Hepatic actions of androgens in the regulation of metabolism

Vita Birzniece[a,b,c,d]

Purpose of review
The purpose of this review is to summarize recent findings on hepatic actions of androgens in the regulation of protein, lipid and glucose metabolism. The rationale for liver-targeted testosterone use will be provided.

Recent findings
Liver-targeted testosterone administration, via the oral route, induces protein anabolic effect by reducing the rate of protein oxidation to a similar extent to that of systemic testosterone administration. Recent evidence indicates that testosterone exerts whole-body anabolic effect through inhibition of nitrogen loss via the hepatic urea cycle. Several hepatic effects of androgens, particularly on glucose metabolism, are direct and take place before any changes in body composition occur. This includes an increase in insulin secretion and sensitivity, and reduction in hepatic glucose output by testosterone. Furthermore, lack of testosterone in the liver exacerbates diet-induced impairment in glucose metabolism. In the liver, androgens induce the full spectrum of metabolic changes through interaction with growth hormone or aromatization to estradiol.

Summary
Liver-targeted testosterone therapy may open up a new approach to achieve whole-body anabolism without systemic side-effects. Aromatizable androgens may be superior to nonaromatizable androgens in inducing a complex spectrum of direct, estrogen-mediated and other hormone-mediated effects of androgens.

Keywords
hepatic glucose output, hepatic urea production, lipid metabolism, oral testosterone, protein metabolism

INTRODUCTION

Sex steroids, not surprisingly, have been linked to the well known sex difference in body composition, distribution of body fat, muscle strength and function. Androgens are the major anabolic hormones, having profound effects on lipid and glucose metabolism, with low testosterone being associated with obesity, metabolic syndrome and diabetes. Therefore, androgens have lately been in focus for treatment of sarcopenia and obesity. A recent position statement states that testosterone replacement in established hypogonadism is warranted; however there are limited data from high-quality randomized controlled trials to justify testosterone treatment in older men, or men with obesity and diabetes who have low testosterone levels without evident gonadal or pituitary failure [1▪]. Thus, as shown also by a meta-analysis, the use of testosterone treatment in men with diabetes or the metabolic syndrome without classical hypogonadism is not supported [2]. However, there are instances, like androgen deprivation (ADT)-induced hypogonadism in prostate cancer patients, when androgen treatment would be beneficial, but systemic administration is contraindicated. Therefore, to guide development of androgen therapy with desirable and well tolerated outcomes in patients, differentiation of tissue-specific androgen action is warranted.

The liver is a major metabolic organ that is sensitive to sex steroids. It is a key visceral organ for controlling energy storage, has high capacity for lipid transport, de-novo lipogenesis, gluconeogenesis and

[a]School of Medicine, Western Sydney University, Sydney, [b]Department of Diabetes and Endocrinology, Blacktown Hospital, Blacktown, [c]Garvan Institute of Medical Research, Sydney and [d]School of Medicine, University of New South Wales, Sydney, New South Wales, Australia

Correspondence to Dr Vita Birzniece, School of Medicine, Western Sydney University, Blacktown Clinical School and Research Centre, Blacktown Hospital, Blacktown 2148, NSW, Australia.
Tel: +61 2 98516059; fax: +61 2 98516050;
e-mail: v.birzniece@westernsydney.edu.au

Curr Opin Endocrinol Diabetes Obes 2018, 25:201–208
DOI:10.1097/MED.0000000000000405

KEY POINTS

- Liver-targeted testosterone therapy may open up a new approach how to induce whole-body anabolism.
- Several effects of androgens, in particularly on glucose metabolism, are direct before any changes in body composition take place.
- Aromatizable androgens may be superior compared with nonaromatizable androgens in inducing the complex spectrum of direct, estrogen-mediated and other hormone-mediated effects of androgens.

substrate oxidation. Androgen receptors are expressed in human hepatocytes [3], hepatic androgen receptor expression is several times higher in male than in female rats, and gradually declines during aging [4]. Therefore, it is conceivable that androgens play a major role in the regulation of hepatic metabolism.

Here, recent findings are summarized on hepatic actions of androgens in the regulation of protein, lipid and glucose metabolism. The rationale for liver-targeted testosterone use will be provided.

EFFECTS OF ANDROGENS ON MUSCLE MASS AND FUNCTION

Androgens are the major anabolic hormones that exert a dose-dependent effect on muscle mass and strength, and improve physical function in men [5,6]. In healthy men, testosterone administration in a dose of approximately six times the physiological rate of testosterone production results in 9-kg increase in lean body mass over 20 weeks [7]. Testosterone also increases muscle strength and potentiates the strength-stimulating effect of exercise [7–9]. Declining levels of testosterone associates with sarcopenia, and testosterone replacement in hypogonadal men does increase muscle mass and strength [10,11]. Thus, androgens stimulate muscle accretion and function; however, tissue-specific mechanisms of the anabolic effect of androgens are not fully understood.

In muscle, androgens stimulate myoblast growth and differentiation, inducing muscle fiber hypertrophy [12]. Muscle-specific androgen receptor knockout models provide evidence of cell type-specific effects of androgen receptor activation in the regulation of muscle anabolism. Surprisingly, studies in myocyte-specific androgen receptor knockout (mARKO) mice show preservation of skeletal muscle mass (except for the highly androgen-sensitive levator ani muscle in which muscle loss is noted [13,14]), whereas global androgen receptor knockout and orchidectomized mARKO mice show a significant reduction in hind limb skeletal muscle mass [13,15]. This indicates that androgen receptor activation only in myocytes is not sufficient to induce muscle anabolism. The effect of androgens on muscle may be mediated through other cell types, such as myofibroblasts, satellite cells, motor neurons or it could be mediated via nongenomic pathways [16–18]. Testosterone aromatization to estradiol may also play a role in maintaining muscle mass [19]. Significantly, muscle mass is decreased in global ARKO males, but normal in ARKO females, indicating that in animal models circulating androgens play a major role in determining sex differences in muscle mass [15]. Local 5α reductase type 1 (*Srd5α1*) expression is of importance for the androgenic activity in muscle, as *Srd5α1* knockout male mice exert reduced forelimb muscle strength but not muscle mass (possibly due to a small sample size) [20]. Thus, the regulation of muscle mass by androgens is complex, involving direct and indirect mechanisms. Recently, evidence for an additional liver-mediated mechanism has been provided and will be discussed here.

ANDROGENS AND LIVER PROTEIN METABOLISM

Protein mass is constantly turning over in a dynamic process of breakdown and synthesis. Amino acids from proteolysis are predominantly resynthesized into new protein, replacing total body protein approximately in every 160 days [21]. About 20% of the amino acid pool is lost via oxidation, representing irreversible loss of protein, a hallmark of catabolism. Testosterone is nitrogen sparing; it reduces the rate of protein oxidation [22], facilitates amino acid availability for reutilization and increases muscle protein accretion [23–25]. Importantly, recent evidence indicates that the testosterone-induced protein anabolic effect is mediated through the liver.

The evidence comes from studies showing that the inhibition of whole-body protein oxidative loss by testosterone is equivalent for both oral (hepatic) and transdermal (systemic) delivery [22]. Oral delivery of nonesterified testosterone exposes the liver to high portal levels of testosterone which undergoes first-pass hepatic metabolism, preventing its appearance in the systemic circulation. Although testosterone administered transdermally enters the systemic circulation reaching both hepatic and extrahepatic tissues. When low-dose (40 mg/day) oral crystalline unconjugated testosterone was administered exposing only liver to testosterone, it reduced whole-body protein loss equivalent to

that seen with systemic testosterone administration. It should be noted that this is an oral crystalline unconjugated testosterone used at a dose that will only expose the liver; therefore, it will not exert systemic adverse effects or hepatotoxicity, as only oral 17 alpha-alkylated androgens are hepatotoxic [26,27]. Such liver-mediated anticatabolic effect of testosterone is evident in postmenopausal women and in hypogonadal men without increasing circulating testosterone levels [22,28]. Thus, the liver is the primary site underpinning the anticatabolic effect of testosterone. But what is the basis for this hepatic mechanism?

Recently, the evidence has been provided that this effect is mediated through the hepatic urea cycle [29▪▪]. The liver is a major site of protein metabolism which is controlled by the urea cycle. Amino acid nitrogen is oxidized into ammonia via the hepatic urea cycle and subsequently eliminated as urea by the kidney, representing a rate-limiting step for an irreversible loss of protein nitrogen [30]. Reduction in urea synthesis is paralleled by an increase in amino acid uptake in muscle [31]. Hormones that cause catabolism, such as glucocorticoids and glucagon, stimulate urea synthesis, whereas the reverse occurs with the anabolic growth hormone (GH) administration [32–35]. As hepatocytes express androgen receptors [3], testosterone may directly affect hepatic protein metabolism. Indeed, restoring testosterone levels into the normal range in hypogonadal men, reduced hepatic urea production by approximately 20% [29▪▪]. Thus, androgens, by inhibiting the hepatic urea cycle reduce hepatic loss of nitrogen, which increases amino acid nitrogen for protein synthesis translating into whole-body anabolism (Fig. 1). This opens up a novel approach to inducing whole-body anabolic effect without systemic exposure to androgens.

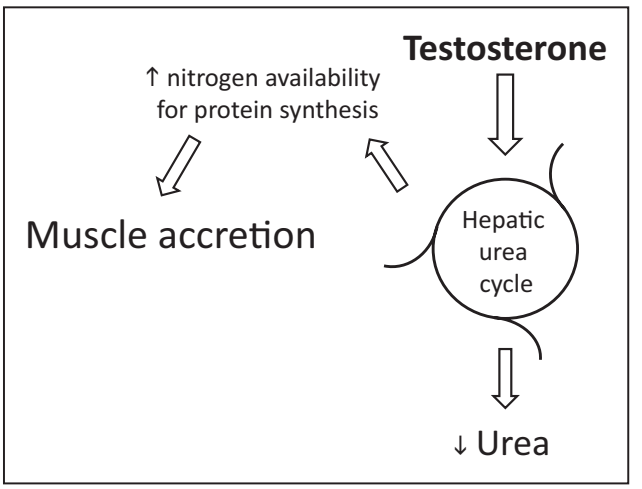

FIGURE 1. Regulation of whole-body protein metabolism by testosterone – liver-mediated mechanism. A proportion of amino acids derived from proteolysis is metabolized irreversibly to urea in the liver and excreted by the kidney. New evidence demonstrates that testosterone specifically reduces hepatic loss of nitrogen, thereby increasing amino acid nitrogen for muscle anabolism. Adapted from [29▪▪].

ANDROGENS AND HEPATIC LIPID METABOLISM

Testosterone plays an important role in regulating body fat [36,37]. Testosterone supplementation reduces fat mass in hypogonadal men and in aging men [11,38–42]. A recent meta-analysis of studies comprising 13 721 men revealed that circulating testosterone was lower in men with nonalcoholic fatty liver disease (NAFLD) than in those without fatty liver [43▪]. As obesity associates with NAFLD and low testosterone levels, the causality of this association is difficult to ascertain from cross-sectional studies. In patients with prostate cancer, ADT increases fat mass, induces metabolic syndrome and liver fat accumulation [44,45]. Furthermore, animal studies suggest that testosterone deficiency enhances diet-induced liver fat accumulation [46], whereas testosterone administration in obese men significantly reduces liver fat [47]. Whether such hepatic effects of testosterone are direct or mediated through modification of whole-body fat metabolism is unclear.

One of the mechanisms by which testosterone regulates the amount of fat is the stimulation of lipid oxidation [48–50]. As fat is oxidized both in the liver and in extrahepatic tissues, such as skeletal muscle, a study was conducted comparing hepatic and systemic effects of testosterone on fat oxidative disposal. Solely liver-targeted testosterone administration in hypogonadal men does not stimulate whole-body fat oxidation, which is however enhanced by transdermal testosterone administration [51]. This indicates that for full effect on fat oxidation, systemic testosterone exposure is required. Other mechanisms by which testosterone affects fat mass involve regulation of lipid storage [52], tissue-dependent inhibition of lipid uptake in visceral adipose tissue [53], inhibition of adipocyte precursor cell differentiation [54] and inhibition of lipoprotein lipase activity in adipocytes [55]. The effect of testosterone on fat mass is also mediated through modulation of other hormones, such as enhancing adrenergic stimulation of lipolysis [56,57], or increasing GH secretion and action that in turn stimulates lipolysis and fat utilization [58].

There is also evidence of a direct androgen effect on hepatic lipid metabolism. Androgen receptor activation is of importance in determining hepatic

lipid deposition. High fat-fed hepatic androgen receptor knockout male mice and aging chow-fed androgen receptor knockout males develop insulin resistance and hepatic steatosis through upregulation of genes involved in fatty acid (FA) synthesis and downregulation of genes involved in fat oxidation [59]. Furthermore, mice with *Srd5A1* knockout, who cannot convert testosterone to Dihydrotestosterone (DHT), show similar changes in gene expression during high-fat feeding [60,61]. Similarly, administration of a 5a-reductase inhibitor in male obese Zucker rats induces hyperinsulinemia and hepatic steatosis, indicative of a direct androgen receptor-mediated effect [61]. However, androgen receptor-independent action on hepatic lipid metabolism by androgens is also present. Mice with nonfunctional androgen receptors and low circulating testosterone show accelerated increase in hepatic steatosis during high-fat feeding, which is reversed by testosterone administration [62].

The effect of androgens on hepatic fat metabolism may also depend on the testosterone-to-estradiol ratio. It has been suggested that in men, the aromatization of testosterone to estradiol is important in reducing fat mass, especially intra-abdominal fat [63]. Importantly, men who cannot produce estradiol from testosterone because of aromatase deficiency develop steatohepatitis [64]. In male mice with aromatase knockout, fatty-liver develops [65,66] in parallel to an increase in de-novo lipogenesis and hepatic FA uptake, as shown by upregulation in hepatic SREBP-1c and CD36 [66]. This indicates that testosterone conversion to estradiol plays a major role in the regulation of hepatic lipid metabolism.

Thus, androgens may exert direct and estrogen-mediated effects on hepatic-lipid metabolism. Human studies indicate that for full androgen effect on lipid utilization, systemic exposure to androgens is required.

ANDROGENS AND LIVER GLUCOSE METABOLISM

It has long been debated whether the effect of androgens on glucose metabolism is direct, or a consequence of changes in fat and muscle mass, or liver fat content, that in turn affects whole-body insulin sensitivity. ADT in prostate cancer patients results in rapid deterioration in glucose metabolism. The risk of new onset diabetes is increased four to five fold in the 1st year of treatment [67]. Importantly, insulin resistance is increased by more than 30% as early as 3 months after initiation of ADT [68]. Acute testosterone withdrawal for as little as 2 weeks reduces insulin sensitivity in young men with idiopathic hypogonadotropic hypogonadism [69]. As changes in glucose metabolism occur before any changes in body composition take place, this points to a direct effect of androgens on glucose metabolism.

Androgen receptor knockout animals show progressive reduction in glucose tolerance and insulin sensitivity with advancing age [70]. These androgen receptor knockout male mice display accelerated weight gain, almost double the amount of triglyceride content in skeletal muscle and liver, hyperinsulinemia and hyperglycemia. Castration in male mice increases circulating glucose levels and hepatic GLUT2 expression, accompanied by an inhibitory effect on serum insulin, Akt phosphorylation, GLUT4 expression and its translocation, glycogen content and glucose uptake in the liver [71,72▪]. The study suggested that regulation of hepatic GLUT2 expression and glycogen production requires androgen receptor activation, whereas both testosterone and estrogen are required for full effect on hepatic Akt phosphorylation [71]. Others report that castration-induced hepatic gluconeogenesis and glucose output associates with an increase in FoxO1 expression, an effect inhibited by testosterone administration [73,74]. Importantly, hepatic androgen receptor knockout male mice develop insulin resistance during high-fat feeding, with decreased phosphoinositide-3 kinase activity and increased phosphenolpyruvate carboxykinase and protein tyrosine phosphatase 1B expression in the liver [59]. Only on a high-fat diet do these animals develop hyperinsulinemia and hyperglycemia, indicating that lack of testosterone in the liver will exacerbate diet-induced impairment in glucose metabolism. Another evidence of a direct androgen effect stems from a study in a human liver cell line, showing that testosterone increases insulin receptor mRNA and insulin sensitivity [75]. Thus, human and animal studies provide evidence of a direct effect of androgens on glucose metabolism.

Testosterone effect on glucose metabolism may be mediated through aromatization, as castration-induced impairment in glucose tolerance and insulin secretion are restored by testosterone but not by the nonaromatizable androgen, DHT [72▪]. In men with central obesity, at the doses used, DHT had no effect on insulin sensitivity in contrast to the beneficial effects of testosterone [41]. Furthermore, male aromatase knockout mice and men with aromatase deficiency show insulin resistance and hyperglycemia, demonstrating the importance of testosterone aromatization in the regulation of glucose metabolism [64,76].

Extrahepatic androgen action is also of great importance in modulating glucose metabolism, in particular, brain and pancreatic androgen receptor activation plays a significant role. Not only does hypogonadism reduce overall activity and

motivation to move, in turn promoting obesity and insulin resistance, selective neuronal androgen receptor deficiency leads to impaired hypothalamic insulin signaling that contributes to increased hepatic glucose production and systemic insulin resistance [72▪,77]. Testosterone also directly stimulates insulin release from the pancreas [78] and inhibits pancreatic beta-cell apoptosis by competing with glucocorticoid signaling [79].

Collectively, although systemic effects of androgens in modifying central androgen receptors or body composition will play a major role in determining effects on glucose metabolism, there is strong evidence pointing to a rapid direct effect on insulin secretion and hepatic glucose metabolism by aromatizable androgens.

OTHER HEPATIC EFFECTS OF ANDROGENS

Sex differences in an extensive set of liver gene expression have been widely recognized [80]. It's been proposed that this sex-specific hepatic gene expression largely depends upon signal transducer and activator of transcription 5b [81]. There are also sex differences in drug-metabolizing enzyme expression in the liver [82], with hepatic cytochrome modulation particularly affected by androgens [83]. Neonatal androgen imprinting is critical in modulating hepatic gene expression, contributing to the defeminization of hepatic steroid-metabolizing enzymes and remodeling of the GH axis [84]. Androgens also play a role in the development of hepatocellular carcinoma with nuclear androgen receptor overexpression and androgen receptors crosstalk with mammalian target of rapamycin, growth factors, endoplasmatic reticulum stress and immune response being proposed as potential mechanisms [85,86]. Hepatotoxicity is a recognized side effect of oral androgens; however, only oral 17 alpha-alkylated androgens exert direct hepatotoxic effect that may even lead to development of peliosis hepatis, adenoma or sarcoma [26,27]. Thus, oral 17 alpha-alkylated androgens should not be used for long-term androgen replacement therapy.

ANDROGENS AND GROWTH HORMONE INTERACTION

Testosterone may exert certain effects by stimulating the GH-IGF-1 system [48,87–90]. In men, testosterone stimulates GH secretion that drives hepatic IGF-1 production [91]. This process requires testosterone conversion to estradiol; nonaromatizable androgens do not stimulate GH secretion [92], and aromatase inhibition or central estrogen antagonism attenuates the stimulation of GH secretion by testosterone [91,93]. Importantly, in men with aromatase deficiency, GH secretion is reduced and is not rescued by systemic estrogen replacement [94]. It has been proposed that in men, the aromatization of testosterone to estradiol is important in reducing fat mass, especially intra-abdominal fat [63]. In this study, Finkelstein *et al.* report that in healthy men with induced hypogonadism, the combination of an aromatase inhibitor with testosterone replacement dampens testosterone-mediated positive effects on fat mass. However, treatment with aromatase inhibitors is also expected to reduce GH secretion and thus, hepatic action of GH. As GH significantly reduces fat mass and promotes hepatic lipid clearance, the effects on whole body and particularly hepatic lipid metabolism in this study may be due to reduced GH secretion and hepatic action, rather than estrogen deficiency *per se* [95–102].

Testosterone also enhances the action of GH on liver and extrahepatic tissues by increasing GH receptor expression [103]. Human studies show that testosterone augments the biological effects of GH, in which to attain the full effect of GH on anabolism and lipid utilization, cotreatment with testosterone is required [48,104,105]. Similarly, in hypogonadal men with GH deficiency, testosterone replacement induced a protein anabolic effect only in the presence of GH [22]. The interaction is bidirectional, as testosterone stimulates muscle *IGF-1* gene expression [106,107], whereas GH increases androgen receptor gene expression in muscle of hypogonadal men [108]. The anabolic effects of GH on physical performance are also potentiated by androgens [10]. These observations provide strong evidence that androgens increase GH secretion and tissue responsiveness to GH.

To optimize whole-body protein anabolism, hepatic interaction of both hormones is required. Only in GH-replete individuals, liver-targeted testosterone administration enhances whole-body protein anabolism by reducing protein oxidative loss through the hepatic urea cycle [22,28,29▪▪]. Solely liver exposure to testosterone stimulates hepatic IGF-1 production [22,28]. Evidence of a direct hepatic androgen effect on IGF-1 comes also from animal studies of male hepatic androgen receptors knockout mice, who present with a marked reduction in circulating IGF-1 [59]. These observations indicate that androgens interact with GH in the liver to stimulate GH effect on hepatic IGF-1 production, thus enhancing whole-body protein anabolism.

CONCLUSION

Androgens exert rapid and direct effects on various aspects of metabolism that are mediated through

the liver. The liver is a primary site in which testosterone and GH interact to regulate protein metabolism, in which both GH and testosterone are required to exert full anabolic effect. The finding of a hepatic urea cycle-mediated anticatabolic effect of testosterone opens a novel approach of targeting the hepatic urea cycle to enhance whole-body protein anabolism. This liver-targeted approach has an advantage in situations in which systemic testosterone treatment poses a health risk in men (ADT in prostate cancer) or virilization in women. Therefore, it has a potential to be developed as a novel, well tolerated and cost-effective treatment for sarcopenia in both men and women. Furthermore, androgen replacement requires testosterone rather than non-aromatizable androgens to induce the full spectrum of testosterone effects which are achieved by androgen receptor activation, aromatization to estradiol and interaction with the GH system.

Acknowledgements

I would like to express my sincere appreciation to Prof Ken Ho and Prof Mark McLean for their scientific guidance and Dr Sue Lynn Lau for constructive comments on the article.

Financial support and sponsorship

No specific financial support has been received for this work.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Yeap BB, Grossmann M, McLachlan RI, *et al.* Endocrine Society of Australia
■ position statement on male hypogonadism (Part 1): Assessment and indications for testosterone therapy. Med J Aust 2016; 205:173–178.

Endocrine Society of Australia position statement: testosterone replacement in established hypogonadism is warranted; however, there are limited data to justify testosterone treatment in older men, or men with obesity and diabetes who have low testosterone levels without evident gonadal or pituitary failure.

2. Grossmann M, Hoermann R, Wittert G, Yeap BB. Effects of testosterone treatment on glucose metabolism and symptoms in men with type 2 diabetes and the metabolic syndrome: a systematic review and meta-analysis of randomized controlled clinical trials. Clin Endocrinol (Oxf) 2015; 83: 344–351.
3. Eagon PK, Elm MS, Stafford EA, Porter LE. Androgen receptor in human liver: characterization and quantitation in normal and diseased liver. Hepatology 1994; 19:92–100.
4. Supakar PC, Roy AK. Role of transcription factors in the age-dependent regulation of the androgen receptor gene in rat liver. Biol Signals 1996; 5:170–179.
5. Bhasin S, Woodhouse L, Casaburi R, *et al.* Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle. J Clin Endocrinol Metab 2005; 90:678–688.
6. Basualto-Alarcon C, Varela D, Duran J, *et al.* Sarcopenia and androgens: a link between pathology and treatment. Front Endocrinol 2014; 5:217.
7. Bhasin S, Woodhouse L, Casaburi R, *et al.* Testosterone dose–response relationships in healthy young men. Am J Physiol Endocrinol Metab 2001; 281:E1172–E1181.
8. Hoffman JR, Kraemer WJ, Bhasin S, *et al.* Position stand on androgen and human growth hormone use. J Strength Cond Res 2009; 23:S1–S59.
9. Bhasin S, Storer TW, Berman N, *et al.* The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med 1996; 335:1–7.
10. Bhasin S, Storer TW, Berman N, *et al.* Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. J Clin Endocrinol Metab 1997; 82:407–413.
11. Snyder PJ, Peachey H, Berlin JA, *et al.* Effects of testosterone replacement in hypogonadal men. J Clin Endocrinol Metab 2000; 85:2670–2677.
12. Herbst KL, Bhasin S. Testosterone action on skeletal muscle. Curr Opin Clin Nutr Metab Care 2004; 7:271–277.
13. Chambon C, Duteil D, Vignaud A, *et al.* Myocytic androgen receptor controls the strength but not the mass of limb muscles. Proc Natl Acad Sci U S A 2010; 107:14327–14332.
14. Ophoff J, Van Proeyen K, Callewaert F, *et al.* Androgen signaling in myocytes contributes to the maintenance of muscle mass and fiber type regulation but not to muscle strength or fatigue. Endocrinology 2009; 150:3558–3566.
15. MacLean HE, Chiu WS, Notini AJ, *et al.* Impaired skeletal muscle development and function in male, but not female, genomic androgen receptor knockout mice. FASEB J 2008; 22:2676–2689.
16. Monks DA, O'Bryant EL, Jordan CL. Androgen receptor immunoreactivity in skeletal muscle: enrichment at the neuromuscular junction. J Comp Neurol 2004; 473:59–72.
17. Chen Y, Zajac JD, MacLean HE. Androgen regulation of satellite cell function. J Endocrinol 2005; 186:21–31.
18. Foradori CD, Weiser MJ, Handa RJ. Nongenomic actions of androgens. Front Neuroendocrinol 2008; 29:169–181.
19. Svensson J, Moverare-Skrtic S, Windahl S, *et al.* Stimulation of both estrogen and androgen receptors maintains skeletal muscle mass in gonadectomized male mice but mainly via different pathways. J Mol Endocrinol 2010; 45:45–57.
20. Windahl SH, Andersson N, Borjesson AE, *et al.* Reduced bone mass and muscle strength in male 5alpha-reductase type 1 inactivated mice. PLoS One 2011; 6:e21402.
21. Consolazio CF, Nelson RA, Matoush LO, *et al.* Nitrogen excretion in sweat and its relation to nitrogen balance requirements. J Nutr 1963; 79:399–406.
22. Birzniece V, Meinhardt UJ, Umpleby MA, *et al.* Interaction between testosterone and growth hormone on whole-body protein anabolism occurs in the liver. J Clin Endocrinol Metab 2011; 96:1060–1067.
23. Ferrando AA, Tipton KD, Doyle D, *et al.* Testosterone injection stimulates net protein synthesis but not tissue amino acid transport. Am J Physiol 1998; 275:E864–E871.
24. Kochakian CD. Comparison of protein anabolic property of various androgens in the castrated rat. Am J Physiol 1950; 160:53–61.
25. Kenyon AT, Knowlton K, Sandiford I, *et al.* A comparative study of the metabolic effects of testosterone propionate in normal men and women and in eunuchoidism. Endocrinology 1940; 26:26–45.
26. Bond P, Llewellyn W, Van Mol P. Anabolic androgenic steroid-induced hepatotoxicity. Med Hypotheses 2016; 93:150–153.
27. Handelsman DJ. Androgen physiology, pharmacology and abuse. In: De Groot LJ, Chrousos G, Dungan K, *et al.*, editors. Endotext. South Dartmouth, MA: MDText.com, Inc; 2000.
28. Birzniece V, Umpleby MA, Poljak A, *et al.* Oral low-dose testosterone administration induces whole-body protein anabolism in postmenopausal women: a novel liver-targeted therapy. Eur J Endocrinol 2013; 169: 321–327.
29. Lam T, Poljak A, McLean M, *et al.* Testosterone prevents protein loss via the
■■ hepatic urea cycle in human. Eur J Endocrinol 2017; 176:489–496.

This is the first study in humans showing that testosterone acts on the liver to reduce hepatic nitrogen loss through the urea cycle.

30. Lieberman M, Marks AD, Smith CM. Marks' basic medical biochemistry. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2009.
31. Grofte T, Wolthers T, Jorgensen JO, *et al.* Hepatic amino- to urea-N clearance and forearm amino-N exchange during hypoglycemic and euglycemic hyperinsulinemia in normal man. J Hepatol 1999; 30:819–825.
32. McLean P, Gurney MW. Effect of adrenalectomy and of growth hormone on enzymes concerned with urea synthesis in rat liver. Biochem J 1963; 87:96–104.
33. Vilstrup H. On urea synthesis – regulation in vivo. Dan Med Bull 1989; 36:415–429.
34. Grofte T, Jensen DS, Greisen J, *et al.* Growth hormone and insulin-like growth factor-I counteracts established steroid catabolism in rats by effects on hepatic amino-N degradation. J Hepatol 2001; 35:700–706.
35. Okun JG, Conway S, Schmidt KV, *et al.* Molecular regulation of urea cycle function by the liver glucocorticoid receptor. Mol Metab 2015; 4:732–740.
36. Bhasin S. Regulation of body composition by androgens. J Endocrinol Invest 2003; 26:814–822.
37. Isidori AM, Giannetta E, Greco EA, *et al.* Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. Clin Endocrinol (Oxf) 2005; 63:280–293.

38. Wang C, Swerdloff RS, Iranmanesh A, *et al.* Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. J Clin Endocrinol Metab 2000; 85:2839–2853.

39. Wang C, Cunningham G, Dobs A, *et al.* Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. J Clin Endocrinol Metab 2004; 89:2085–2098.

40. Snyder PJ, Peachey H, Hannoush P, *et al.* Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J Clin Endocrinol Metab 1999; 84:2647–2653.

41. Marin P, Holmang S, Gustafsson C, *et al.* Androgen treatment of abdominally obese men. Obes Res 1993; 1:245–251.

42. Marin P, Holmang S, Jonsson L, *et al.* The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. Int J Obes Relat Metab Disord 1992; 16:991–997.

43. ■ Jaruvongvanich V, Sanguankeo A, Riangwiwat T, Upala S. Testosterone, sex hormone-binding globulin and nonalcoholic fatty liver disease: a systematic review and meta-analysis. Ann Hepatol 2017; 16:382–394.

The recent meta-analysis revealed that circulating testosterone was lower in men with nonalcoholic fatty-liver disease than in those without fatty liver.

44. Gabbi C, Carubbi F, Losi L, *et al.* Nonalcoholic fatty liver disease induced by leuprorelin acetate. J Clin Gastroenterol 2008; 42:107–110.

45. Faris JE, Smith MR. Metabolic sequelae associated with androgen deprivation therapy for prostate cancer. Curr Opin Endocrinol Diabetes Obes 2010; 17:240–246.

46. Nikolaenko L, Jia Y, Wang C, *et al.* Testosterone replacement ameliorates nonalcoholic fatty liver disease in castrated male rats. Endocrinology 2014; 155:417–428.

47. Hoyos CM, Yee BJ, Phillips CL, *et al.* Body compositional and cardiometabolic effects of testosterone therapy in obese men with severe obstructive sleep apnoea: a randomised placebo-controlled trial. Eur J Endocrinol 2012; 167:531–541.

48. Gibney J, Wolthers T, Johannsson G, *et al.* Growth hormone and testosterone interact positively to enhance protein and energy metabolism in hypopituitary men. Am J Physiol Endocrinol Metab 2005; 289: E266–E271.

49. Arslanian S, Suprasongsin C. Testosterone treatment in adolescents with delayed puberty: changes in body composition, protein, fat, and glucose metabolism. J Clin Endocrinol Metab 1997; 82:3213–3220.

50. Mauras N, Hayes V, Welch S, *et al.* Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. J Clin Endocrinol Metab 1998; 83:1886–1892.

51. Birzniece V, Meinhardt UJ, Handelsman DJ, Ho KK. Testosterone stimulates extra-hepatic but not hepatic fat oxidation (Fox): comparison of oral and transdermal testosterone administration in hypopituitary men. Clin Endocrinol (Oxf) 2009; 71:715–721.

52. De Pergola G. The adipose tissue metabolism: role of testosterone and dehydroepiandrosterone. Int J Obes Relat Metab Disord 2000; 24(Suppl 2): S59–S63.

53. Marin P, Lonn L, Andersson B, *et al.* Assimilation of triglycerides in subcutaneous and intraabdominal adipose tissues in vivo in men: effects of testosterone. J Clin Endocrinol Metab 1996; 81:1018–1022.

54. Dieudonne MN, Pecquery R, Leneveu MC, Giudicelli Y. Opposite effects of androgens and estrogens on adipogenesis in rat preadipocytes: evidence for sex and site-related specificities and possible involvement of insulin-like growth factor 1 receptor and peroxisome proliferator-activated receptor gamma2. Endocrinology 2000; 141:649–656.

55. Ramirez ME, McMurry MP, Wiebke GA, *et al.* Evidence for sex steroid inhibition of lipoprotein lipase in men: comparison of abdominal and femoral adipose tissue. Metabolism 1997; 46:179–185.

56. Xu XF, De Pergola G, Bjorntorp P. Testosterone increases lipolysis and the number of beta-adrenoceptors in male rat adipocytes. Endocrinology 1991; 128:379–382.

57. Rebuffe-Scrive M, Marin P, Bjorntorp P. Effect of testosterone on abdominal adipose tissue in men. Int J Obes 1991; 15:791–795.

58. Eakman GD, Dallas JS, Ponder SW, Keenan BS. The effects of testosterone and dihydrotestosterone on hypothalamic regulation of growth hormone secretion. J Clin Endocrinol Metab 1996; 81:1217–1223.

59. Lin HY, Yu IC, Wang RS, *et al.* Increased hepatic steatosis and insulin resistance in mice lacking hepatic androgen receptor. Hepatology 2008; 47:1924–1935.

60. Dowman JK, Hopkins LJ, Reynolds GM, *et al.* Loss of 5alpha-reductase type 1 accelerates the development of hepatic steatosis but protects against hepatocellular carcinoma in male mice. Endocrinology 2013; 154: 4536–4547.

61. Livingstone DE, Barat P, Di Rollo EM, *et al.* 5alpha-Reductase type 1 deficiency or inhibition predisposes to insulin resistance, hepatic steatosis, and liver fibrosis in rodents. Diabetes 2015; 64:447–458.

62. Kelly DM, Nettleship JE, Akhtar S, *et al.* Testosterone suppresses the expression of regulatory enzymes of fatty acid synthesis and protects against hepatic steatosis in cholesterol-fed androgen deficient mice. Life Sci 2014; 109:95–103.

63. Finkelstein JS, Lee H, Burnett-Bowie SA, *et al.* Gonadal steroids and body composition, strength, and sexual function in men. N Engl J Med 2013; 369:1011–1022.

64. Maffei L, Murata Y, Rochira V, *et al.* Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment. J Clin Endocrinol Metab 2004; 89:61–70.

65. Hewitt KN, Pratis K, Jones ME, Simpson ER. Estrogen replacement reverses the hepatic steatosis phenotype in the male aromatase knockout mouse. Endocrinology 2004; 145:1842–1848.

66. Amano A, Kondo Y, Noda Y, *et al.* Abnormal lipid/lipoprotein metabolism and high plasma testosterone levels in male but not female aromatase-knockout mice. Arch Biochem Biophys 2017; 622:47–58.

67. Derweesh IH, Diblasio CJ, Kincade MC, *et al.* Risk of new-onset diabetes mellitus and worsening glycaemic variables for established diabetes in men undergoing androgen-deprivation therapy for prostate cancer. BJU Int 2007; 100:1060–1065.

68. Harrington JM, Schwenke DC, Epstein DR, Bailey DE Jr. Androgen-deprivation therapy and metabolic syndrome in men with prostate cancer. Oncol Nurs Forum 2014; 41:21–29.

69. Yialamas MA, Dwyer AA, Hanley E, *et al.* Acute sex steroid withdrawal reduces insulin sensitivity in healthy men with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 2007; 92:4254–4259.

70. Lin HY, Xu Q, Yeh S, *et al.* Insulin and leptin resistance with hyperleptinemia in mice lacking androgen receptor. Diabetes 2005; 54:1717–1725.

71. Muthusamy T, Murugesan P, Balasubramanian K. Sex steroids deficiency impairs glucose transporter 4 expression and its translocation through defective Akt phosphorylation in target tissues of adult male rat. Metabolism 2009; 58:1581–1592.

72. ■ Dubois V, Laurent MR, Jardi F, *et al.* Androgen deficiency exacerbates high-fat diet-induced metabolic alterations in male mice. Endocrinology 2016; 157:648–665.

Castration-induced impairment in glucose tolerance and insulin secretion are restored by testosterone but not by the nonaromatizable androgen.

73. Xia F, Xu X, Zhai H, *et al.* Castration-induced testosterone deficiency increases fasting glucose associated with hepatic and extra-hepatic insulin resistance in adult male rats. Reprod Biol Endocrinol 2013; 11:106.

74. Pal M, Gupta S. Testosterone supplementation improves glucose homeostasis despite increasing hepatic insulin resistance in male mouse model of type 2 diabetes mellitus. Nutr Diabetes 2016; 6:e236.

75. Parthasarathy C, Renuka VN, Balasubramanian K. Sex steroids enhance insulin receptors and glucose oxidation in Chang liver cells. Clin Chim Acta 2009; 399:49–53.

76. Takeda K, Toda K, Saibara T, *et al.* Progressive development of insulin resistance phenotype in male mice with complete aromatase (CYP19) deficiency. J Endocrinol 2003; 176:237–246.

77. Yu IC, Lin HY, Liu NC, *et al.* Neuronal androgen receptor regulates insulin sensitivity via suppression of hypothalamic NF-kappaB-mediated PTP1B expression. Diabetes 2013; 62:411–423.

78. Morimoto S, Fernandez-Mejia C, Romero-Navarro G, *et al.* Testosterone effect on insulin content, messenger ribonucleic acid levels, promoter activity, and secretion in the rat. Endocrinology 2001; 142:1442–1447.

79. Harada N, Katsuki T, Takahashi Y, *et al.* Androgen receptor silences thioredoxin-interacting protein and competitively inhibits glucocorticoid receptor-mediated apoptosis in pancreatic beta-Cells. J Cell Biochem 2015; 116:998–1006.

80. Zhang Y, Klein K, Sugathan A, *et al.* Transcriptional profiling of human liver identifies sex-biased genes associated with polygenic dyslipidemia and coronary artery disease. PLoS One 2011; 6:e23506.

81. Clodfelter KH, Holloway MG, Hodor P, *et al.* Sex-dependent liver gene expression is extensive and largely dependent upon signal transducer and activator of transcription 5b (STAT5b): STAT5b-dependent activation of male genes and repression of female genes revealed by microarray analysis. Mol Endocrinol 2006; 20:1333–1351.

82. Li J, Wan Y, Na S, *et al.* Sex-dependent regulation of hepatic CYP3A by growth hormone: roles of HNF6, C/EBPalpha, and RXRalpha. Biochem Pharmacol 2015; 93:92–103.

83. Liu Y, Santillo MF, Flynn TJ, Ferguson MS. Sex hormone modulation of both induction and inhibition of CYP1A by genistein in HepG2/C3A cells. In Vitro Cell Dev Biol Anim 2015; 51:426–431.

84. Ramirez MC, Luque GM, Ornstein AM, Becu-Villalobos D. Differential neonatal testosterone imprinting of GH-dependent liver proteins and genes in female mice. J Endocrinol 2010; 207:301–308.

85. Zhang H, Li X, Yang Y, *et al.* Significance and mechanism of androgen receptor (AR) overexpression and AR-mTOR crosstalk in hepatocellular carcinoma. Hepatology 2017. [Epub ahead of print]

86. Kanda T, Yokosuka O. The androgen receptor as an emerging target in hepatocellular carcinoma. J Hepatocell Carcinoma 2015; 2:91–99.

87. Yang S, Xu X, Bjorntorp P, Eden S. Additive effects of growth hormone and testosterone on lipolysis in adipocytes of hypophysectomized rats. J Endocrinol 1995; 147:147–152.

88. Saggese G, Cesaretti G, Franchi G, Startari L. Testosterone-induced increase of insulin-like growth factor I levels depends upon normal levels of growth hormone. Eur J Endocrinol 1996; 135:211–215.

89. Mauras N. Growth hormone and sex steroids. Interactions in puberty. Endocrinol Metab Clin North Am 2001; 30:529–544.
90. Mauras N, Rini A, Welch S, *et al.* Synergistic effects of testosterone and growth hormone on protein metabolism and body composition in prepubertal boys. Metabolism 2003; 52:964–969.
91. Weissberger AJ, Ho KK. Activation of the somatotropic axis by testosterone in adult males: evidence for the role of aromatization. J Clin Endocrinol Metab 1993; 76:1407–1412.
92. Veldhuis JD, Metzger DL, Martha PM Jr, *et al.* Estrogen and testosterone, but not a nonaromatizable androgen, direct network integration of the hypothalamo-somatotrope (growth hormone)-insulin-like growth factor I axis in the human: evidence from pubertal pathophysiology and sex-steroid hormone replacement. J Clin Endocrinol Metab 1997; 82:3414–3420.
93. Veldhuis JD, Mielke KL, Cosma M, *et al.* Aromatase and 5alpha-reductase inhibition during an exogenous testosterone clamp unveils selective sex steroid modulation of somatostatin and growth hormone secretagogue actions in healthy older men. J Clin Endocrinol Metab 2009; 94:973–981.
94. Rochira V, Zirilli L, Maffei L, *et al.* Tall stature without growth hormone: four male patients with aromatase deficiency. J Clin Endocrinol Metab 2010; 95:1626–1633.
95. Takahashi Y, Iida K, Takahashi K, *et al.* Growth hormone reverses nonalcoholic steatohepatitis in a patient with adult growth hormone deficiency. Gastroenterology 2007; 132:938–943.
96. Fukuda I, Hizuka N, Yasumoto K, *et al.* Metabolic co-morbidities revealed in patients with childhood-onset adult GH deficiency after cessation of GH replacement therapy for short stature. Endocr J 2008; 55:977–984.
97. Nishizawa H, Iguchi G, Murawaki A, *et al.* Nonalcoholic fatty liver disease in adult hypopituitary patients with GH deficiency and the impact of GH replacement therapy. Eur J Endocrinol 2012; 167:67–74.
98. Barclay JL, Nelson CN, Ishikawa M, *et al.* GH-dependent STAT5 signaling plays an important role in hepatic lipid metabolism. Endocrinology 2011; 152:181–192.
99. Fan Y, Menon RK, Cohen P, *et al.* Liver-specific deletion of the growth hormone receptor reveals essential role of growth hormone signaling in hepatic lipid metabolism. J Biol Chem 2009; 284:19937–19944.
100. Zhao JT, Cowley MJ, Lee P, *et al.* Identification of novel GH-regulated pathway of lipid metabolism in adipose tissue: a gene expression study in hypopituitary men. J Clin Endocrinol Metab 2011; 96:E1188–1196.
101. Christ ER, Cummings MH, Albany E, *et al.* Effects of growth hormone (GH) replacement therapy on very low density lipoprotein apolipoprotein B100 kinetics in patients with adult GH deficiency: a stable isotope study. J Clin Endocrinol Metab 1999; 84:307–316.
102. Birzniece V, Barrett PHR, Ho KKY. Tamoxifen reduces hepatic VLDL production and GH secretion in women: a possible mechanism for steatosis development. Eur J Endocrinol 2017; 177:137–143.
103. Yu YM, Domene HM, Sztein J, *et al.* Developmental changes and differential regulation by testosterone and estradiol of growth hormone receptor expression in the rabbit. Eur J Endocrinol 1996; 135:583–590.
104. Keenan BS, Richards GE, Ponder SW, *et al.* Androgen-stimulated pubertal growth: the effects of testosterone and dihydrotestosterone on growth hormone and insulin-like growth factor-I in the treatment of short stature and delayed puberty. J Clin Endocrinol Metab 1993; 76:996–1001.
105. Johannsson G, Gibney J, Wolthers T, *et al.* Independent and combined effects of testosterone and growth hormone on extracellular water in hypopituitary men. J Clin Endocrinol Metab 2005; 90:3989–3994.
106. Sculthorpe N, Solomon AM, Sinanan AC, *et al.* Androgens affect myogenesis in vitro and increase local IGF-1 expression. Med Sci Sports Exerc 2012; 44:610–615.
107. Brill KT, Weltman AL, Gentili A, *et al.* Single and combined effects of growth hormone and testosterone administration on measures of body composition, physical performance, mood, sexual function, bone turnover, and muscle gene expression in healthy older men. J Clin Endocrinol Metab 2002; 87:5649–5657.
108. Hayes VY, Urban RJ, Jiang J, *et al.* Recombinant human growth hormone and recombinant human insulin-like growth factor I diminish the catabolic effects of hypogonadism in man: metabolic and molecular effects. J Clin Endocrinol Metab 2001; 86:2211–2219.