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Testosterone deficiency induces markedly decreased serum triglycerides, increased small dense LDL, and hepatic steatosis mediated by dysregulation of lipid assembly and secretion in mice fed a high-fat diet

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ABSTRACT

Objective. Although low serum testosterone (T) is associated with metabolic disorders, the mechanism of this association is unclear. The objective of the present study was to investigate the combined effects of T deficiency and a high-fat diet (HFD) on hepatic lipid homeostasis in mice.

Materials/Methods. Orchiectomized (ORX) mice and sham-operated (SHAM) mice were randomly divided into five groups: SHAM mice fed a standard diet (SD), SHAM mice fed HFD, ORX mice fed SD, ORX mice fed HFD, and ORX mice fed HFD with T supplementation. After 4 weeks of treatment, we investigated the synthesis and secretion of lipids in the liver and detailed serum lipoprotein profiles in each group.

Results. ORX mice fed HFD showed increased hepatic steatosis, markedly decreased serum triglyceride (TG) and TG-VLDL content, and increased serum very small-LDL content. Gene expression analysis revealed that ORX mice fed HFD showed significantly decreased expression of microsomal triglyceride transfer protein, lipin-1, peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ coactivator 1-α, and significantly increased sterol regulatory element-binding protein-1, diacylglycerol acyltransferase-2 and fatty acid synthase. Reduction of hepatic AMPK phosphorylation was observed in ORX mice fed HFD. These perturbations in ORX mice fed HFD were normalized to the levels of SHAM mice fed HFD by T supplementation.

Conclusion. T deficiency is associated with failure of lipid homeostasis mediated by altered expression of genes involved in hepatic assembly and secretion of lipids.

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Abbreviations: T, testosterone; AR, androgen receptor; HFD, high-fat diet; SD, standard diet; ORX, orchiectomized; SHAM, sham-operated; PPAR, peroxisome proliferator-activated receptor; SREBP-1, sterol regulatory element-binding protein 1; DGAT-2, diacylglycerol acyltransferase 2; FAS, fatty acid synthase; MTP, microsomal transfer protein; Arf-1, ADP-ribosylation factor-1; PGC-1α, PPAR-γ coactivator 1-α; HPLC, high-performance liquid chromatography; RT-PCR, reverse transcription-polymerase chain reaction.

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1. Introduction

Serum testosterone (T) concentrations decline progressively with aging in men. Decreased serum T concentrations in aging men cause typical symptoms, including erectile dysfunction, cognitive decline, dysthymia, and sleep disorder, resulting in significant detriment in their quality of life [1]. In addition, low serum T has been linked to metabolic disorders, including hepatic steatosis, metabolic syndrome, and type 2 diabetes [2–5]. Recently, Kim et al. [6] reported that a low concentration of serum total T is independently associated with nonalcoholic fatty liver disease.

The effects of T are mediated by the androgen receptor (AR). The total AR-knockout (ARKO) mice exhibit increased adiposity [7,8]. Lin et al. [9] reported that hepatic-ARKO mice fed a high-fat diet (HFD) developed hepatic steatosis. It has been reported that castration of hypertensive rats induced a decrease in serum triglyceride (TG) and very-low-density lipoprotein (VLDL) [10]. Studies of orchiectomized (ORX) mice have also suggested that T is an important regulator of lipid homeostasis [11–13]. These findings from clinical and animal studies suggest that T deficiency contributes to the development and progression of hepatic steatosis. However, little is known about the effects of T deficiency on the expression of genes involved in the synthesis and secretion of TG and VLDL in the liver such as sterol regulatory element-binding protein 1 (SREBP-1), peroxisome proliferator-activated receptor-α (PPAR-α), and microsomal triglyceride transfer protein (MTP), and detailed serum lipid profiles.

The objective of the present study was to investigate the combined effects of T deficiency and HFD on hepatic lipid homeostasis in mice.

2. Methods

2.1. Animals and experimental design

All experimental procedures were approved by the Committee for Animal Research, Kyoto Prefectural University of Medicine. Six-week-old C57BL/6 J male mice were purchased from Shimizu Laboratory Supplies (Kyoto Japan) and were housed in a specific pathogen-free controlled environment. Mice were either ORX or sham-operated (SHAM) at 7 weeks of age. Mice were fed an HFD (HFD32, 507.6 kcal/100 g, fat kcal 56.7%; CLEA Japan, Tokyo, Japan) or a standard diet (SD; 344.9 kcal/100 g, fat kcal 4.6%; CLEA Japan) for 4 weeks starting at 8 weeks of age. To study the effects of T deficiency and HFD, male mice were divided into 5 groups, each with 6 mice: SHAM mice fed SD (SHAM/SD), ORX mice fed SD (ORX/SD), SHAM mice fed HFD (SHAM/HFD), ORX mice fed HFD (ORX/HFD), and ORX mice fed HFD with T supplementation (ORX/HFD+T). T was administrated to ORX/HFD mice as a subcutaneous injection depot of testosterone enanthate (3.6 μg/g body weight; ISEI Japan) diluted in sesame oil once in every 6 days [14]. At the end of the experiment, after an overnight fast, mice were killed by administration of an overdose of sodium aminobarbital. After blood collection by cardiac puncture, the liver was removed. The liver was either fixed with 10% buffered formaldehyde for histological examination or immediately frozen in liquid nitrogen for lipid analysis, and mRNA and protein extraction.

2.2. Analytical procedures

Blood glucose concentrations were measured using a glucometer (GLUTEST ACE, Sanwa Kagaku Kenkyusho, Nagoya, Japan). Serum insulin concentrations were measured using an insulin enzyme immunoassay system, the Morinaga Ultra Sensitive Mouse Insulin Assay Kit (Morinaga Institute of Biological Science, Kanagawa, Japan). Serum T concentrations of HFD-fed mice at 6 days after T administration were analyzed by liquid chromatography–tandem mass spectrometry (LS-MS/MS) [15] at ASKA Pharma Medical (Kanagawa, Japan).

2.3. Determination of TG and cholesterol levels in serum and liver

Serum TG and cholesterol concentrations were determined using the enzymatic assay systems Choletest®TG and Choletest®CHO (SEKISUI MEDICAL, Tokyo, Japan), respectively. For determination of hepatic TG and cholesterol concentrations, total lipids were extracted from the liver by the Folch method [16], and then hepatic TG and cholesterol contents were measured using the same enzymatic assay.

2.4. Analysis of lipoproteins by high-performance liquid chromatography (HPLC)

Serum lipoproteins were analyzed using an HPLC service (®LipoSEARCH) at Skylight Biotech (Akita, Japan), as previously described [17,18]. In brief, 10 μL of whole serum was injected into two connected columns (300×7.8 mm) of TSKgel LipopropakXL (Tosoh, Tokyo, Japan) and lipoproteins were separated with 0.05 mol/L Tris-buffered acetate (pH 8.0) containing 0.3 mol/L sodium acetate, 0.05% sodium azide, and 0.005% Brij-35 at a flow rate of 0.7 mL/min. The column effluent was split equally into two lines by a P-460 MicroSplitter (Upchurch Scientific, Oak Harbor, WA, USA); one effluent portion was mixed with cholesterol reagent (Determiner L TC/Kyowa Medex, Tokyo, Japan) and the other with TG reagent (Determiner L TG/Kyowa Medex). We defined 3 VLDL subclasses, 4 LDL subclasses, and 5 HDL subclasses using 20 component peaks categorized on the basis of lipoprotein particle size (diameter).

2.5. RNA isolation, first-strand cDNA synthesis, and gene expression analysis

Livers of fasting mice were resected and immediately frozen using liquid nitrogen-cooled tongs. Then, liver tissue was ground finely with a liquid nitrogen-cooled mortar and pestle and homogenized in ice-cold TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and total RNA was isolated as described in the manufacturer's instructions. Total RNA (0.5 μg) was reverse-transcribed using PrimeScript RT Master Mix (TaKaRa Bio, Shiga, Japan) for first-strand cDNA synthesis utilizing an oligonucleotide dT primer and random hexamer priming according to the manufacturer's recommendations. The RT reaction was performed for 15 min at 37 °C, and the inactivation of RT was for 5 s at 85 °C.

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