Life Sciences 109 (2014) 95–103

Contents lists available at ScienceDirect

Life Sciences

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

Testosterone suppresses the expression of regulatory enzymes of fatty acid synthesis and protects against hepatic steatosis in cholesterol-fed androgen deficient mice ☆

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ARTICLE INFO

Article history:
Received 28 February 2014
Accepted 7 June 2014
Available online 20 June 2014

Keywords:
Testosterone
Lipid deposition
Androgen receptor
Fatty liver
Lipogenesis

ABSTRACT

Aims: Non-alcoholic fatty liver disease and its precursor hepatic steatosis is common in obesity and type-2 diabetes and is associated with cardiovascular disease (CVD). Men with type-2 diabetes and/or CVD have a high prevalence of testosterone deficiency. Testosterone replacement improves key cardiovascular risk factors. The effects of testosterone on hepatic steatosis are not fully understood.
Main methods: Testicular feminised (Tfm) mice, which have a non-functional androgen receptor (AR) and very low serum testosterone levels, were used to investigate testosterone effects on high-cholesterol diet-induced hepatic steatosis.
Key findings: Hepatic lipid deposition was increased in Tfm mice and orchidectomised wild-type littermates versus intact wild-type littermate controls with normal androgen physiology. Lipid deposition was reduced in Tfm mice receiving testosterone treatment compared to placebo. Oestrogen receptor blockade significantly, but only partially, reduced the beneficial effects of testosterone treatment on hepatic lipid accumulation. Expression of key regulatory enzymes of fatty acid synthesis, acetyl-CoA carboxylase alpha (ACACA) and fatty acid synthase (FASN) were elevated in placebo-treated Tfm mice versus placebo-treated littermates and Tfm mice receiving testosterone treatment. Tfm mice on normal diet had increased lipid accumulation compared to littermates but significantly less than cholesterol-fed Tfm mice and demonstrated increased gene expression of hormone sensitive lipase, stearyl-CoA desaturase-1 and peroxisome proliferator-activated receptor-gamma but FASN and ACACA were not altered.
Significance: An action of testosterone on hepatic lipid deposition which is independent of the classic AR is implicated. Testosterone may act in part via an effect on the key regulatory lipogenic enzymes to protect against hepatic steatosis.

Introduction

In parallel with the global increase in the prevalence of type-2 diabetes (T2D) and obesity, there has been a sharp rise in the incidence of non-alcoholic fatty liver disease (NAFLD) and its precursor hepatic steatosis (Collantes et al., 2004). Indeed, it has been previously reported that approximately 70% of patients who are either obese or who have T2D, will go on to develop NAFLD (Roden, 2006; Farrell and Larter, 2006), an elevated incidence of cardiovascular events and cardiovascular risk factors (Targher et al., 2005, 2006, 2007, 2010; McKimmie et al., 2008).

Evidence suggests that testosterone deficiency is a cardiovascular risk factor and that low circulating levels of testosterone are associated with T2D, visceral obesity, insulin resistance, hyperinsulinemia, dyslipidemia and carotid atherosclerosis (Jones, 2010). Testosterone replacement therapy (TRT) in hypogonadal men has beneficial effects on lipid and carbohydrate metabolism and inflammation (Kelly and Jones, 2013a). Furthermore, TRT to the normal range reduces insulin resistance and central adiposity and improves glycaemic control and

☆ Presented in part at the Annual meeting of the Endocrine Society in Washington, June 2009; Boston, June 2011; Houston, June 2012; and the Society for Endocrinology annual meeting in Harrogate 2010.

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http://dx.doi.org/10.1016/j.lfs.2014.06.007
0024-3205/

cholesterol levels in hypogonadal men with T2D and/or the MetS (Boyanov et al., 2003; Kapoor et al., 2006; Heufelder et al., 2009; Cornoldi et al., 2010; Kalinchenko et al., 2010; Jones et al., 2011). The relationship between hepatic steatosis and serum testosterone concentrations has not yet received sufficient attention. Some population-based studies have reported an association between low serum testosterone concentrations and hepatic steatosis (Völzke et al., 2010; Kim et al., 2012; Tian et al., 2012; Kley et al., 1975), while others report no significant differences in serum testosterone concentrations (Myking et al., 1987) or after adjustment for age (Tian et al., 2012). Treatment of obese male patients who had obstructive sleep apnoea with 18 weeks of testosterone therapy resulted in a significant decrease in liver fat assessed by CT imaging (Hoyos et al., 2012). However, a recent brief report investigating the effect of testosterone administration on mobility limitation in older men with low testosterone additionally described no effect of TRT on liver fat assessed by MRI (Huang et al., 2013).

A limited number of animal studies have also investigated the effects of testosterone on hepatic steatosis. Orchidectomised male mice receiving 4 weeks high-fat diet feeding had significantly elevated hepatic lipid deposition and triglyceride content compared to sham operated controls (Senmaru et al., 2013). This was accompanied by altered expression of genes involved in hepatic lipid assembly and secretion. Testosterone supplementation normalised hepatic steatosis and mitigated the aberrant gene expression seen in orchidectomised littermates. Hepatic androgen receptor knockout (hARKO) mice receiving high-fat diet developed hepatic steatosis and insulin resistance in male but not in female mice via increased de novo lipogenesis, although it was not determined whether this was a result of AR dysfunction or an obesity-related testosterone decline (Lin et al., 2008). Therefore, the role of testosterone in the molecular mechanisms of hepatic steatosis remains relatively unexamined and the underlying mechanisms of the beneficial action of TRT are currently unclear.

The aim of the present study was to determine whether testosterone deficiency is associated with increased hepatic steatosis in the Tfm mouse following feeding on a high-cholesterol diet, to investigate the effects of testosterone treatment on hepatic lipid homeostasis and determine the role of the AR in this animal model of hepatic steatosis.

Materials and methods

The Tfm mouse model

The Tfm mouse exhibits a natural mutation in the gene encoding the classical AR which results in a truncated receptor protein which lacks both DNA- and steroid-binding domains rendering the receptor non-functional (Charest et al., 1991; He et al., 1991). In addition, serum levels of testosterone are markedly (10-fold) reduced in the Tfm mouse compared to normal XY littermate controls due primarily to the loss of 17α-hydroxylase, a key enzyme necessary for testosterone synthesis (Murphy and O'Shaughnessy, 1991; Jones et al., 2003).

In this study we utilised archived tissue from two previously published studies to investigate the influence of testosterone and the role of the AR in hepatic lipid deposition (Nettleship et al., 2007) and to investigate hepatic gene expression in selected repeated animal groups (Kelly et al., 2012). Archival tissue from experiment 1 (Nettleship et al., 2007) was primarily processed and stored for histological analysis at the time of collection and was therefore not of sufficient quality and quantity to allow subsequent gene and protein expression analysis. Tissue from experiment 2 was processed and stored for both histological and gene and protein expression analysis. Animals were caged under standard conditions in a temperature and humidity controlled room on a 12 h light:12 h darkness cycle. Water and food were unrestricted throughout the study. All procedures were carried out under the jurisdiction of the UK Home Office project licences, governed by the UK Animals Scientific Procedures Act 1986.

Experiment 1: the effect of testosterone and the influence of the AR on hepatic lipid deposition

At 8 weeks of age, 2 groups of mice, Tfm mice (n = 32) and XY littermates (n = 16), were randomly assigned to different experimental groups. Animals underwent either sham operation or surgical orchidectomy and were allowed to recover. At 9 weeks of age, surgically prepared mice were sub-divided into the following groups: sham operated XY littermates receiving once fortnightly intramuscular injection of 10 μl saline as a placebo injection (XY + P, n = 8), orchidectomised XY littermates receiving once fortnightly intramuscular injection of 10 μl placebo (XY + O, n = 8), sham operated Tfm mice receiving once fortnightly intramuscular injection of 10 μl placebo (Tfm + P, n = 8), sham operated Tfm mice receiving once fortnightly intramuscular injection of 10 μl of 100 mg/ml testosterone (Sustanon100® which contains; testosterone propionate 20 mg/ml, testosterone phenylpropionate 40 mg/ml, and testosterone isocaproate 40 mg/ml, Organon Laboratories Ltd, Cambridge, UK) (Tfm + S100, n = 8) reported to produce serum testosterone levels within the normal range (Nettleship et al., 2007), sham operated Tfm mice receiving once fortnightly intramuscular injection of 10 μl 250 mg/ml testosterone (Sustanon 250®) (Tfm + S250), and sham operated Tfm mice receiving 10 μl of Sustanon100® in conjunction with 30 μl of 50 mg/ml oestrogen receptor α (ERα) antagonist, fulvestrant (Faslodex®, AstraZeneca, Cheshire, UK) at 15 times the human dose (Tfm + S100 + F, n = 8), as shown in Table 1. At 10-weeks of age all mice were fed a cholesterol-enriched diet, ad libitum, (42% butterfat, 1.25% cholesterol and 0.5% cholate — Special diet services, UK) for a period of 28-weeks (Nettleship et al., 2007).

Experiment 2: the effect of testosterone and the influence of the AR on hepatic gene expression

At 9 weeks of age, mice were randomly assigned to the following groups: XY littermates receiving once fortnightly intramuscular injection of 10 μl placebo (XY + P, n = 12), Tfm mice receiving once fortnightly intramuscular injection of 10 μl placebo (Tfm + P, n = 12), and Tfm mice receiving once fortnightly intramuscular injection of 10 μl of Sustanon 100® (Tfm + S100, n = 12). At 10-weeks of age mice were fed a cholesterol-enriched diet, ad libitum, for 28-weeks. In addition, 2 animal groups receiving normal chow diet (XY + ND, n = 12; Tfm + ND, n = 12) for the duration of the study were included in this experiment as shown in Table 1 (Kelly et al., 2012).

Tissue collection

At the end of the feeding period all animals were sacrificed via a Home Office approved schedule 1 method. Following midline sternotomy, the rib cage was opened and whole blood was collected from the chest cavity after severance of the thoracic aorta and the liver removed. The liver tissue was then washed with physiological saline solution and either embedded in optical cutting temperature compound (Agar Scientific Limited, Essex, UK) or transferred into RNA later (Qiagen, UK), snap frozen and stored at −80 °C until processing for analysis.

Quantification of lipid deposition in the liver

Serial 8 μm frozen sections of the liver (100 per mouse) were cut on a cryostat and 10 (every tenth section) sections of the liver tissue were used per animal for analysis. The frozen 8 μm cryosections of the liver tissue were allowed to air dry onto charged slides (Surgipath, UK) at room temperature and then stained with Oil Red O as described previously (Nettleship et al., 2007). Following Oil red O staining, liver sections were digitally photographed using Nikon E400 microscope with settings standardised between images. Quantification of the lipid-stained areas was performed using Image J (USA) software package. A 2 × 2 grid was placed over each section and the top right hand