Coenzyme A and Its Thioester Pools in Obese Zucker and Zucker Diabetic Fatty Rats

Shigeru Chohnan 1,*, Shiori Matsuno 1, Kei Shimizu 1, Yuka Tokutake 2, Daisuke Kohari 1 and Atsushi Toyoda 1

1 Department of Food and Life Sciences, Ibaraki University College of Agriculture, 3-21-1 Chuo, Ami, Ibaraki 300-0393, Japan; shiori.matsuno.afb@gmail.com (S.M.); sksk19900928@yahoo.co.jp (K.S.); daisuke.kohari.abw@vc.ibaraki.ac.jp (D.K.); atsushi.toyoda.0516@vc.ibaraki.ac.jp (A.T.)
2 Department of Applied Life Science, United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai, Fuchu, Tokyo 183-8509, Japan; y.tokutake.afb@gmail.com
* Correspondence: shigeru.chohnan.agr@vc.ibaraki.ac.jp; Tel.: +81-29-888-8672

Received: 13 January 2020; Accepted: 3 February 2020; Published: 6 February 2020

Abstract: Feeding behavior is closely related to hypothalamic malonyl-CoA level in the brain and diet-induced obesity affects total CoA pools in liver. Herein, we performed a comprehensive analysis of the CoA pools formed in thirteen tissues of Zucker and Zucker diabetic fatty (ZDF) rats. Hypothalamic malonyl-CoA levels in obese rats remained low and were almost the same as those of lean rats, despite obese rats having much higher content of leptin, insulin, and glucose in their sera. Regardless of the fa-genotypes, larger total CoA pools were formed in the livers of ZDF rats and the size of hepatic total CoA pools in Zucker rats showed almost one tenth of the size of ZDF rats. The decreased total CoA pool sizes in Zucker rats was observed in the brown adipose tissues, while ZDF-fatty rats possessed 6% of total CoA pool in the lean rats in response to fa deficiency. This substantially lower CoA content in the obese rats would be disadvantageous to non-shivering thermogenesis. Thus, comparing the intracellular CoA behaviors between Zucker and ZDF rats, as well as the lean and fatty rats of each strain would help to elucidate features of obesity and type 2 diabetes in combination with result (s) of differential gene expression analysis and/or comparative genomics.

Keywords: coenzyme A; acetyl-CoA; malonyl-CoA; Zucker rat; ZDF rat

1. Introduction

Coenzyme A (CoA) participates in numerous metabolic pathways as an acyl carrier in cells. This cofactor is synthesized through five enzymatic steps [1]. Pantothenate kinase (PanK) is a key enzyme catalyzing the first step in the production of 5′-phosphopantothenate. Four PanK homologs are encoded in mouse chromosomes and the expression pattern of the PANK genes is found to differ in tissues [1]. For example, PANK1 and PANK3 are prevalently expressed in murine liver, while PANK2 and PANK3 are predominant in the testis and brain [2]. Recently, human PANK4 was revealed to be a pseudogene whose product does not display PanK activity [3]. Nevertheless, active PanKs provide CoA in tissues and are regulated by non-esterified CoA (CoASH) and acyl-CoAs. In addition, hypothalamic malonyl-CoA displayed a positive response to feeding behavior, leading to the depression of orexigenic neuropeptides and an increase in anorexigenic peptides [4]. As a result, this observation was applied to the feeding behavior in socially defeat-stressed mice [5]. Previously, we conducted a comprehensive analysis of CoA pools in thirteen tissues of Wistar rats [6,7]. The liver, heart, and brown adipose tissue have larger total CoA pools consisting of CoASH, acetyl-CoA, and malonyl-CoA than other tissues. In obese rats that are continuously fed a high-fat diet (HFD), the hepatic total CoA pool size shrank to
one fifth of that of rats fed a high-carbohydrate diet (HCD) and a high-protein diet (HPD). However, CoA pools in the heart and brown adipose tissue were not affected by diet composition. In the brain, diet-induced obesity caused by HFD resulted in elevated malonyl-CoA level in the hypothalamus, indicating an energy-sufficient status. Furthermore, malonyl-CoA level exceeding that of acetyl-CoA was observed in the skeletal muscle of obese rats. Thus, CoA pools were found to be acutely influenced by the quality of diet and feeding, especially in pools of the hypothalamus, skeletal muscle, and liver.

Metabolic diseases, such as obesity and type 2 diabetes, are continuously increasing worldwide and have now become a serious problem. Model animal research contributes to the understanding of these diseases via basic and clinical studies. Because Zucker [8–10] and Zucker diabetic fatty (ZDF) [11] rats are widely used as model animals of obesity and type 2 diabetes, respectively, they were employed in the present study. Zucker rats have a missense mutation (fa) in the leptin receptor (Lepr) gene [12–14] and because of this point mutation, the homozygous (fa/fa) mutants exhibit hyperphagia, defective non-shivering thermogenesis, and energy deposition in the adipose tissue, which consequently lead to obesity [15]. In addition, obese rats display endocrinological defects such as insulin resistance, dyslipidemia, glucose intolerance, and hyperinsulinemia. ZDF rats are derived from the Zucker strain and exhibit non-insulin-dependent diabetes mellitus together with obesity [11,16]. Fatty rats are thus widely used in research on type 2 diabetes and they display insulin resistance, hyperglycemia, hyperlipidemia, moderate hypertension, and progressive renal injury [17].

In this study, we sought to provide information on acetyl-CoA, malonyl-CoA, CoASH, and the total CoA pools in 13 tissues of rats with genetically-induced obesity and diabetes mellitus. Additionally, we compared the data obtained from Zucker and ZDF rats.

2. Materials and Methods

2.1. Experimental Animals

Male Zucker-fatty (Crlj:Zuc-Leprfa, Leprfa/Leprfa), Zucker-lean (Crlj:Zuc-Leprfa, Leprfa/+ or +/+), ZDF-fatty (ZDF-Leprfa/Crlj, Leprfa/Leprfa), and ZDF-lean (ZDF-Leprfa/Crlj, Leprfa/+ or +/+ rats (6 rats/group; age, 11 weeks old) were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan). Rats were individually housed in single cages (276 × 445 × 204 mm, polycarbonate, CLEA Japan, Inc., Tokyo, Japan) and were fed standard laboratory pellet chow (MF diet from Oriental Yeast Co. Ltd., Tokyo, Japan) and water ad libitum under a 12-h light (6:00 am to 6:00 pm)/12-h dark (6:00 pm to 6:00 am) cycle at 22 ± 1 °C. After a one-week acclimation period, rats were starved for 16 h with free access to drinking water. The cerebral cortex, hippocampus, hypothalamus, cerebellum, and medulla oblongata from the brain of rats and eight types of tissue, namely the liver, spleen, kidney (right side), heart, skeletal muscle (right soleus muscle), perirenal adipose tissue, brown adipose tissue, and epididymal adipose tissue, were removed. These tissues were stored at −80 °C until use.

This study was carried out in accordance with the guidelines of the Animal Care and Use Committee of Ibaraki University (Approval number 112) and the guidelines of the Ministry of Education, Culture, Sports, Science, and Technology, Japan (Notice No.71).

2.2. Extraction and Determination of the CoA Pool in Tissues

The prepared tissues were immersed in 400 µL of 0.6 M sulfuric acid per 100 mg of tissue, and homogenized [6,7]. Following storage of the extract at 4 °C overnight to inactivate the endogenous enzymes, the homogenized tissues were centrifuged at 9000×g at 4 °C for 10 min, and 1 M Tris (1/20 volume of supernatant) was added to the supernatant. The acidic extract was adjusted to approximately pH 6.5 with NaOH on ice. Thereafter, the solution was maintained at −80 °C overnight. After thawing, precipitation was removed by centrifugation, and acetyl-CoA, malonyl-CoA, and CoASH in the extract were determined using the acyl-CoA cycling method described below [18,19].

All four enzymes used in this method were prepared in our laboratory. Malonate decarboxylase, a multifunctional enzyme, was partially purified from Pseudomonas putida JCM 20089 (formerly IAM
The other enzymes, namely acetate kinase (AckA from *Escherichia coli* W3110), citrate synthase (CitZ from *Bacillus subtilis* str. 168), and phosphate acetyltransferase (Pta from *B. subtilis* str. 168), were cloned and expressed in *E. coli* JM109 as His-tagged enzymes [22]. The reaction mixture used to measure the sum of acetyl-CoA and malonyl-CoA contained 50 mM Tris-HCl (pH 7.2), 1 mM 2-mercaptoethanol, 10 mM MgSO$_4$, 50 mM malonate, 10 mM ATP, 1 U of malonate decarboxylase, and the tissue extracts containing acetyl-CoA and/or malonyl-CoA (2.5 to 80 pmol) in 400 µL. The cycling reaction was initiated by adding malonate decarboxylase. After a 20-min incubation at 30 °C, 1 U of the recombinant acetate kinase was added to the mixture. After an additional 20 min, 0.2 mL of 2.5 M neutralized hydroxylamine was added, and the incubation was continued for an additional 20 min at 30 °C. The reaction was terminated by adding 0.6 mL of 10 mM ferric chloride dissolved in 25 mM trichloroacetic acid-1 M HCl. The A$_{540}$ of the acetohydroxamate formed was measured. Each assay was performed in duplicate.

Malonyl-CoA and CoASH in the tissue extracts were separately determined by eliminating acetyl-CoA with citrate synthase (CS) and converting CoASH to acetyl-CoA with phosphate acetyltransferase (PAT), respectively, prior to carrying out measurements with the acyl-CoA cycling method. The reaction mixture for CS treatment contained 50 mM Tris-HCl (pH 7.2), 2 mM oxaloacetate, 5 µg of the recombinant CS, and the tissue extracts in 1 mL. The reaction was carried out at 25 °C for 20 min and terminated by placing the reaction tube on an ice slush. One milliliter reaction mixture for PAT treatment contained 50 mM Tris-HCl (pH 7.2), 0.1 mM acetylphosphate, 0.8 µg of the recombinant His-tagged PAT, and the tissue extracts. After incubation at 25 °C for 20 min, the reaction was terminated by eliminating the His-tagged enzyme through Ni sepharose 6 Fast Flow (GE Healthcare UK Ltd., Buckinghamshire, UK). The remaining malonyl-CoA in the CS treatment and the acetyl-CoA derived from CoASH were determined using the acyl-CoA cycling method.

2.3. Analysis of Blood Serum

Glucose, triglycerides, non-esterified fatty acids (NEFAs), total ketone bodies (T-KB), total cholesterol (T-CHO), insulin, and leptin in the sera of Zucker and ZDF rats were analyzed at Nagahama Life Science Laboratory (Oriental Yeast Co. Ltd., Shiga, Japan). Briefly, glucose, triglycerides, NEFAs, T-KB, and T-CHO were assayed by enzymatic methods while insulin and leptin were measured by ELISA.

2.4. Statistical Analysis

Body weight, tissue weight, blood serum, and CoA pools in each tissue were compared between Zucker and ZDF, lean and fatty rats, and their interaction by Two-way ANOVA using R Ver. 3.5.2 (R Development Core Team). Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Analysis of Body Weight, Tissue Weight, and Blood Serum

As shown in Table 1, Zucker- and ZDF-fatty rats exhibited hyperphagia. Compared to lean rats, daily food intake and drinking by Zucker-fatty rats were 1.7- and 1.8-fold higher during one week of habitation, thereby leading to obesity. In ZDF-fatty rats, the level of food intake per day was 2.4-fold higher than that in lean rats. Additionally, daily drinking was markedly elevated to a value 5.0-fold higher (151 ± 4 mL/day). Body weight of Zucker-fatty rats was clearly increased, while ZDF-fatty rats had a weight loss of 6.72 ± 1.37 g during one week of habitation.

The livers, kidneys, perirenal adipose tissues, brown adipose tissues, and epididymal adipose tissues of both fatty rats were enlarged; however, no differences were found between fatty and lean rats in the spleen and heart. The increased weights observed in the three adipose tissues from fatty rats were more marked in Zucker-fatty rats while the gain in kidney was more prominent in ZDF-fatty rats.
was substantially higher (251 ± 7.9-fold higher than that of Zucker-lean rats. In contrast, ZDF-fatty rats had an insulin level of 2.06 ± 0.07 ng/mL, which was as low as that of Zucker-lean rats. Conversely, their blood glucose level was as high as that of Zucker-fatty rats. Serum lipids such as triglycerides, NEFAs, and T-CHO were much higher in obese rats than lean rats. Additionally, a significant difference was found between fatty and lean rats in T-KB levels. Thus, Zucker-fatty rats presented typical obesity with hyperglycemia, hyperlipidemia, hyperinsulinemia, and hyperleptinemia while ZDF-fatty rats exhibited symptoms of abnormal polydipsia caused by diabetes as shown in Table 1. The values of these metabolic parameters in the bred Zucker and ZDF rats were close to the results previously reported by Jonas et al. [23].

### Table 2. Biochemical analysis of blood serum.

| Zucker | ZDF | Two-way ANOVA |
|--------|-----|---------------|
|        | Lean| Fatty | Lean| Fatty | Str. | fa | Str. × fa |
| Glucose (mg/dL) | 140 ± 4| 251 ± 9| 114 ± 5| 295 ± 20| ns | *** | ** |
| Triglycerides (mg/dL) | 40.7 ± 3.5| 542 ± 59| 19.8 ± 1.5| 237 ± 40| *** | *** | *** |
| NEFAs (µEq/L) | 286 ± 29| 604 ± 61| 261 ± 14| 598 ± 39| ns | *** | ns |
| T-KB (µmol/L) | 830 ± 36| 1032 ± 233| 726 ± 45| 1170 ± 186| ns | * | ns |
| T-CHO (mg/dL) | 67.7 ± 4.1| 102 ± 5| 64.3 ± 1.0| 137 ± 6| ** | *** | ** |
| Insulin (ng/mL) | 1.54 ± 0.19| 12.1 ± 3.9| 0.602 ± 0.072| 2.06 ± 0.75| * | * | * |
| Leptin (ng/mL) | 2.11 ± 0.07| 73.0 ± 3.4| 0.595 ± 0.034| 12.7 ± 1.7| *** | *** | *** |

All data are expressed as mean ± SEM (n = 6). Statistical significances between Zucker and ZDF (Str.), lean and fatty rats (fa), and their interaction (Str. × fa) were derived by two-way ANOVA: ns, not significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001.

#### 3.2. Analysis of CoA Pools

The sizes of acetyl-CoA, malonyl-CoA, and CoASH, and the total CoA pools (sum of acetyl-CoA, malonyl-CoA, and CoASH) in the brain tissues of Zucker and ZDF rats were analyzed (Table 3). Although each CoA pool in the brain tissues of the Zucker-fatty rats was unambiguously decreased.

---

**Table 1. Body weight, food intake, drinking, and tissue weights in rats.**

| Zucker | ZDF | Two-way ANOVA |
|--------|-----|---------------|
|        | Lean| Fatty | Lean| Fatty | Str. | fa | Str. × fa |
| Body weight (g) | 305.3 ± 5.3| 429.0 ± 2.9| 286.2 ± 2.2| 344.7 ± 5.8| *** | *** | *** |
| 11 weeks | 327.5 ± 6.8| 459.6 ± 2.7| 297.2 ± 2.1| 338.0 ± 5.6| *** | *** | *** |
| Body weight gain (g/week) | 22.2 ± 2.0| 30.5 ± 1.9| 10.9 ± 0.8| −6.72 ± 1.37| *** | ** | *** |
| Food intake (g/day) | 23.3 ± 1.0| 39.1 ± 1.0| 17.0 ± 0.2| 41.5 ± 2.8| ns | *** | * |
| Drinking (mL/day) | 29.6 ± 1.7| 52.0 ± 5.9| 30.1 ± 0.7| 151 ± 4| *** | *** | *** |
| Liver (g) | 9.53 ± 0.46| 18.1 ± 1.0| 8.50 ± 0.08| 16.5 ± 0.5| * | *** | ns |
| Spleen (g) | 0.459 ± 0.020| 0.490 ± 0.029| 0.524 ± 0.012| 0.504 ± 0.017| ns | ns | ns |
| Kidney (g) | 1.23 ± 0.03| 1.41 ± 0.04| 1.18 ± 0.05| 1.65 ± 0.05| * | *** | ** |
| Heart (g) | 0.999 ± 0.056| 1.06 ± 0.03| 1.13 ± 0.04| 1.11 ± 0.03| * | ns | ns |
| Skeletal muscle (g) | 0.171 ± 0.008| 0.128 ± 0.007| 0.144 ± 0.003| 0.136 ± 0.004| ns | *** | *** |
| Perirenal adipose tissue (g) | 3.79 ± 0.23| 18.4 ± 0.7| 1.64 ± 0.09| 9.35 ± 0.33| *** | *** | *** |
| Brown adipose tissue (g) | 0.533 ± 0.031| 1.76 ± 0.09| 0.344 ± 0.027| 0.782 ± 0.048| *** | *** | *** |
| Epididymal adipose tissue (g) | 4.65 ± 0.31| 14.2 ± 0.3| 2.58 ± 0.08| 6.33 ± 0.36| *** | *** | *** |

All data are expressed as mean ± SEM (n = 6). ZDF - Zucker diabetic fatty. Statistical significances between Zucker and ZDF (Str.), lean and fatty rats (fa), and their interaction (Str. × fa) were derived by two-way ANOVA: ns, not significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001.
same or a smaller size than the respective lean rats, the medulla oblongata in the Zucker-fatty rats and the cerebral cortex in the ZDF-fatty rats clearly had larger total CoA pools. Conversely, the pool sizes of the cerebral cortex in the Zucker-fatty rats and the medulla oblongata in the ZDF-fatty rats were smaller. By comparing the total CoA pools between both strains, ZDF rats were found to possess a larger total CoA pool (i.e., approximately 10 nmol/mg or higher) in tissues, except in the hypothalamus. In the hypothalamus, the acetyl-CoA pools were larger in ZDF rats but markedly decreased in obese rats. Conversely, the malonyl-CoA pools, which are closely related to feeding behavior, had almost the same values in lean and fatty rats, although obese rats exhibited hypophagia.

Table 3. CoA pools in rat tissues.

| Tissues         | Species | Lean   | Zucker   | ZDF     | Two-way ANOVA |
|-----------------|---------|--------|---------|---------|---------------|
|                 | Str. fa | Str. × fa |
| Cerebral cortex | M       | 0.267 ± 0.018 | 0.230 ± 0.006 | 0.299 ± 0.035 | 0.155 ± 0.011 | ns ** | * |
| CoA             | M       | 0.69 ± 1.04 | 4.52 ± 1.22 | 5.62 ± 1.47 | 13.2 ± 0.5 | ns ** | *** |
| Total           |         | 6.52 ± 1.11 | 6.29 ± 1.19 | 8.48 ± 1.16 | 15.4 ± 0.6 | ns    |          |
| Hippocampus     | M       | 0.243 ± 0.014 | 0.214 ± 0.015 | 0.276 ± 0.021 | 0.211 ± 0.014 | ns    |          |
| CoA             | M       | 1.04 ± 0.22 | 1.47 ± 0.19 | 11.7 ± 0.6 | 9.58 ± 1.90 | ns    |          |
| Total           | M       | 2.92 ± 0.37 | 3.08 ± 0.27 | 14.3 ± 0.7 | 11.9 ± 2.0 | ns    |          |
| Hypothalamus    | M       | 1.36 ± 0.10 | 1.14 ± 0.07 | 2.29 ± 0.07 | 1.49 ± 0.08 | ns    |          |
| CoA             | M       | 0.173 ± 0.013 | 0.152 ± 0.012 | 0.137 ± 0.006 | 0.133 ± 0.007 | * ns |          |
| Total           | M       | 1.31 ± 0.18 | 1.06 ± 0.10 | 1.86 ± 0.20 | 1.06 ± 0.16 | ns    |          |
| Cerebellum      | M       | 3.74 ± 0.19 | 2.83 ± 0.07 | 2.68 ± 0.16 | 2.25 ± 0.17 | * ns |          |
| CoA             | M       | 0.511 ± 0.042 | 0.480 ± 0.025 | 1.54 ± 0.06 | 1.50 ± 0.06 | *** * |          |
| Total           | M       | 2.55 ± 0.45 | 1.31 ± 0.11 | 10.9 ± 1.0 | 9.33 ± 0.94 | ns    |          |
| Liver           | M       | 3.28 ± 0.39 | 2.37 ± 0.15 | 5.78 ± 0.98 | 5.83 ± 0.37 | *** ns |          |
| CoA             | M       | 0.918 ± 0.103 | 0.745 ± 0.077 | 1.58 ± 0.20 | 2.42 ± 0.17 | * * ** |          |
| Total           | M       | 6.15 ± 2.32 | 9.20 ± 2.72 | 80.8 ± 9.4 | 105 ± 8     | *** * |          |
| Medulla oblongata | M   | 0.074 ± 0.017 | 0.119 ± 0.020 | 0.264 ± 0.013 | 0.187 ± 0.014 | ns    |          |
| CoA             | M       | 2.32 ± 1.24 | 7.83 ± 1.86 | 6.61 ± 1.48 | 4.67 ± 1.13 | ns    |          |
| Total           | M       | 3.40 ± 1.46 | 9.26 ± 1.93 | 9.31 ± 1.48 | 6.62 ± 1.14 | ns    |          |
| Spleen          | A       | 3.28 ± 0.39 | 2.37 ± 0.15 | 5.78 ± 0.98 | 5.83 ± 0.37 | *** ns |          |
| CoA             | M       | 0.918 ± 0.103 | 0.745 ± 0.077 | 1.58 ± 0.20 | 2.42 ± 0.17 | * * ** |          |
| Kidney          | M       | 1.09 ± 0.06 | 0.791 ± 0.090 | 1.90 ± 0.10 | 1.84 ± 0.11 | *** ns |          |
| CoA             | M       | 0.561 ± 0.048 | 0.567 ± 0.026 | 0.865 ± 0.038 | 0.909 ± 0.046 | *** ns |          |
| Total           | M       | 2.12 ± 0.38 | 1.65 ± 0.55 | 1.53 ± 0.28 | 1.18 ± 0.31 | ns    |          |
| Heart           | M       | 3.78 ± 0.44 | 3.00 ± 0.60 | 4.30 ± 0.30 | 3.91 ± 0.43 | ns    |          |
| CoA             | M       | 4.79 ± 0.56 | 3.21 ± 0.64 | 11.7 ± 0.6 | 8.93 ± 0.35 | *** ** |          |
| Total           | M       | 6.66 ± 0.45 | 1.47 ± 0.34 | 0.703 ± 0.028 | 0.699 ± 0.043 | * * |          |
| Skeletal muscle | M       | 15.9 ± 3.5 | 24.7 ± 5.3 | 42.1 ± 4.5 | 31.9 ± 2.8 | *** ns |          |
| CoA             | M       | 23.3 ± 4.1 | 29.4 ± 6.0 | 54.4 ± 5.0 | 41.5 ± 3.1 | *** ns |          |
| Total           | M       | 0.993 ± 0.127 | 0.660 ± 0.117 | 0.703 ± 0.113 | 0.19 ± 0.14 | ns    |          |
| Perirenal       | M       | 1.52 ± 0.16 | 1.61 ± 0.13 | 1.94 ± 0.28 | 2.00 ± 0.13 | * ns |          |
| CoA             | M       | 5.89 ± 0.43 | 6.26 ± 0.80 | 6.28 ± 1.08 | 9.70 ± 0.99 | * ns |          |
| Total           | M       | 8.40 ± 0.43 | 8.53 ± 0.82 | 8.93 ± 1.13 | 12.8 ± 1.0 | * * * |          |
| Adipose tissue  | M       | 0.191 ± 0.050 | 0.166 ± 0.030 | 0.197 ± 0.023 | 0.06 ± 0.016 | ns * |          |
| CoA             | M       | 0.395 ± 0.094 | 0.231 ± 0.044 | 0.398 ± 0.150 | 0.219 ± 0.108 | ns    |          |
| Total           | M       | 0.690 ± 0.152 | 0.418 ± 0.077 | 0.666 ± 0.174 | 0.304 ± 0.125 | ns * |          |
| Brown adipose   | M       | 1.44 ± 0.29 | 0.406 ± 0.102 | 1.47 ± 0.15 | 0.394 ± 0.044 | ns *** |          |
| CoA             | M       | 3.65 ± 0.66 | 3.43 ± 1.01 | 4.44 ± 5.3 | 2.02 ± 0.49 | *** * |          |
| Total           | M       | 6.01 ± 0.69 | 4.10 ± 0.87 | 47.0 ± 5.4 | 2.82 ± 0.48 | *** *** |          |

“A”, “M”, “CoA”, and “Total” indicate acetyl-CoA, malonyl-CoA, CoASH, and total CoA (defined as the sum of the three CoA pools), respectively. All data are expressed as nmol/mg of tissue and mean ± SD (n = 6). Statistical significance between Zucker and ZDF (Str.), lean and fatty rats (fa), and their interaction (Str. × fa) were derived by two-way ANOVA: ns, not significant; *, p < 0.05; **, p < 0.01; ****, p < 0.001. nd, not detected.
The liver, heart, and brown adipose tissue of Wistar rats had substantially larger total CoA pools (i.e., exceeding 10 nmol/g of tissue) among the thirteen tissues measured [6,7]. In particular, the liver could form a large total CoA pool of approximately 100 nmol/g of tissue under starvation in HCD-fed rats [7]. The present study revealed that the total CoA pools were substantially reduced in Zucker rats. In contrast, ZDF rats had large total CoA pools of 88.2 ± 10.5 nmol/g and 114 ± 8 nmol/g in the tissue of lean and fatty rats, respectively, owing to the remarkably high levels of CoASH. These values were close to those of the livers of the HCD- or HPD-fed Wistar rats. In addition to the clearly enlarged liver recognized in obese rats, their total CoA content was found to be higher than that in lean rats. The phenomena of no significant difference in total CoA pools between lean rats and fatty rats and the considerably larger sizes in ZDF than Zucker rats were also observed in the heart. The characteristic of fat-deficiency was found in the total CoA pool for the brown adipose tissues of ZDF rats. The content of acetyl-CoA, malonyl-CoA, and CoASH in fatty rats were much lower, with 26.8%, 36.2%, and 4.5% of the values found in lean rats, respectively. Consequently, the total CoA pool was reduced to 6.0%. These low content of acylated CoAs were close to the respective pool sizes in the epididymal adipose tissue, which is one of the tissues that had the lowest CoA contents together with the perirenal adipose tissue. Thus, it is noteworthy that all CoA pools in the brown adipose tissue of ZDF-fatty rats were abnormally reduced. Although the reduction of the total CoA pool was observed in Zucker obese rats, originally, the total CoA pool in the lean rats had a small size of 6.01 ± 0.69 nmol/g of tissue.

In skeletal muscles, the malonyl-CoA pools were larger than the acetyl-CoA pools in all four rats. The higher contents of malonyl-CoA might interfere with the incorporation of fatty acids by carnitine acyltransferase I into the mitochondria and the subsequent β-oxidation. Because free CoA was not detected in the spleens of Zucker rats and ZDF-fatty rats, reduced sizes of the total CoA pools were observed. In the kidneys, the total CoA pools were not affected by fat-deficiency and strain.

4. Discussion

In the hypothalamus, malonyl-CoA functions as a mediator that controls feeding behavior by downregulating orexigenic neuropeptides (neuropeptide Y and agouti-related peptide) and upregulating anorexigenic neuropeptides (proopiomelanocortin and cocaine- and amphetamine regulated-transcript) [4]. Malonyl-CoA is modulated by the phosphorylation and dephosphorylation of acetyl-CoA carboxylase (ACC), which is one of the target proteins of AMP-activated protein kinase (AMPK) [24]. Leptin is known to activate ACC by inhibiting AMPK activity and then enhance hypothalamic malonyl-CoA level in mice [25,26]. Thus, leptin, which is secreted from white adipose tissues, suppresses food intake through the hypothalamic malonyl-CoA signaling system described above. In the present study, Zucker- and ZDF-fatty rats, which have a deficient leptin receptor, have a largely excess amount of leptin in their blood sera (Table 2). However, almost the same sizes of malonyl-CoA pools were observed in the fa/fa Zucker and ZDF strains as those of the respective lean rats (Table 3). The results obtained with the leptin receptor-deficient rats supported the finding that the governance of malonyl-CoA content by phosphorylation and dephosphorylation of AMPK in the hypothalamus occurs mainly downstream of the leptin signal. AMPK is rigorously regulated by the intracellular level of AMP, which is increased in the energy-starved state [27]. Wolfgang and his co-workers reported that the regulation of AMPK and ACC via the response to glucose in ob/ob mice still functioned in a leptin-independent manner [26]. Therefore, the hypothalamic malonyl-CoA levels in these fatty rats were as low as those in lean rats, suggesting that although brain cells have higher levels of blood glucose, cells in the hypothalamus of obese rats could detect energy starvation. This phenomenon would indicate the inhibition of glucose incorporation via insulin-resistance in the hypothalamus. Insulin receptors are expressed in the hypothalamus [28], while glucose transporter 2 (GLUT2) mainly mediates glucose incorporation with the insulin-independent system [29,30]. Unlike ob/ob mice, hypothalamic malonyl-CoA behavior alone cannot explain the relationship between energy balance and appetite in fat-deficient rats that display insulin resistance in addition to leptin resistance. Neuropeptide Y is known to be abundant in obese Zucker rats [31–33]. Hence, the regulation of
hypothalamic malonyl-CoA level through AMPK, which recognizes the change in energy status, may be disrupted, and the increase in ghrelin may predominantly function as a mediator of hyperphagia in Zucker rats [34,35].

The hepatic total CoA pool, mainly the CoASH constituent, is increased owing to its use in \(\beta\)-oxidation in the fasted state [6,36]. The livers of Wistar rats fed HFD for 4 weeks were enlarged while their hepatic total CoA pools were greatly reduced to almost one fifth of those of the HCD-fed rats according to the decrease in the CoASH pool [7]. These findings imply that hepatic CoA metabolism was greatly affected by the quality of the diets and body energy states. In the present study, hypertrophy was observed in the livers of Zucker- and ZDF-fatty rats (i.e., the genetically-induced obese rats) (Table 1). Interestingly, an extremely small size of the total CoA pools (i.e., approximately 10 nmol/g of tissue) was found in Zucker-lean and fatty rats (Table 3). This observation might be an intrinsic characteristic of hepatic metabolism in Zucker rats, regardless of the \(fa\) genotype. The hepatic total CoA pool sizes in both ZDF-obese and lean rats had nearly 10-fold higher values than Zucker rats (Table 3). Thus, it is interesting that Zucker rats had hepatic total CoA pools similar to Wistar obese rats fed an HFD. ZDF rats also had almost the same size as Wistar rats fed HCD, regardless of their fatty or lean appearance. In addition, the compositions of the CoASH and acyl-CoA contents in Zucker and ZDF rats resembled those of Wistar rats fed HFD and HCD, respectively [7]. Based on the CoA metabolites, the hepatic metabolism of Zucker and ZDF rats displayed inherent differences. At the molecular level, the hepatic total CoA pool size is balanced with CoA synthesis by PanK1, the mainly expressed PanK isoform in murine liver [37,38], and its degradation by nudix hydrolase 7 [39–41]. These enzyme activities are modulated by the action of peroxisome proliferator-activated receptor \(\alpha\) (PPAR\(\alpha\)) [41–43]. Hepatic PPAR\(\alpha\) content in Zucker obese rats is statistically lower than that of lean rats [44]. However, Zucker obese rats and lean rats displayed an equivalent total CoA pool. Although the total CoA pool size was much larger in ZDF rats than Zucker rats, a similar result was obtained between ZDF and Zucker rats. Analysis of the hepatic CoA pools in Zucker and ZDF rats revealed a discrepancy between in vivo CoA behavior and the response of PPAR\(\alpha\) to CoA biosynthesis on fatty acid degradation in the fasted state. On the other hand, hepatic hypertrophy and hyperlipidemia were observed in both obese rats, and concomitantly, serum leptin and insulin were higher in fatty rats than in lean rats (Tables 1 and 2). It has been reported that leptin inhibits the production of triacylglycerol [45], whereas insulin promotes it [46,47]. In obese Zucker rats (\(fa/\)), approximately 2 to 4 times more triacylglycerol than in lean rats (+/+ or \(fa/+\)) was accumulated in the liver, followed by its secretion into circulation [48]. Since the leptin receptor did not function in the fatty rats, the turnover of triacylglycerol was more active in the synthesis than in the degradation by the action of insulin. Consequently, the accumulation of triacylglycerol was observed in the liver of obese rats. In the present study, this phenomenon occurred in ZDF-fatty rats as well as in Zucker-fatty rats, and this result reflected the amounts of insulin in sera well. In addition, the significantly lower total CoA levels in Zucker rats were clearly disadvantageous for \(\beta\)-oxidation, indicating that Zucker rats were in an in vivo environment that favored the accumulation of triacylglycerol (Table 3). Previous findings did not reveal the hepatic CoA metabolism and hypothalamic CoA metabolism in these obese rats.

Zucker- and ZDF-fatty rats demonstrated progressive renal failure [49–52]. Although renal hypertrophy was observed in these obese rats, there was no important difference between lean and fatty rats in CoA contents, suggesting that renal intracellular metabolisms were seldom damaged, at least until 12-weeks old (Table 3).

Malonyl-CoA is often present as a minor CoA molecular species in cells. However, its level was found to exceed that of acetyl-CoA in the skeletal muscles of HFD-fed obese Wistar rats [7]. Herein, we found the same result in the skeletal muscles of Zucker and ZDF rats. This predominance of malonyl-CoA relative to acetyl-CoA in skeletal muscles might be established by other genetic factor(s), such as deficiency of liver kinase B1 (LKB1) or AMPK, and not the obesity induced by their \(fa\)-deficiencies. This is because even lean rats possessed larger malonyl-CoA than acetyl-CoA pools.
LKB1 is a major AMPK kinase in skeletal muscle [53,54]. Accordingly, phosphorylated-AMPK levels were reduced and ACCs were activated in LKB1 knockout and dominant negative mice [54–56].

Compared to HCD-fed rats, diet-induced obesity in Wistar rat resulted in unchanged total CoA pool size in the brown adipose tissue [7]. Here, we found that genetically-induced obesity affected the total CoA pool sizes in brown adipose tissues (Table 3). The Zucker and ZDF obese rats had extremely small pools of total CoA (4.10 ± 0.87 and 2.82 ± 0.48 nmol/g of tissue) and acetyl-CoA (0.406 ± 0.102 and 0.394 ± 0.044 nmol/g of tissue), while Wistar rats tended to form large pools of total CoA (20–60 nmol/g of tissue) and acetyl-CoA (5–10 nmol/g) [6,7]. Zucker-lean rats possessed a small total CoA pool similar to Zucker-fatty rats, implying that those with the fa/+ or +/+ had already acquired abnormal CoA metabolisms in their brown adipose tissue and liver, which were subsequently manifested by the leptin-receptor deficient rats. PPARα was found to be abundant in the brown adipose tissue of Zucker rats. Conversely, obese rats expressed the receptor at a significantly lower level than lean rats [44]. This finding is inconsistent with our observation in Zucker rats, but aligned well with the relationship between ZDF-lean and fatty rats based on CoA behaviors. Brown adipose tissue plays a crucial role in thermogenesis via the mitochondrial proton conductance pathway mediated by uncoupling protein 1. The enlarged brown adipose tissue of Zucker-fatty rat displayed reduced activity in the mitochondrial proton conductance pathway [57]. Our data demonstrate that the substantially smaller acetyl-CoA pool in Zucker-fatty rat provided a further disadvantage to this non-shivering thermogenesis. This is because NADH and FADH2 could form an imbalance in H⁺ between the intermembrane space and matrix in mitochondria via electron transport, which is generated from acetyl-CoA through the TCA cycle.

PanK was recently reported to be closely related to cellular CoA biosynthesis, as well as indirectly related to insulin sensitivity and glucose homeostasis via micro RNAs 103 and 107 located in intron 5 of the three PANK genes [58–60]. Therefore, CoASH, acetyl-CoA, and malonyl-CoA are key metabolic regulators in addition to their role as intermediates in many pathways.

5. Conclusions

Zucker- and ZDF-fatty rats are widely used as the respective animal models of obesity and diabetes caused by mutation of the leptin receptor. Although ZDF rats are derived from Zucker rats, the sizes of their hepatic total CoA pool and the contents of their three molecular species were found to considerably differ. In addition, a negative influence on CoA behavior was found in the brown adipose tissue of fa/fa rats alone. Conversely, Zucker rats, even those that were lean and displayed the fa/+ or +/+ genotype, displayed abnormal CoA metabolism in their livers and brown adipocytes. Based on CoA contents, the obesity in Zucker rats cannot be clearly explained by functional deficiency in the leptin receptor alone. In the future, not only the comparison between Zucker-fatty and Zucker-lean rats, and ZDF-fatty and ZDF-lean rats (i.e., intra-strain comparison), but also that between Zucker-lean and ZDF-lean rats, and Zucker-fatty and ZDF-fatty rats (inter-strain comparison) on genetical and physiological data will be important for elucidating obesity and type 2 diabetes mellitus. Moreover, monitoring of the in vivo behaviors of CoASH and its derivatives in the hypothalamus, liver, skeletal muscle, and brown adipose tissue together with differential gene expression analysis and/or comparative genomics will aid in the rational explanation of obesity and type 2 diabetes in the respective Zucker and ZDF rat models.

Author Contributions: Conceptualization, S.C. and A.T. formal analysis, Y.T. and D.K. investigation, S.M., K.S. and Y.T. writing-original draft preparation, S.C. and A.T. writing-review and editing, S.C. project administration, S.C. funding acquisition, S.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas (Research in a proposed research area) “Molecular Basis and Disorders of Control of Appetite and Fat Accumulation” 23126501 from MEXT.

Acknowledgments: We would like to thank Yuta Ogata and Wataru Iio for their technical assistance and useful advice on the study. We greatly appreciate the English language review performed by Editage (https://www.editage.jp/).
Conflicts of Interest: The authors declare no conflict of interest.

References

1. Leonardi, R.; Zhang, Y.M.; Rock, C.O.; Jackowski, S. Coenzyme A: Back in action. Prog. Lipid Res. 2005, 44, 125–153. [CrossRef] [PubMed]
2. Leonardi, R.; Zhang, Y.M.; Lykidis, A.; Rock, C.O.; Jackowski, S. Localization and regulation of mouse pantothenate kinase 2. FEBS Lett. 2007, 581, 4639–4644. [CrossRef] [PubMed]
3. Yao, J.; Subramanian, C.; Rock, C.O.; Jackowski, S. Human pantothenate kinase 4 is a pseudo-pantothenate kinase. Protein Sci. 2019, 28, 1031–1047. [CrossRef] [PubMed]
4. Hu, Z.; Cha, S.H.; Chohnan, S.; Lane, M.D. Hypothalamic malonyl-CoA as a mediator of feeding behavior. Proc. Natl. Acad. Sci. USA 2003, 100, 12624–12629. [CrossRef] [PubMed]
5. Iio, W.; Tokutake, Y.; Matsukawa, N.; Tsukahara, T.; Chohnan, S.; Toyoda, A. Anorexic behavior and elevation of hypothalamic malonyl-CoA in socially defeated rats. Biochem. Biophys. Res. Commun. 2012, 421, 301–304. [CrossRef] [PubMed]
6. Tokutake, Y.; Onizawa, N.; Katoh, H.; Toyoda, A.; Chohnan, S. Coenzyme A and its thioester pools in fasted and fed rat tissues. Biochem. Biophys. Res. Commun. 2010, 402, 158–162. [CrossRef]
7. Tokutake, Y.; Iio, W.; Onizawa, N.; Ogata, Y.; Kohari, D.; Toyoda, A.; Chohnan, S. Effect of diet composition on coenzyme A and its thioester pools in various rat tissues. Biochem. Biophys. Res. Commun. 2012, 423, 781–784. [CrossRef] [PubMed]
8. Kava, R.; Greenwood, M.R.C.; Johnson, P.R. Zucker (fa/fa) rat. ILAR News 1990, 32, 4–8. [CrossRef]
9. Zucker, L.M.; Zucker, T.F. Fatty, a new mutation in the rat. J. Hered. 1961, 52, 275–278. [CrossRef]
10. Zucker, T.F.; Zucker, L.M. Fatty accretion and growth in the rat. J. Nutr. 1963, 80, 6–19.
11. Peterson, R.G.; Shaw, W.N.; Neel, M.-A.; Little, L.A.; Eichberg, J. Zucker diabetic fatty rat as a model for non-insulin-dependent diabetes mellitus. ILAR J. 1990, 32, 16–19. [CrossRef]
12. Chua, S.C., Jr.; Chung, W.K.; Wu-Peng, X.S.; Zhang, Y.; Liu, S.M.; Tartaglia, L.; Leibel, R.L. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 1996, 271, 994–996. [CrossRef] [PubMed]
13. Chua, S.C., Jr.; White, D.W.; Wu-Peng, X.S.; Liu, S.M.; Okada, N.; Kershaw, E.E.; Chung, W.K.; Power-Kehoe, L.; Chua, M.; Tartaglia, L.A.; et al. Phenotype of fatty due to Gln269Pro mutation in the leptin receptor (Lepr). Diabetes 1996, 45, 1141–1143. [CrossRef] [PubMed]
14. Phillips, M.S.; Liu, Q.; Hammond, H.A.; Dugan, V.; Hey, P.J.; Caskey, C.J.; Hess, J.F. Leptin receptor missense mutation in the fatty Zucker rat. Nat. Genet. 1996, 13, 18–19. [CrossRef] [PubMed]
15. Alexandre de Artiñano, A.; Miguel Castro, M. Experimental rat models to study the metabolic syndrome. Br. J. Nutr. 2009, 102, 1246–1253. [CrossRef] [PubMed]
16. Clark, J.B.; Palmer, C.J.; Shaw, W.N. The diabetic Zucker fatty rat. Proc. Soc. Exp. Biol. Med. 1983, 173, 68–75. [CrossRef] [PubMed]
17. Kong, L.; Wu, H.; Cui, W.; Zhou, W.; Luo, P.; Sun, J.; Yuan, H.; Miao, L. Advances in murine models of diabetic nephropathy. J. Diabetes Res. 2013, 2013, 797548. [CrossRef]
18. Chohnan, S.; Takamura, Y. A simple micromethod for measurement of CoASH and its use in measuring intracellular levels of CoASH and short chain acyl-CoAs in Escherichia coli K12 cells. Agric. Biol. Chem. 1991, 55, 87–94.
19. Takamura, Y.; Kitayama, Y.; Arakawa, A.; Yamanaka, S.; Tosaki, M.; Ogawa, Y. Malonyl-CoA:acetyl-CoA cycling. A new micromethod for determination of acyl-CoAs with malonate decarboxylase. Biochim. Biophys. Acta 1985, 834, 1–7.
20. Chohnan, S.; Fujio, T.; Takaki, T.; Yonekura, M.; Nishihara, H.; Takamura, Y. Malonate decarboxylase of Pseudomonas putida is composed of five subunits. FEMS Microbiol. Lett. 1998, 169, 37–43. [CrossRef]
21. Takamura, Y.; Kitayama, Y. Purification and some properties of malonate decarboxylase from Pseudomonas ovalis: An oligomeric enzyme with bifunctional properties. Biochim. Int. 1981, 3, 483–491.
22. Ogata, Y.; Chohnan, S. Prokaryotic type III pantothenate kinase enhances coenzyme A biosynthesis in Escherichia coli. J. Gen. Appl. Microbiol. 2015, 61, 266–269. [CrossRef] [PubMed]
23. Jonas, M.; Edelman, E.R.; Groothuis, A.; Baker, A.B.; Seifert, P.; Rogers, C. Vascular neointimal formation and signaling pathway activation in response to stent injury in insulin-resistant and diabetic animals. Circ. Res. 2005, 97, 725–733. [CrossRef] [PubMed]

24. Hu, Z.; Dai, Y.; Prentki, M.; Chohnan, S.; Lane, M.D. A role for hypothalamic malonyl-CoA in the control of food intake. J. Biol. Chem. 2005, 280, 39681–39683. [CrossRef] [PubMed]

25. Gao, S.; Kinzig, K.P.; Aja, S.; Scott, K.A.; Keung, W.; Kelly, S.; Strynadka, K.; Chohnan, S.; Smith, W.W.; Tamashiro, K.L.; et al. Leptin activates hypothalamic acetyl-CoA carboxylase to inhibit food intake. Proc. Natl. Acad. Sci. USA 2007, 104, 17358–17363. [CrossRef]

26. Wolfgang, M.J.; Cha, S.H.; Sidhaye, A.; Chohnan, S.; Cline, G.; Shulman, G.I.; Lane, M.D. Regulation of hypothalamic malonyl-CoA by central glucose and leptin. Proc. Natl. Acad. Sci. USA 2007, 104, 19285–19290. [CrossRef]

27. Gowans, G.J.; Hawley, S.A.; Ross, F.A.; Hardie, D.G. AMP is a true physiological regulator of AMP-activated protein kinase by both allosteric activation and enhancing net phosphorylation. Cell Metab. 2013, 18, 556–566. [CrossRef]

28. Fernandez, A.M.; Torres-Alemán, I. The many faces of insulin-like peptide signalling in the brain. Nat. Rev. Neurosci. 2012, 13, 225–239. [CrossRef]

29. Eny, K.M.; Wolever, T.M.S.; Fontaine-Bisson, B.; El-Sohemy, A. Genetic variant in the glucose transporter type 2 (GLUT 2): Expression in specific brain nuclei. Brain Res. 1994, 638, 221–226. [CrossRef]

30. Beck, B. Neuropeptides and obesity. Nutrition 2000, 16, 916–923. [CrossRef]

31. Beck, B.; Burlet, A.; Nicolas, J.P.; Burlet, C. Hypothalamic neuropeptide Y (NPY) in obese Zucker rats: Implications in feeding and sexual behaviors. Physiol. Behav. 1990, 47, 449–453. [CrossRef]

32. Beck, B.; Burlet, A.; Nicolas, J.P.; Burlet, C. Hyperphagia in obesity is associated with a central peptidergic dysregulation in rats. J. Nutr. 1990, 120, 806–811. [CrossRef] [PubMed]

33. Beck, B.; Richy, S.; Stricker-Krongrad, A. Ghrelin and body weight regulation in the obese Zucker rat in relation to feeding state and dark/light cycle. Exp. Biol. Med. 2003, 228, 1124–1131. [CrossRef]

34. Beck, B.; Richy, S.; Stricker-Krongrad, A. Feeding response to ghrelin agonist and antagonist in lean and obese Zucker rats. Life Sci. 2004, 76, 473–478. [CrossRef]

35. Beck, B.; Richy, S.; Stricker-Krongrad, A. Feeding response to ghrelin agonist and antagonist in lean and obese Zucker rats. Life Sci. 2004, 76, 473–478. [CrossRef]

36. Leonardi, R.; Rehg, J.E.; Rock, C.O.; Jackowski, S. Pantothenate kinase 1 is required to support the metabolic control of liver. Mol. Pharmacol. 2005, 69, 1045–1052. [CrossRef] [PubMed]

37. Rock, C.O.; Calder, R.B.; Karim, M.A.; Jackowski, S. Pantothenate kinase regulation of the intracellular concentration of coenzyme A. J. Biol. Chem. 2000, 275, 1377–1383. [CrossRef]

38. Zhang, Y.-M.; Rock, C.O.; Jackowski, S. Feedback regulation of murine pantothenate kinase 3 by coenzyme A and coenzyme A thioesters. J. Biol. Chem. 2005, 280, 32594–32601. [CrossRef]

39. Gasmi, L.; McLennan, A.G. The mouse Nudt7 gene encodes a peroxisomal nudix hydrolase specific for coenzyme A and its derivatives. Biochem. J. 2001, 357, 33–38. [CrossRef]

40. Leonardi, R.; Rock, C.O.; Jackowski, S. Pank1 deletion in leptin-deficient mice reduces hyperglycaemia and hyperinsulinemia and modifies global metabolism without affecting insulin resistance. Diabetologia 2014, 57, 1466–1475. [CrossRef]

41. Reilly, S.J.; Tillander, V.; Ofman, R.; Alexson, S.E.H.; Hunt, M.C. The nudix hydrolase 7 is an acyl-CoA diphosphatase involved in regulating peroxisomal coenzyme A homeostasis. J. Biochem. 2008, 144, 655–663. [CrossRef] [PubMed]

42. Makia, N.L.; Goldstein, J.A. CYP2C8 is a novel target of peroxisome proliferator-activated receptor in human liver. Mol. Pharmacol. 2016, 89, 154–164. [CrossRef] [PubMed]

43. Ramaswamy, G.; Karim, M.A.; Murti, K.G.; Jackowski, S. PPARα controls the intracellular coenzyme A concentration via regulation of PANK1a gene expression. J. Lipid Res. 2004, 45, 17–31. [CrossRef] [PubMed]

44. Oana, F.; Takeda, H.; Hayakawa, K.; Matsuzawa, A.; Akahane, S.; Isaji, M.; Akahane, M. Physiological difference between obese (fa/fa) Zucker rats and lean Zucker rats concerning adiponectin. Metabolism 2005, 54, 995–1001. [CrossRef] [PubMed]
45. Shimabukuro, M.; Koyama, K.; Chen, G.; Wang, M.-Y.; Trieu, F.; Lee, Y.; Newgard, C.B.; Unger, R.H. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc. Natl. Acad. Sci. USA* 1997, 94, 4637–4641. [CrossRef] [PubMed]

46. Foretz, M.; Guichard, C.; Ferré, P.; Foufelle, F. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. *Proc. Natl. Acad. Sci. USA* 1997, 94, 4637–4641. [CrossRef] [PubMed]

47. Shimomura, I.; Bashmakov, Y.; Ikemoto, S.; Horton, J.D.; Brown, M.S.; Goldstein, J.L. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. *Proc. Natl. Acad. Sci. USA* 1999, 96, 13656–13661. [CrossRef]

48. Himeno, K.; Seike, M.; Fukuchi, S.; Masaki, T.; Sakata, T.; Yoshimatsu, H. Heterozygosity for leptin receptor (fa) accelerates hepatic triglyceride accumulation without hyperphagia in Zucker rats. *Obes. Res. Clin. Pract.* 2009, 3, 1–52. [CrossRef]

49. Coimbra, T.M.; Janssen, U.; Gröne, H.J.; Ostendorf, T.; Kunter, U.; Schmidt, H.; Brabant, G.; Floege, J. Early events leading to renal injury in obese Zucker (fatty) rats with type II diabetes. *Kidney Int.* 2000, 57, 167–182. [CrossRef]

50. Etgen, G.J.; Oldham, B.A. Profiling of Zucker diabetic fatty rats in their progression to the overt diabetic state. *Metabolism* 2000, 49, 684–688. [CrossRef]

51. Mizuno, M.; Sada, T.; Kato, M.; Koike, H. Renoprotective effect of blockade of angiotensin II AT1 receptors in an animal model of type 2 diabetes. *Hypertens. Res.* 2002, 25, 271–278. [CrossRef] [PubMed]

52. Vora, J.P.; Zimsen, S.M.; Houghton, D.C.; Anderson, S. Evolution of metabolic and renal changes in the ZDF/Drt-fa rat model of type II diabetes. *J. Am. Soc. Nephrol.* 1996, 7, 113–117. [PubMed]

53. Sakamoto, K.; Goransson, O.; Hardie, D.G.; Alessi, D.R. Activity of LKB1 and AMPK-related kinases in skeletal muscle: Effects of contraction, phenformin, and AICAR. *Am. J. Physiol. Endocrinol. Metab.* 2004, 287, E310–E317. [CrossRef] [PubMed]

54. Sakamoto, K.; McCarthy, A.; Smith, D.; Green, K.A.; Grahame Hardie, D.; Ashworth, A.; Alessi, D.R. Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction. *EMBO J.* 2005, 24, 1810–1820. [CrossRef]

55. Miura, S.; Kai, Y.; Tadaishi, M.; Tokutake, Y.; Sakamoto, K.; Bruce, C.R.; Febbraio, M.A.; Kita, K.; Chohnan, S.; Ezaki, O. Marked phenotypic differences of endurance performance and exercise-induced oxygen consumption between AMPK and LKB1 deficiency in mouse skeletal muscle: Changes occurring in the diaphragm. *Am. J. Physiol. Endocrinol. Metab.* 2013, 305, E213–E229. [CrossRef]

56. Thomson, D.M.; Porter, B.B.; Tall, J.H.; Kim, H.J.; Barrow, J.R.; Winder, W.W. Skeletal muscle and heart LKB1 deficiency causes decreased voluntary running and reduced muscle mitochondrial marker enzyme expression in mice. *Am. J. Physiol. Endocrinol. Metab.* 2007, 292, E196–E202. [CrossRef]

57. Marchington, D.; Rothwell, N.J.; Stock, M.J.; York, D.A. Energy balance, diet-induced thermogenesis and brown adipose tissue in lean and obese (fa/fa) Zucker rats after adrenalectomy. *J. Nutr.* 1983, 113, 1395–1402. [CrossRef]

58. Mourelatos, Z.; Dostie, J.; Paushkin, S.; Sharma, A.; Charroux, B.; Abel, L.; Rappaport, J.; Mann, M.; Dreyfuss, G. miRNPs: A novel class of ribonucleoproteins containing numerous microRNAs. *Genes Dev.* 2002, 16, 720–728. [CrossRef]

59. Trajkovski, M.; Hausser, J.; Soutschek, J.; Bhat, B.; Akin, A.; Zavolan, M.; Heim, M.H.; Stoffel, M. MicroRNAs 103 and 107 regulate insulin sensitivity. *Nature* 2011, 474, 649–653. [CrossRef]

60. Wilfred, B.R.; Wang, W.X.; Nelson, P.T. Energizing miRNA research: A review of the role of miRNAs in lipid metabolism, with a prediction that miR-103/107 regulates human metabolic pathways. *Mol. Genet. Metab.* 2007, 91, 209–217. [CrossRef]