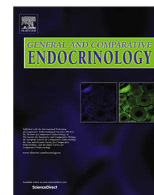
General and Comparative Endocrinology 259 (2018) 115–121

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen



Chronic dexamethasone exposure markedly decreased the hepatic triglyceride accumulation in growing goats

Qu Chen, Liqiong Niu, Canfeng Hua, Yali Geng, Liuping Cai, Shiyu Tao, Yingdong Ni *, Ruqian Zhao

Key Laboratory of Animal Physiology & Biochemistry, Nanjing Agricultural University, Nanjing 210095, PR China

ARTICLE INFO

Article history:
Received 13 September 2017
Revised 5 November 2017
Accepted 15 November 2017
Available online 16 November 2017

Keywords:
Chronic stress
Dexamethasone
Triglyceride metabolism
Liver
Goat

ABSTRACT

Chronic stress seriously threatens welfare and health in animals and humans. Consecutive dexamethasone (Dex) injection was used to mimic chronic stress previously. In order to investigate the effect of chronic stress on hepatic lipids metabolism, in this study, 10 healthy male goats were randomly allocated into two groups, one received a consecutive injection of Dex via intramuscularly for 3 weeks (Dex group), the other received the same volume of saline as the control group (Con group). Hepatic health and triglyceride (TG) metabolism were analyzed and compared between two groups. The data showed that a significant decrease of TG in plasma and the liver was significantly decreased by Dex ($P < .05$), while the hepatic nonesterified fatty acid (NEFA) concentration was increased compared to the Con group ($P < .05$). Consistent with the decrease of TG level, the activity of hepatic lipoprotein lipase (LPL) and hepatic lipase (HL) enzymes activities were significantly enhanced by Dex. Real-time PCR results showed that the mRNA expression of sterol regulatory element binding transcription factor 1 (SREBP-1), acyl-CoA dehydrogenase long chain (ACADL) and acyl-CoA synthetase bubblegum family member 1 (ACSBG1) genes in liver was significantly up-regulated by chronic Dex injection ($P < .05$), whereas perilipin 2 (PLIN2) and adipose triglyceride lipase (ATGL) mRNA expression was significantly decreased by Dex ($P < .05$). In addition, no obvious damages were observed in the liver in both Con and Dex groups demonstrating by the sirius red staining, HE staining as well as several biochemical parameters related to the functional status of hepatocytes. Our data indicate that chronic Dex exposure decreases TG levels in the circulation and the liver through activating lipolysis and inhibiting lipogenesis without causing hepatic damages in the growing goats.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Glucocorticoids (GCs), the stress hormones, are steroid hormones secreted by the adrenal cortex under a state of stress in mammals and other vertebrates. It's well documented that the immediate effects of stress hormones are beneficial in a particular short-term situation, however, long-term exposure to stress creates a high level of these hormones, which may lead to metabolic disorders and diseases. Dexamethasone (Dex), a synthetic GC, has been used as a potent anti-inflammatory and immunosuppressive agents in treatment of different inflammatory disorders (Du et al., 2014; Pang et al., 2012). Long-term exposure to Dex also causes severe the metabolic disorders particularly causing imbalance of glucose and lipids homeostasis. However, the relevant studies is still limited in ruminant animals.

Triglyceride (TG) is the main storage form of fat in vivo. In adipose cells, lipogenesis and lipolysis are in progress at the same time, and decomposition or deposition of fat is determined by mutual velocity of these 2 processes, moreover the process of fat accumulation is regulated by the lipase and lipolytic enzyme activity as well as the concentration of effective substrate (Baldner et al., 1985; Ingle et al., 1972). The basic lipid metabolism process of ruminants are similar to non-ruminants, like other mammals, liver is one of the major organs involved in the TG metabolism. Sterol response element-binding proteins (SREBPs) are a family of membrane-bound transcription factors containing a helix-loop-helix-leucinezipper, and it plays an important role in fatty acid metabolism (McPherson and Gauthier, 2004). SREBP-1 plays a crucial role in the development of fatty liver, and the disruption of SREBP-1 caused a significant reduction in hepatic expression of a battery of lipogenic genes (Yahagi et al., 2002). Perilipin2 (PLIN2), also known as adipose differentiation-related protein (ADRP), or adipophilin, is a member of the PAT family involved in lipid droplet (LD) formation in the liver and peripheral tissues (Miura et al.,

* Corresponding author.
E-mail address: niyingdong@njau.edu.cn (Y. Ni).

https://doi.org/10.1016/j.ygcen.2017.11.011
0016-6480/© 2017 Elsevier Inc. All rights reserved.

2002). In adipocytes, perilipin protects the LDs from lipases under basal conditions. Imamura et al. have confirmed PLIN2 overexpression can cause fat deposition in NIH-3T3 and Swiss-3T3 cells (Imamura et al., 2002). Lipoprotein lipase (LPL) is a multifunctional protein and plays a major role in the metabolism and transport of lipids (Wang and Eckel, 2009). LPL is a rate-limiting enzyme that it can hydrolyze core TG in circulating chylomicrons and very low density lipoproteins (VLDLs). Weinstock et al. found whole-body deficiency of LPL results in severe hypertriglyceridemia in mice (Weinstock et al., 1995). Hepatic lipase (HL) is a lipolytic enzyme, mainly synthesized in hepatic parenchymal cells (Jansen et al., 2002). Previous studies showed LPL effectively hydrolyzes TG in chylomicra and in large VLDL, but has little or no activity against high density lipoprotein (HDL) in the plasma environment, whereas HL can hydrolyze TG in HDL (Clay et al., 1989). Clay et al. found rabbits have low HL activity and LDL which are enlarged and enriched in TG (Clay et al., 1989).

Chronic stress can induce adrenal hyperplasia and increased cortisol levels. GCs can affect fat metabolism in vivo, a pronounced elevation of circulating GCs levels can display central obesity, fatty liver development, and insulin resistance (Shibli-Rahhal et al., 2006). The effect of glucocorticoids on adipose tissue is still a matter of controversy, but most data have shown GCs can induce lipolysis. Previous studies showed in rat epididymal fat cells and 3T3-L1 preadipocyte, Dex stimulated fat decomposition with dose-dependence and time-dependence, the gene expression of hormone-sensitive lipase (HSL) (Slavin et al., 1994) and adipose triglyceride lipase (ATGL) (Villena et al., 2004) were increased. Furthermore Xu et al. found Dex induced phosphorylation and down-regulation of perilipin that modulates lipolysis (Xu et al., 2009). Hence these results demonstrated that GCs can directly stimulate lipolysis. In addition lipolysis caused by Dex can be inhibited by glucocorticoid receptor (GR) antagonist RU486 (Russell and Tisdale, 2005), the data indicated that GCs influenced transcription of the genes related to lipolysis probably through combination with its receptors. Adipose tissue decomposition result in increased circulating nonesterified fatty acid (NEFA) and it will be accumulation in the liver, and up-regulates lipase activity to induce fatty liver disease.

At present the researches are mainly focused on rodents and mankind, the studies are less involved in ruminants. Besides there is little report on hepatic lipolysis after Dex treatment. This study preliminarily investigated the mechanism of hepatic lipolysis induced by Dex in goats, and paved the way for further research in the future.

2. Materials and methods

2.1. Ethics statement

The Institutional Animal Care and Use Committee (IACUC) of Nanjing Agricultural University approved all animal procedures. The "Guidelines on Ethical Treatment of Experimental Animals" (2006) No. 398 set by the Ministry of Science and Technology, China and the Regulation regarding the Management and Treatment of Experimental Animals (2008) No. 45 set by the Jiangsu Provincial People's Government, was strictly followed during the slaughter and sampling procedures.

2.2. Animals and experimental procedures

In brief, 10 healthy male goats (body weight 25 ± 1.0 kg) were raised in individual pens with free access to water and fed twice daily at 08:00 and 18:00, respectively. Animals were accustomed to all procedures of sampling and treatment before treatments. The dose of Dex was determined based on the previous study by Emikpe et al. (2013). Ten goats were randomly assigned to two groups: one group was injected intramuscularly with saline as Con group, another group was injected intramuscularly with Dex 0.2 mg/kg injection at 7:30 before morning feeding for 21 days.

2.3. Samples collection

Samples of plasma were obtained on days 1, 7, 14, and 21 of the experiment shortly before the injection and morning feeding from jugular vein. Samples of liver were collected at the end of the experiment. After an overnight fasting, all goats were weighed and killed by injections of xylazine [0.5 mg (kg body mass)−1; Xylosol; Ogris Pharme, Wels, Austria] and pentobarbital [50 mg (kg body mass)−1; Release; WDT, Garbsen, Germany]. Immediately after death, liver tissue samples were frozen immediately in liquid nitrogen and then used for extraction of RNA and proteins.

2.4. Plasma and liver parameters detection

Plasma TG, AST and ALT were measured using an automatic biochemical analyzer (7020, HITACHI, Tokyo, Japan), strictly following the manufacturer's instructions. Liver TG was detected by using TG kit (Jiancheng Co. Ltd, Nanjing, China), strictly following the manufacturer's instructions. Liver NEFA was detected by using NEFA kit (Jiancheng Co. Ltd, Nanjing, China), strictly following the manufacturer's instructions. Enzymes activity of LPL and HL were detected by using LPL/HL kit (Jiancheng Co. Ltd, Nanjing, China), strictly following the manufacturer's instructions. Liver GSH-Px was detected by using GSH-Px kit (Jiancheng Co. Ltd, Nanjing, China), strictly following the manufacturer's instructions. Liver MDA was detected by using MDA kit (Jiancheng Co. Ltd, Nanjing, China), strictly following the manufacturer's instructions. CAT was detected by using CAT kit (Jiancheng Co. Ltd, Nanjing, China), strictly following the manufacturer's instructions. Liver T-AOC was detected by using T-AOC kit (Jiancheng Co. Ltd, Nanjing, China), strictly following the manufacturer's instructions.

2.5. RNA isolation, cDNA synthesis and real-time PCR

Liver tissue was quickly collected, immediately frozen in liquid nitrogen and stored at −80 °C until RNA isolation. Total RNA was extracted from liver samples with TRIzol reagent (15596026; Invitrogen, Shanghai, China). The concentration and quality of the RNA were measured with a NanoDropND-1000 Spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA). Then 2 μg of total RNA was treated with RNase-Free DNase (M6101; Promega, Madison, WI, USA) and reverse transcribed according to manufacturer's instructions. Two micro liters of diluted cDNA (1:40, v/v) was used for real-time PCR, which was performed in an Mx3000P (Stratagene, USA). GAPDH, which is not affected by the experimental factors, was chosen as the reference gene. All the primers were listed in Table 1, were synthesized by Tsingke Company (Nanjing, China). The method of 2−Ct was used to analyze the real-time PCR results, and gene mRNA levels were expressed as the fold change relative to the mean value of the control group.

2.6. Western blotting analysis

One hundred milligrams of frozen liver was minced and homogenized in 1 ml of ice-cold RIPA buffer containing the protease inhibitor cocktail Complete EDTA-free (Roche, Penzberg, Germany). The homogenates were centrifuged at 12,235*g* for 20 min at 4 °C and then the supernatant fraction was collected. The protein concentration was determined using a BCA Protein Assay kit (Pierce, Rockford, IL, USA). Eighty micrograms of protein extract from each

Download English Version:

https://daneshyari.com/en/article/8631150

Download Persian Version:

https://daneshyari.com/article/8631150

Daneshyari.com