\#\#\#}$
Cholesterol (μmol/L)	1.64 ± 0.09	2.72 ± 0.13***	1.65 ± 0.10	3.02 ± 0.34$^{\#\#\#}$
FFA (mmol/L)	0.26 ± 0.04	0.49 ± 0.10***	0.29 ± 0.06	0.60 ± 0.11$^{\#}$
Insulin (pmol/L)	407.2 ± 172.5	728.1 ± 146.7**	448.6 ± 113.9	3212.6 ± 4118.4$^{\#\#}$
Hepatic lipids (μmol/g liver)				
TAG	9.16 ± 1.06	13.53 ± 1.87***	6.01 ± 1.03	34.36 ± 10.60$^{\#\#}$
DAG	1.74 ± 0.17	2.03 ± 0.21*	1.76 ± 0.23	2.10 ± 0.17$^{\#}$
FFA	1.55 ± 0.21	1.57 ± 0.21	1.55 ± 0.14	1.51 ± 0.19
Phospholipids	37.78 ± 1.14	36.33 ± 2.14	39.27 ± 0.97	35.54 ± 1.79$^{\#\#}$
Total cholesterol	5.60 ± 0.36	5.37 ± 0.29	6.25 ± 0.75	4.94 ± 0.65$^{\#\#}$
CE	1.56 ± 0.36	1.32 ± 0.21	1.68 ± 0.31	1.54 ± 0.45

Values represent means ± SD (n = 4–8). $^{*, **, ***}$ Significantly different from WI rats ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). $^{\#, \#\#, \#\#\#}$ Significantly different from ZL rats ($^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$). In the absence of a superscript, the difference in the means is not significant ($P > 0.05$).

WI, Wistar rat; GK, Goto-Kakizaki rat; ZL, Lean Zucker (?/+) rat; ZF, Obese Zucker (*fa*/*fa*) rat. TAG, triacylglycerols; DAG, diacylglycerols; FFA, unesterified fatty acids; CE, cholesteryl esters.

Table 3 Fatty acid profiles of hepatic lipids in GK and ZF rats

Fatty acids	WI	GK	ZL	ZF
TAG		(mol%)		
16:0	29.27 ± 1.67	30.62 ± 0.76	27.78 ± 1.08	37.89 ± 1.90$^{\#\#\#}$
16:1n-7	2.87 ± 0.45	4.31 ± 0.46***	2.25 ± 0.43	9.22 ± 0.69$^{\#\#\#}$
18:0	9.25 ± 1.57	7.81 ± 1.10*	4.91 ± 0.77	2.67 ± 0.63$^{\#\#\#}$
18:1n-9	15.44 ± 1.26	16.06 ± 0.64	18.44 ± 1.00	30.85 ± 1.20$^{\#\#\#}$
18:1n-7	3.90 ± 0.39	5.18 ± 0.43***	3.08 ± 0.36	5.55 ± 0.93$^{\#\#\#}$
18:2n-6	26.13 ± 1.83	21.96 ± 1.71**	30.03 ± 1.62	10.49 ± 1.54$^{\#\#\#}$
18:3n-3	1.03 ± 0.13	0.82 ± 0.05**	1.34 ± 0.12	0.63 ± 0.15$^{\#\#\#}$
20:3n-9	0.24 ± 0.04	0.22 ± 0.03	0.38 ± 0.06	0.19 ± 0.09$^{\#\#\#}$
20:3n-6	0.52 ± 0.08	0.48 ± 0.04	0.38 ± 0.04	0.19 ± 0.04$^{\#\#\#}$
20:4n-6	4.60 ± 0.77	5.56 ± 0.44*	2.20 ± 0.32	0.34 ± 0.05$^{\#\#\#}$
20:5n-3	1.35 ± 0.14	1.50 ± 0.19	1.70 ± 0.26	0.39 ± 0.07$^{\#\#\#}$
22:5n-3	1.99 ± 0.38	2.07 ± 0.30	2.77 ± 0.28	0.58 ± 0.14$^{\#\#\#}$
22:6n-3	3.40 ± 0.60	3.41 ± 0.28	4.74 ± 0.32	1.03 ± 0.26$^{\#\#\#}$
Total (μmol/g liver)	27.48 ± 3.20	40.59 ± 5.60***	18.02 ± 3.02	103.1 ± 31.81$^{\#\#\#}$
Phosapholipids		(mol%)		
16:0	21.24 ± 0.75	19.49 ± 0.70***	20.71 ± 0.95	20.32 ± 0.89
16:1n-7	0.85 ± 0.11	1.15 ± 0.15**	0.67 ± 0.12	2.30 ± 0.21$^{\#\#\#}$
18:0	21.17 ± 0.58	21.89 ± 0.26**	22.14 ± 0.73	24.59 ± 1.47$^{\#\#}$
18:1n-9	3.47 ± 0.22	2.94 ± 0.20***	3.62 ± 0.17	5.31 ± 0.32$^{\#\#\#}$
18:1n-7	4.10 ± 0.36	5.00 ± 0.19***	3.17 ± 0.44	3.26 ± 0.68
18:2n-6	18.34 ± 0.82	11.96 ± 0.52***	20.24 ± 0.98	19.43 ± 1.18
18:3n-3	0.21 ± 0.13	0.07 ± 0.01*	0.12 ± 0.01	0.09 ± 0.02$^{\#\#}$
20:3n-9	0.15 ± 0.03	0.16 ± 0.02	0.42 ± 0.11	0.35 ± 0.06
20:3n-6	1.34 ± 0.19	1.19 ± 0.17	1.10 ± 0.12	3.29 ± 0.21$^{\#\#\#}$
20:4n-6	21.40 ± 1.03	27.47 ± 1.30***	21.21 ± 0.99	13.51 ± 0.40$^{\#\#\#}$
20:5n-3	1.16 ± 0.11	0.74 ± 0.17***	1.01 ± 0.13	1.48 ± 0.25$^{\#\#}$
22:5n-3	1.30 ± 0.14	1.53 ± 0.09**	1.20 ± 0.13	1.10 ± 0.18
22:6n-3	5.29 ± 0.43	6.43 ± 0.32***	4.40 ± 0.49	4.97 ± 0.84
Total (μmol/g liver)	54.58 ± 2.08	49.41 ± 1.20***	63.02 ± 3.00	60.58 ± 2.20
DAG		(mol%)		
16:0	11.25 ± 1.25	11.69 ± 1.82	10.65 ± 1.57	16.83 ± 2.96$^{\#\#\#}$
16:1n-7	1.82 ± 0.36	3.66 ± 0.54***	1.15 ± 0.17	6.13 ± 0.55$^{\#\#\#}$
18:0	8.84 ± 1.06	7.71 ± 1.39	8.15 ± 0.71	7.50 ± 0.67
18:1n-9	4.75 ± 0.61	5.52 ± 0.47*	4.61 ± 1.09	8.86 ± 0.66$^{\#\#\#}$
18:1n-7	6.93 ± 0.44	9.23 ± 0.35***	6.97 ± 0.55	7.18 ± 0.55
18:2n-6	57.14 ± 1.37	51.49 ± 2.91***	59.78 ± 2.95	45.85 ± 2.91$^{\#\#\#}$
18:3n-3	0.34 ± 0.05	0.27 ± 0.05*	0.36 ± 0.03	0.28 ± 0.07$^{\#}$
20:3n-9	0.91 ± 0.26	1.01 ± 0.22	0.65 ± 0.13	0.60 ± 0.06

20:3n-6	1.52 ± 0.31	1.83 ± 0.21*	0.95 ± 0.10	2.59 ± 0.28$^{\#\#\#}$
20:4n-6	5.32 ± 0.51	6.12 ± 0.40**	5.61 ± 0.61	3.29 ± 0.22$^{\#\#\#}$
20:5n-3	0.39 ± 0.16	0.43 ± 0.12	0.27 ± 0.07	0.26 ± 0.13
22:5n-3	0.26 ± 0.22	0.33 ± 0.14	0.29 ± 0.17	0.22 ± 0.18
22:6n-3	0.55 ± 0.29	0.71 ± 0.16	0.56 ± 0.37	0.40 ± 0.26
Total (μmol/g liver)	3.48 ± 0.34	4.05 ± 0.41*	3.52 ± 0.46	4.20 ± 0.33$^{\#\#}$
FFA		(mol%)		
16:0	40.32 ± 2.93	39.02 ± 2.35	44.59 ± 4.66	42.48 ± 5.87
16:1n-7	1.21 ± 0.24	1.36 ± 0.29	0.87 ± 0.10	2.32 ± 0.26$^{\#\#\#}$
18:0	25.01 ± 1.13	25.46 ± 1.60	24.89 ± 1.30	26.40 ± 3.43
18:1n-9	4.49 ± 0.72	4.09 ± 0.15	4.22 ± 0.95	5.78 ± 2.05
18:1n-7	3.02 ± 0.83	4.32 ± 0.35**	2.30 ± 0.19	2.36 ± 0.56
18:2n-6	12.37 ± 0.59	10.20 ± 0.71**	11.66 ± 1.16	9.86 ± 1.34$^{\#}$
18:3n-3	0.76 ± 0.40	0.54 ± 0.15	0.93 ± 0.22	1.14 ± 0.43
20:3n-9	0.81 ± 0.72	0.80 ± 0.42	0.33 ± 0.09	1.06 ± 1.23
20:3n-6	0.93 ± 0.20	0.70 ± 0.08*	0.42 ± 0.09	1.45 ± 0.21$^{\#\#\#}$
20:4n-6	8.27 ± 1.28	10.58 ± 0.58**	8.02 ± 1.13	5.32 ± 0.81$^{\#\#}$
20:5n-3	0.77 ± 0.16	0.48 ± 0.06***	0.49 ± 0.09	0.76 ± 0.28$^{\#}$
22:5n-3	0.55 ± 0.23	0.78 ± 0.15*	0.38 ± 0.29	0.24 ± 0.32
22:6n-3	1.51 ± 0.26	1.68 ± 0.27	0.88 ± 0.51	0.83 ± 0.50
Total (μmol/g liver)	1.51 ± 0.21	1.57 ± 0.21	1.55 ± 0.14	1.51 ± 0.19
CE		(mol%)		
16:0	45.40 ± 3.02	41.70 ± 7.46	47.86 ± 7.89	52.61 ± 5.30
16:1n-7	3.74 ± 0.58	5.81 ± 1.13**	2.36 ± 0.40	6.50 ± 1.64$^{\#\#}$
18:0	14.75 ± 2.95	13.16 ± 2.55	13.43 ± 1.91	15.35 ± 4.88
18:1n-9	13.27 ± 2.28	13.38 ± 1.80	12.44 ± 3.30	10.60 ± 2.61
18:1n-7	2.32 ± 0.31	3.10 ± 0.26**	1.84 ± 0.28	1.99 ± 0.64
18:2n-6	12.02 ± 1.03	11.41 ± 1.61	14.17 ± 3.21	7.84 ± 2.34$^{\#\#}$
18:3n-3	1.24 ± 0.28	0.77 ± 0.20**	0.45 ± 0.23	0.09 ± 0.17$^{\#\#}$
20:3n-9	2.25 ± 0.71	2.06 ± 0.68	2.76 ± 2.66	2.17 ± 0.69
20:3n-6	0.16 ± 0.10	0.14 ± 0.13	0.05 ± 0.11	0.03 ± 0.07
20:4n-6	3.59 ± 0.48	7.01 ± 3.01*	4.26 ± 1.16	2.37 ± 0.81$^{\#\#}$
20:5n-3	1.27 ± 0.26	1.46 ± 0.48	0.38 ± 0.20	0.45 ± 0.30
22:5n-3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:6n-3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total (μmol/g liver)	1.56 ± 0.36	1.32 ± 0.21	1.68 ± 0.31	1.54 ± 0.45

Values represent means ± SD (n = 4–6). $^{*, **, ***}$Significantly different from WI rats ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). $^{\#, \#\#, \#\#\#}$ Significantly different from ZL rats ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$). In the absence of a superscript, the difference in the means is not significant ($P > 0.05$).

Fatty acids are designated by the numbers of carbon atoms and double bonds; palmitic acid, 16:0;

palmitoleic acid, 16:1n-7; stearic acid, 18:0; oleic acid, 18:1n-9; *cis*-vaccenic acid, 18:1n-7; linoleic acid, 18:2n-6; α-linolenic acid, 18:3n-3; 5,8,11-eicosatrienoic acid, 20:3n-9; 8,11,14-eicosatrienoic acid, 20:3n-6; arachidonic acid, 20:4n-6; 5,8,11,14,17-eicosapentaenoic acid, 20:5n-3; 7,10,13,16,19-docosapentaenoic acid, 22:5n-3; 4,7,10,13,16,19-docosahexaenoic acid, 22:6n-3.

Table 4 Gene expression in the liver

Gene	WI	GK	ZL	ZF
Lipogenesis				
Fas	1.00 ± 0.36	0.72 ± 0.24	1.00 ± 0.42	8.96 ± 2.30$^{\#\#\#}$
Acc1	1.00 ± 0.23	0.94 ± 0.10	1.00 ± 0.25	4.34 ± 0.85 $^{\#\#\#}$
G6pd	1.00 ± 0.31	1.98 ± 0.36 ***	1.00 ± 0.15	1.94 ± 0.59 $^{\#\#}$
Acly	1.00 ± 0.29	1.60 ± 0.49**	1.00 ± 0.08	4.19 ± 0.92$^{\#\#\#}$
Me1	1.00 ± 0.28	1.98 ± 0.55**	1.00 ± 0.36	4.07 ± 0.80$^{\#\#\#}$
Gpat1	1.00 ± 0.14	0.73 ± 0.09**	1.00 ± 0.14	3.31 ± 0.29$^{\#\#\#}$
Gpat4	1.00 ± 0.20	0.87 ± 0.09	1.00 ± 0.21	0.95 ± 0.24
Lipin2	1.00 ± 0.28	1.50 ± 0.11**	1.00 ± 0.21	0.88 ± 0.13
Lipin3	1.00 ± 0.57	0.65 ± 0.10	1.00 ± 0.24	1.25 ± 0.20
Dgat1	1.00 ± 0.22	1.08 ± 0.30	1.00 ± 0.34	1.61 ± 0.59
Dgat2	1.00 ± 0.24	0.84 ± 0.11	1.00 ± 0.05	1.78 ± 0.35$^{\#\#}$
Fatty acid modification				
Scd1	1.00 ± 0.29	1.93 ± 0.29***	1.00 ± 0.91	8.88 ± 1.37$^{\#\#\#}$
Elovl5	1.00 ± 0.16	1.60 ± 0.23***	1.00 ± 0.12	1.19 ± 0.26
Elovl6	1.00 ± 0.50	1.21 ± 0.34	1.00 ± 0.36	29.32 ± 11.70$^{\#\#}$
Fatty acid trafficking				
Fat/Cd36	1.00 ± 0.29	0.72 ± 0.15*	1.00 ± 0.22	1.97 ± 0.47$^{\#\#\#}$
Fatp2	1.00 ± 0.19	0.99 ± 0.15	1.00 ± 0.08	0.94 ± 0.19
Fatp4	1.00 ± 0.18	1.27 ± 0.32	1.00 ± 0.29	1.07 ± 0.34
Fatp5	1.00 ± 0.17	0.78 ± 0.04*	1.00 ± 0.18	1.04 ± 0.39
Fabppm	1.00 ± 0.21	1.02 ± 0.14	1.00 ± 0.18	0.93 ± 0.36
Fabp1	1.00 ± 0.21	0.97 ± 0.19	1.00 ± 0.18	0.75 ± 0.22
Acsl1	1.00 ± 0.14	0.96 ± 0.07	1.00 ± 0.16	0.91 ± 0.12
Acsl3	1.00 ± 0.19	0.89 ± 0.16	1.00 ± 0.26	0.95 ± 0.45
Acsl5	1.00 ± 0.26	0.73 ± 0.26	1.00 ± 0.32	1.74 ± 0.30$^{\#\#}$
Fatty acid degradation				
Cpt1a	1.00 ± 0.41	1.58 ± 0.34*	1.00 ± 0.51	0.53 ± 0.29
Mcad	1.00 ± 0.14	1.06 ± 0.09	1.00 ± 0.12	0.94 ± 0.16
Lcad	1.00 ± 0.13	1.09 ± 0.10	1.00 ± 0.07	1.21 ± 0.20$^{\#}$
Vlcad	1.00 ± 0.20	1.06 ± 0.18	1.00 ± 0.10	1.08 ± 0.12
Acox1	1.00 ± 0.23	1.10 ± 0.11	1.00 ± 0.22	1.01 ± 0.12
Ucp2	1.00 ± 0.24	1.43 ± 0.19**	1.00 ± 0.11	0.99 ± 0.19
Acc2	1.00 ± 0.19	1.07 ± 0.12	1.00 ± 0.32	1.01 ± 0.32
Mcd	1.00 ± 0.33	1.09 ± 0.07	1.00 ± 0.14	0.85 ± 0.13
Lipoprotein metabolism				

Apoc3	1.00 ± 0.17	0.76 ± 0.11*	1.00 ± 0.15	1.37 ± 0.15$^{\#\#}$
Mtp	1.00 ± 0.09	0.92 ± 0.11	1.00 ± 0.34	1.23 ± 0.16
Glucose metabolism				
Gck	1.00 ± 0.39	1.27 ± 0.45	1.00 ± 0.29	1.34 ± 0.55
G6pase	1.00 ± 0.59	1.74 ± 0.30*	1.00 ± 0.47	0.78 ± 0.26
Pepck	1.00 ± 0.27	1.39 ± 0.12**	1.00 ± 0.11	0.54 ± 0.12$^{\#\#\#}$
Lpk	1.00 ± 0.18	1.19 ± 0.20	1.00 ± 0.28	3.52 ± 0.41$^{\#\#\#}$
Glut2	1.00 ± 0.19	1.18 ± 0.10	1.00 ± 0.34	1.23 ± 0.05
Others				
Irs-1	1.00 ± 0.26	1.27 ± 0.09*	1.00 ± 0.05	0.79 ± 0.19$^{\#}$
Irs-2	1.00 ± 0.21	0.86 ± 0.25	1.00 ± 0.45	0.34 ± 0.08$^{\#\#}$
Acot1	1.00 ± 0.24	1.48 ± 0.36**	1.00 ± 0.15	1.17 ± 0.23
Cyp7a1	1.00 ± 0.51	1.11 ± 0.25	1.00 ± 0.79	1.05 ± 0.26

Values represent means ± SD (n = 6–14). *, **, *** Significantly different from WI rats ($^{*}P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). $^{\#}$, $^{\#\#}$, $^{\#\#\#}$ Significantly different from ZL rats ($^{\#}P < 0.05$; $^{\#\#}$ $P < 0.01$; $^{\#\#\#}$ $P < 0.001$). In the absence of a superscript, the difference in the means is not significant ($P > 0.05$).

Fig. 1

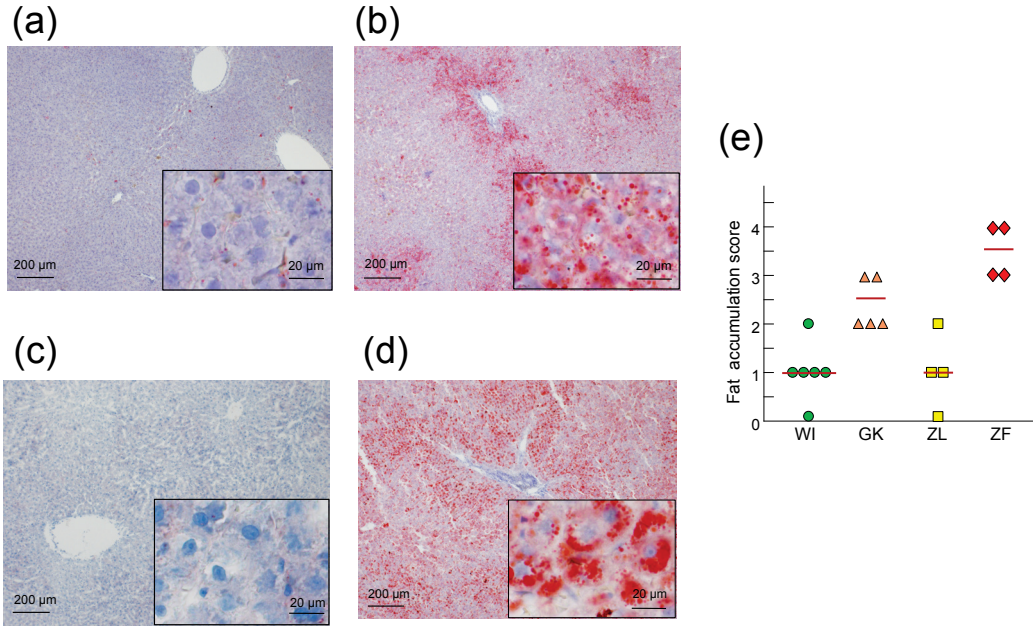


Fig. 2

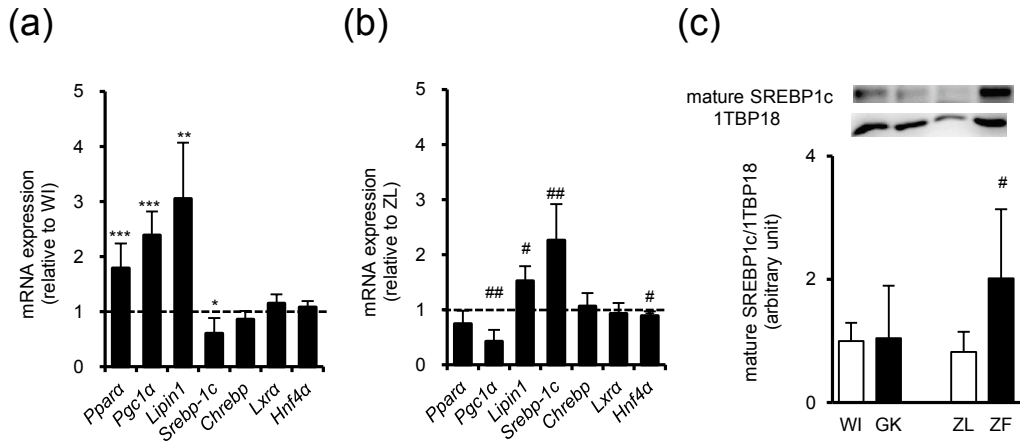

(a)
(b)
(c)
mRNA expression (relative to WI)
mRNA expression (relative to ZL)
mature SREBP1c
1TBP18
mature SREBP1c/1TBP18 (arbitrary unit)
Pparα
Pgc1α
Lipin1
Srebp-1c
Chrebp
Lxrα
Hnf4α
WI
GK
ZL
ZF

Fig. 3

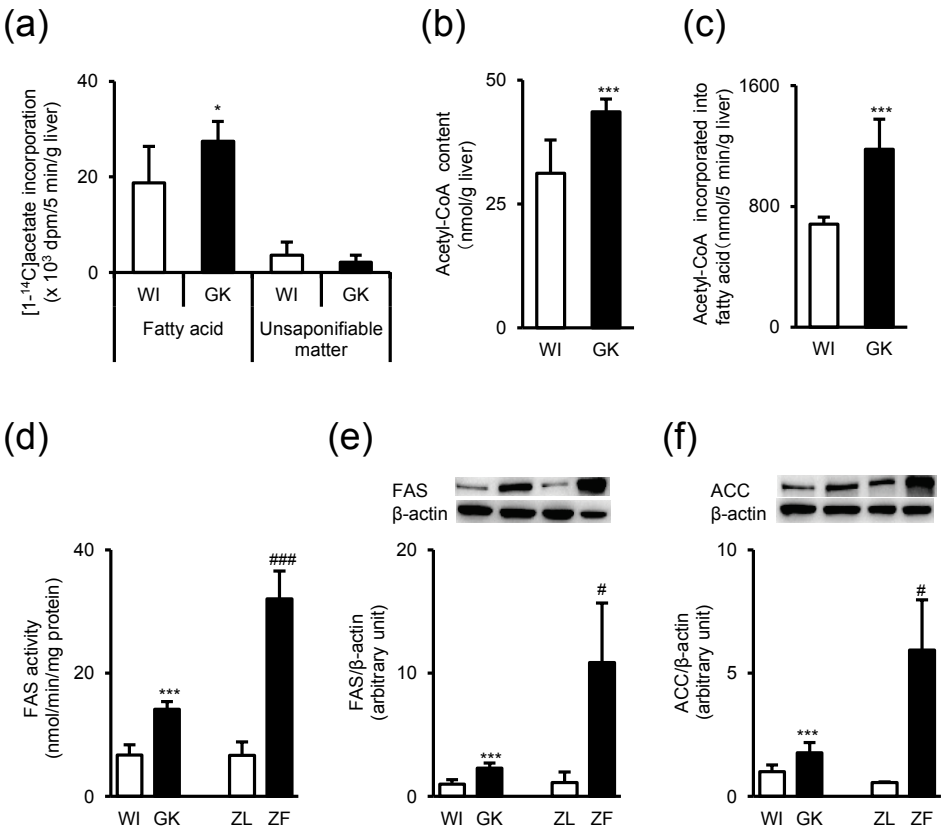

(a)
[1-14C]acetate incorporation (x 10^3 dpm/5 min/g liver)
40
20
0
*
WI
GK
WI
GK
Fatty acid
Unsaponifiable matter
(b)
Acetyl-CoA content (nmol/g liver)
50
25
0
***
WI
GK
(c)
Acetyl-CoA incorporated into fatty acid (nmol/5 min/g liver)
1600
800
0
***
WI
GK
(d)
FAS activity (nmol/min/mg protein)
40
20
0
***
###
WI
GK
ZL
ZF
(e)
FAS
β-actin
FAS/β-actin (arbitrary unit)
20
10
0
***
#
WI
GK
ZL
ZF
(f)
ACC
β-actin
ACC/β-actin (arbitrary unit)
10
5
0
***
#
WI
GK
ZL
ZF

Fig. 4

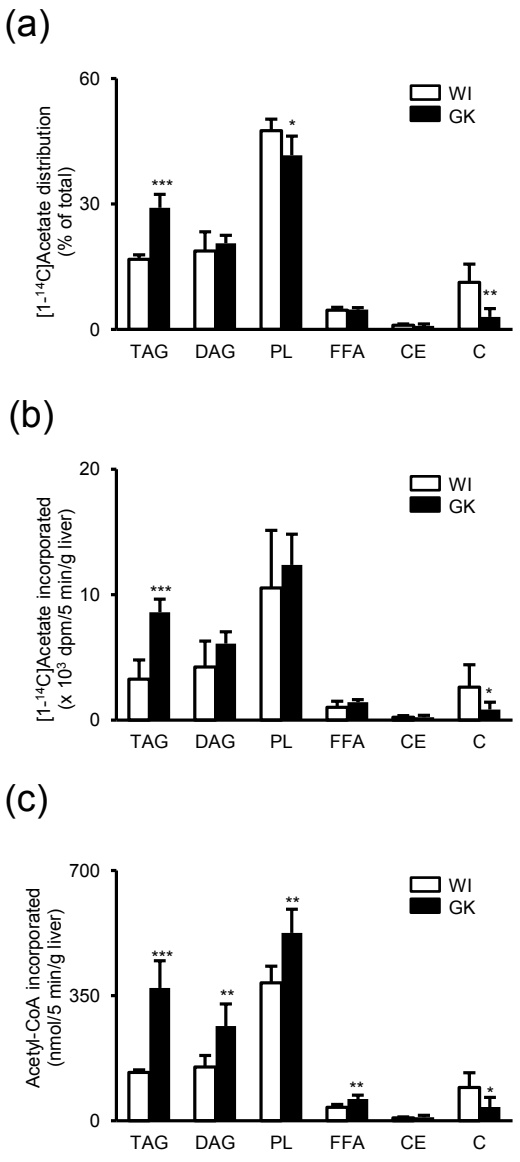
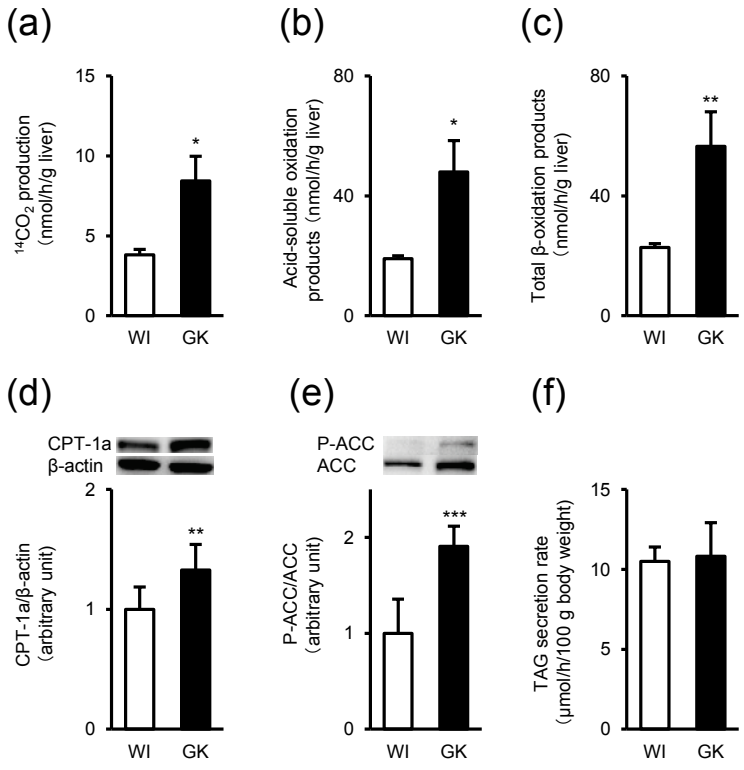

(a)
60
30
0
[1-14C]Acetate distribution (% of total)
WI
GK
***
*
**
TAG
DAG
PL
FFA
CE
C
(b)
20
10
0
[1-14C]Acetate incorporated (x 10³ dpm/5 min/g liver)
WI
GK
***
*
TAG
DAG
PL
FFA
CE
C
(c)
700
350
0
Acetyl-CoA incorporated (nmol/5 min/g liver)
WI
GK
***
**
**
**
*
TAG
DAG
PL
FFA
CE
C

Fig. 5


(a)
(b)
(c)
$^{14}CO_2$ production (nmol/h/g liver)
Acid-soluble oxidation products (nmol/h/g liver)
Total β-oxidation products (nmol/h/g liver)
WI
GK
(d)
(e)
(f)
CPT-1a
β-actin
CPT-1a/β-actin (arbitrary unit)
P-ACC
ACC
P-ACC/ACC (arbitrary unit)
TAG secretion rate (μmol/h/100 g body weight)