Tamoxifen reduces hepatic VLDL production and GH secretion in women: a possible mechanism for steatosis development

Short title: Tamoxifen inhibits VLDL production

Vita Birzniece [1, 2, 3], **P Hugh R Barrett** [4], **Ken KY Ho** [1,5]

[1] Garvan Institute of Medical Research, Sydney NSW 2010, Australia

[2] School of Medicine, Western Sydney University, Penrith NSW 2751, Australia

[3] School of Medicine, University of New South Wales, Sydney NSW 2052, Australia

[4] School of Biomedical Sciences, Faculty of Health and Medical Sciences, and Faculty of Engineering and Mathematical Sciences, The University of Western Australia, Perth WA 6009, Australia

[5] Centres of Health Research, Princess Alexandra Hospital, Brisbane QLD 4102, Australia

Key Words*:* SERMs, fatty liver, IGF-1, ApoB, estradiol

Word Count: Text: 2406, Abstract: 248, Tables: 1, Figures: 2

Corresponding Author:

Dr Vita Birzniece; School of Medicine, Western Sydney University, 2751 NSW, Australia, phone: +61298516059, fax: +61298516050, email: v.birzniece@westernsydney.edu.au

Disclosure information: All authors have nothing to declare.

This work was supported by the National Health and Medical Research Council of Australia (grant number GNT1064365).

The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12611001093976).

Abstract

Context: Growth hormone (GH) stimulates hepatic synthesis of very-low-density lipoproteins (VLDL), whereas hepatic steatosis develops as a result of GH deficiency. Steatosis is also a complication of tamoxifen treatment, the cause of which is not known. As tamoxifen inhibits the secretion and action of GH, we hypothesize that it induces steatosis by inhibiting hepatic VLDL export.

Aim: To investigate whether tamoxifen reduces hepatic VLDL secretion.

Design: Eight healthy, normolipidemic women (age 64.4 ± 2.1 years) were studied in random sequence at baseline, after 2 weeks of tamoxifen (20 mg/d) and after 2 weeks of estradiol valerate (EV; 2 mg/d) treatments, separated by a 4-week washout period. The kinetics of apolipoprotein B (apoB), the structural protein of VLDL particles, were measured using a stable isotope $2H^3$-leucine turnover technique. VLDL-apoB fractional catabolic rate (FCR) was determined using a multicompartment model. VLDL-apoB secretion was estimated as the product of FCR and VLDL-apoB concentration. GH response to arginine stimulation, circulating levels of IGF-1, FFA, and TG, along with TG content in VLDL were measured.

Results: Tamoxifen significantly ($p<0.05$) reduced VLDL-apoB concentration and secretion by 27.3±7.8% and 29.8±10.2%, respectively. In contrast EV did not significantly change VLDL-apoB concentration or secretion. Tamoxifen but not EV significantly reduced ($p<0.05$) GH response to arginine stimulation. Both treatments significantly lowered ($p<0.05$) circulating IGF-1.

Conclusion: Inhibition of VLDL secretion may contribute to the development of fatty liver during tamoxifen therapy. As GH stimulates VLDL secretion, the development of steatosis may arise secondarily from GH insufficiency induced by tamoxifen.

Introduction

Hepatic steatosis develops in up to 50 % of patients with breast cancer treated with tamoxifen [1-4]. However, the mechanism by which tamoxifen induces fatty liver is unknown.

Steatosis is a common complication of growth hormone deficiency (GHD). Fatty liver, assessed by fatty infiltration on imaging studies or liver biopsy based on standardized grading system, is found in up to three quarters of GHD adults in Japan [5, 6]. Up to 30 % of patients with GHD develop steatosis and GH therapy reverses this based on histological evaluation and normalization of liver enzymes [6-8]. Hepatic steatosis develops within 3 years of the diagnosis of hypothalamic or pituitary dysfunction, especially with obesity [9]. Furthermore, in patients with non-alcoholic fatty liver disease (NAFLD), low GH levels are associated with a more advanced stage of NAFLD [10]. Evidence supporting a pathophysiologic role of GH comes from the observation that a loss of GH receptor function in the liver leads to steatosis [11]. Liver-specific deletion of the GH receptor causes abnormal intrahepatic lipid metabolism, which is not corrected by IGF-1 administration [12]. GH stimulates fat oxidation and the synthesis of very-low-density lipoproteins (VLDL) for hepatic export of triglycerides (TGs) [13, 14]. Thus, GH plays a vital role in regulating hepatic lipid metabolism.

Our research on the GH system has provided strong evidence that estrogens regulate the secretion and the action of GH [15-17]. SERM are synthetic estrogens, compounds that possess tissue-specific antagonist and agonist properties. In the case of tamoxifen, it acts centrally as an estrogen receptor antagonist and peripherally on the liver as an agonist [18, 19]. Because estrogens drive GH secretion centrally, tamoxifen markedly reduces GH secretion [17]. Estrogens inhibit hepatic GHR signalling and when taken by the oral route antagonises the metabolic actions of GH on the liver, resulting in a fall in IGF-1 and fatty acid oxidation [20-24]. The fall in circulating IGF-1 by oral estrogens can trigger a secondary increase in GH secretion from loss of central feedback inhibition. Tamoxifen exerts hepatic effects similar to those of estrogens. However,

unlike estrogens, tamoxifen does not cause a secondary increase in GH because its central action as an antagonist prevents the increase in GH secretion [17]. Thus, while both inhibit the hepatic actions of GH, tamoxifen evokes a more severe GH deficient state from additional inhibition of GH secretion.

These findings suggest that steatosis developing from tamoxifen may arise secondarily from a GH deficient state caused by its suppression of the system. Unlike with tamoxifen, steatosis is not a recognised complication of oral estrogen use, suggesting that the underlying mechanism is unlikely to arise from an estrogen agonist effect on the liver. Because estrogen and tamoxifen exert contrasting central effects, it is conceivable that steatosis may develop from impaired VLDL production as a result of reduced GH secretion during tamoxifen treatment.

The aim of this study was to investigate whether hepatic VLDL production is inhibited by tamoxifen. We have compared VLDL dynamics during tamoxifen and estradiol valerate treatment in post-menopausal women using stable isotope methodology.

Methods

Subjects

Eight healthy women (mean age 64.4 ± 2.1 years; mean BMI 23.7 ± 1.2 kg/m^2) were recruited from the community through advertisements. Study participants were normolipidemic, in good general health and had normal haematological tests, renal and hepatic function. Exclusion criteria included BMI ≥ 30 kg/m^2, hypothalamic or pituitary disorders, diabetes mellitus, cancer and chronic renal or hepatic illnesses, or taking any medications known to interfere with endocrine systems. Study participants were instructed to follow their usual diet and physical activity throughout the study. St. Vincent's Hospital Human Research Ethics Committee approved the study, which was conducted in accordance with the principles of the Declaration

of Helsinki. The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12611001093976).

Study design

This was an open label study of tamoxifen (Genox®) and estradiol valerate administration. The dose of tamoxifen was 20 mg/day and for estradiol valerate (EV) 2 mg/day. Study medications were administered in randomized order for two weeks each, with a 4-week washout in between. Study participants were studied in the Clinical Research Facility, Garvan Institute of Medical Research after an overnight fast. On each visit, including baseline (no intervention), assessment of VLDL turnover was performed, GH response to arginine stimulation, and serum IGF-1, free fatty acids (FFA), TG levels were measured. Study bloods were collected and serum samples were obtained by centrifugation, and stored at -80°C until analysis.

VLDL turnover

We quantified hepatic VLDL secretion by stable isotope $2H^3$-leucine turnover technique [25]. It is assumed that plasma alpha-KIC is in equilibrium with intrahepatic leucine and reflects the enrichment of hepatic leucine transfer ribonucleic acid, which is necessary for the production of apoB, the principal structural apolipoprotein of VLDL. The steady state isotope enrichment of the precursor pool occurs within 30-45 min of $2H^3$-leucine infusion and remains constant throughout the study.

After an overnight fast, blood samples were taken before and during the 3-hour primed (1.34 mg/kg) constant infusion of $2H^3$-leucine at a rate of 1.34 mg/kg/hour. EDTA blood samples were centrifuged immediately and plasma stored at 4ºC for up to 8 hours. The VLDL fraction was then isolated from plasma through ultracentrifugation (50,000 rpm at 20ºC for 16 hours) and recovered by aspiration.

Laboratory methods for measurement of VLDL-apoB turnover have been described previously by Chan and co-authors [26]. We quantified the concentration of VLDL-apoB by the Lowry method. ApoB was precipitated with 50% isopropanol and delipidated by 100% isopropanol, made soluble in alkaline deoxycholate solution and protein estimated by the Lowry method. Isotopic enrichment of VLDL and plasma was measured by gas chromatography mass spectrometry (GCMS) as previously described [26].

VLDL-apoB fractional catabolic rate (FCR) is that fraction of the VLDL-apoB pool irreversibly cleared from the plasma per day. VLDL-apoB secretion rate was estimated as the product of FCR and VLDL-apoB concentration. Pool size is the mass of tracee in the VLDL pool determined as the product of plasma volume and trace concentration.

The multicompartment model included compartment 1, which reflected the plasma tracer/tracee ratio of the leucine tracer in plasma. Compartment 2 represents intrahepatic delay that accounts for the time required for the synthesis and secretion of VLDL-apoB into plasma. Compartment 3 represents the plasma VLDL-apoB. The rate constant out of compartment 3 is equivalent to the VLDL-apoB FCR. The SAAM II software (The Epsilon Group, Charlottesville, VA) was used to fit the model to the tracer data.

Arginine stimulation test

Subjects rested on a bed for at least 30 min before the baseline blood samples were taken. Thirty grams of L-Arginine hydrochloride (Phebra Pty Ltd, Australia) was infused over 30 min period. Blood samples for GH level measurements were taken at 0 and 30, 60 and 90 min after commencement of arginine infusion.

Analytical methods

All samples for any individual were measured in the same assay run for each analyte. Serum GH was measured by Immulite 2000 (L2KGRH, WHO NIBSC 2nd IS 98/574; Siemens Medical Solution Diagnostics, Los Angeles, CA, USA). The inter-assay and intra-assay CVs for GH at

3 ng/ml were 5.8% and 5.7%, respectively. Serum IGF-1 was measured by RIA after acid ethanol extraction as previously described [17]. The CVs for IGF-1 were 8.3% at 14.7 nmol/l and 7.4% at 28.6 nmol/l. TG was measured using Enzyme Colorimetric method by Cobas 701 auto-analyser (Roche Diagnostics, Indianapolis, IN, USA). The inter-assay CV for TG was 2.6% at 2.9 mmol/l. 17β-estradiol was measured by electrochemiluminescence immunoassay with inter-assay CV of 4.7% at 166 pmol/L.

Statistical analysis

Treatment effects were assessed by factorial ANOVA followed by paired t-tests with Bonferroni's correction where appropriate. The GH response to arginine data were logarithmically transformed and analysed by repeated measures ANOVA. Results were expressed as mean with standard errors (SEM) and a p value of less than 0.05 was considered to be significant. Statistical analysis was undertaken using the statistical software package SPSS (IBM SPSS Statistics 21, NY, US).

Results

VLDL metabolism

Tamoxifen, but not EV, significantly ($p<0.05$) lowered mean VLDL-apoB concentration (Δ -27.3 ± 7.8%; Fig 1 & Table 1). The change in VLDL-apoB concentration between tamoxifen and EV treatments was significantly different ($p<0.05$). Tamoxifen significantly lowered VLDL-apoB secretion rate by 29.8 ± 10.2% ($p<0.05$) while EV treatment did not significantly affect this. Neither tamoxifen nor EV significantly change the FCR of VLDL-apoB.

Both tamoxifen and EV did not significantly change plasma or VLDL triglyceride concentrations. The VLDL-TG to VLDL-apoB ratio, a measure of the TG content of VLDL particles, also did not change significantly with tamoxifen or EV treatments (Table 1).

171 ***Effects on GH and IGF-1***

172 Tamoxifen significantly reduced the peak GH response to arginine stimulation by 45.3 ± 8.9%
173 ($p<0.01$; Table 1). EV did not significantly affect the peak GH response to stimulation (Fig 2).
174 Compared with baseline, administration of tamoxifen or EV significantly reduced circulating
175 IGF-1 concentrations (Δ -14.8 ± 5.3% and -21.4 ± 4.7%, respectively; $p<0.05$; Table 1). IGF-1
176 levels following tamoxifen and EV treatments were not significantly different.

177 ***Other measurements***

178 FFA concentrations did not change significantly with either treatments. Mean circulating
179 estradiol levels increased significantly during treatment with EV ($p<0.01$; Table 1).

180

181 **Discussion**

182 We investigated the effects of tamoxifen and estradiol valerate on hepatic VLDL metabolism
183 and on the GH system in healthy postmenopausal women using isotopic tagging of apoB, the
184 principal structural protein of VLDL. VLDL-apoB concentration was significantly lower during
185 tamoxifen than during EV treatment. Tamoxifen but not EV significantly reduced hepatic
186 VLDL secretion while both treatments did not affect the FCR. Neither tamoxifen, nor EV
187 significantly modified the TG content of VLDL. Tamoxifen but not EV treatment significantly
188 reduced the peak GH response to stimulation. Both tamoxifen and EV significantly reduced
189 IGF-1 levels to a similar extent. In summary, tamoxifen but not EV reduced hepatic VLDL
190 production and reduced GH secretion.

191 VLDL is composed of a core of triglycerides, surrounded by cholesterol, cholesteryl esters,
192 phospholipids, and apolipoproteins, of which apoB is the principal apolipoprotein. VLDL
193 secretion rate depends on availability of intrahepatic lipid substrate. Fatty acids taken up by the
194 liver are either oxidized or reesterified to triglycerides and exported from the liver as VLDL

195 particles. Therefore, a block in either beta-oxidation or VLDL export leads to TG accumulation
196 and the development of a fatty liver.

197 GH plays a pivotal role in hepatic lipid metabolism. Rodent studies reveal that loss of hepatic
198 GH receptor function leads to TG accumulation in the liver [11]. Several clinical studies have
199 described the occurrence of steatosis in patients with GH deficiency which resolves with GH
200 therapy [7, 8]. Because GH stimulates hepatic fatty acid oxidation [13] and VLDL secretion [14, 27], it
201 is likely that steatosis develops in GHD patients from suppression of fatty acid oxidation and
202 of VLDL production.

203 Our previous work has provided strong evidence that tamoxifen exerts a more profound
204 suppressive effect on the GH system than classical estrogen such as EV. While both tamoxifen
205 and EV antagonise the action of GH on the liver, tamoxifen exerts an additional central effect
206 of inhibiting GH secretion as observed in the GH response to arginine, confirming our earlier
207 report [17]. We investigated whether the double blow effect which creates a more profound GH
208 deficient state reduced VLDL production. Our findings provide evidence that steatosis develops
209 during tamoxifen most likely from impaired VLDL export arising from the induction of a
210 profound GH deficient state.

211 Studies have demonstrated that estrogens may stimulate VLDL production depending on the
212 route of administration. Oral but not transdermal estrogen enhance the fractional secretion and
213 production rate of VLDL in particular the light fraction of VLDL particles [28, 29]. As oral but not
214 parenteral route of estrogen delivery stimulates GH secretion [17, 20], the increase in GH output
215 may in turn stimulate hepatic VLDL production. We did not observe a significant increase in
216 GH secretion or VLDL production during EV administration although there was a small trend.
217 Steatosis is not a recognised complication of oral estrogen use. The finding that VLDL secretion
218 is either unaffected or may be stimulated by oral estrogens supports the hypothesis that

suppression of GH-stimulated VLDL export is a plausible mechanism for the development of steatosis.

It is not clear from clinical studies whether GH or IGF-1 regulates hepatic VLDL metabolism. However rodent studies provide little evidence that IGF-1 has a major role in regulating VLDL metabolism in the liver. In-vitro studies report no direct effect of IGF-1 on VLDL secretion in isolated rat hepatocytes, contrary to that of GH [27, 30]. Steatosis which develops as a consequence of liver-specific deletion of the GH receptor is not improved by IGF-1 treatment [12]. Steatosis does not develop in liver specific IGF-1-deficient mice [31]. Thus the collective evidence point to a direct non-IGF-1 mediated action of GH on VLDL secretion.

The intracellular biochemical mechanisms by which VLDL secretion is reduced by tamoxifen is not known. The assembly and secretion of apoB-containing lipoproteins is strictly dependent on the microsomal triglyceride transfer protein (MTP), which shuttles triglycerides into the nascent VLDL particle [32, 33]. Animal studies show that continuous GH infusion stimulates hepatic MTP mRNA and protein levels [34]. Thus tamoxifen, by inhibiting GH secretion, could reduce MTP synthesis in the liver, reducing VLDL formation.

A direct effect on hepatocytes of tamoxifen independent of GH, cannot be ruled out. It's been proposed from *in vitro* experiments that tamoxifen induces hepatic steatosis by impairing beta-oxidation and respiration, or by increasing fatty acid synthesis through up-regulation of SREBP-1c pathway [35, 36]. Other studies report no direct effect of tamoxifen on hepatocyte fatty acid oxidation, whereas systemic treatment of tamoxifen in mice substantially increases hepatic triglyceride content and activates fatty acid synthesis [37]. *In vitro* incubation of VLDL with tamoxifen resulted in increased TG content in VLDL particles [38], however studies investigating direct effect of tamoxifen on hepatic VLDL production are scarce. Studies comparing the *in vitro* effects of tamoxifen and estradiol have not been reported. Thus, the possibility that tamoxifen direct regulates VLDL turnover and assembly cannot be excluded.

This study has some limitations, including a relatively small sample size, although each patient completed each treatment and as such served as their own control. In addition, the duration of the tracer study was relatively short, limiting the identification of kinetic heterogeneity within the VLDL-apoB fraction. Longer term studies may provide more precise measures of VLDL-apoB kinetics. However, as the primary determinant of VLDL-apoB concentration is secretion rate it is unlikely that small errors in FCR impact on our findings.

In summary, tamoxifen significantly reduced hepatic VLDL secretion in healthy postmenopausal women. Tamoxifen inhibited GH secretion, inducing a GH-deficient state. As GH is a potent stimulant of hepatic lipid metabolism and VLDL secretion, we propose that the reduction in VLDL secretion may arise in part from profound suppression of the GH-IGF-1 axis. Diminished hepatic VLDL secretion may contribute to the development of fatty liver during tamoxifen therapy.

Acknowledgements

This work was supported by the National Health and Medical Research Council of Australia (NHMRC). PHRB is a Senior Research Fellow of the NHMRC. We thank Alphapharm for providing Genox®, research nurses for clinical assistance, Surya Sutanto, and the Endocrinology Laboratory, Royal Prince Alfred Hospital, Sydney, Australia.

References

1. Ogawa Y, Murata Y, Nishioka A, Inomata T & Yoshida S. Tamoxifen-induced fatty liver in patients with breast cancer. *Lancet* 1998 **351** 725.
2. Murata Y, Ogawa Y, Saibara T, Nishioka A, Fujiwara Y, Fukumoto M, Inomata T, Enzan H, Onishi S & Yoshida S. Unrecognized hepatic steatosis and non-alcoholic steatohepatitis in adjuvant tamoxifen for breast cancer patients. *Oncol Rep* 2000 **7** 1299-1304.
3. Nishino M, Hayakawa K, Nakamura Y, Morimoto T & Mukaihara S. Effects of tamoxifen on hepatic fat content and the development of hepatic steatosis in patients with breast cancer: high frequency of involvement and rapid reversal after completion of tamoxifen therapy. *AJR Am J Roentgenol* 2003 **180** 129-134.
4. Liu CL, Huang JK, Cheng SP, Chang YC, Lee JJ & Liu TP. Fatty liver and transaminase changes with adjuvant tamoxifen therapy. *Anticancer Drugs* 2006 **17** 709-713.
5. Ichikawa T, Hamasaki K, Ishikawa H, Ejima E, Eguchi K & Nakao K. Non-alcoholic steatohepatitis and hepatic steatosis in patients with adult onset growth hormone deficiency. *Gut* 2003 **52** 914.
6. Nishizawa H, Iguchi G, Murawaki A, Fukuoka H, Hayashi Y, Kaji H, Yamamoto M, Suda K, Takahashi M, Seo Y, 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.
7. Takahashi Y, Iida K, Takahashi K, Yoshioka S, Fukuoka H, Takeno R, Imanaka M, Nishizawa H, Takahashi M, Seo Y, et al. Growth hormone reverses nonalcoholic steatohepatitis in a patient with adult growth hormone deficiency. *Gastroenterology* 2007 **132** 938-943.
8. Fukuda I, Hizuka N, Yasumoto K, Morita J, Kurimoto M & Takano K. 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.
9. Adams LA, Feldstein A, Lindor KD & Angulo P. Nonalcoholic fatty liver disease among patients with hypothalamic and pituitary dysfunction. *Hepatology* 2004 **39** 909-914.
10. Ichikawa T, Nakao K, Hamasaki K, Furukawa R, Tsuruta S, Ueda Y, Taura N, Shibata H, Fujimoto M, Toriyama K, et al. Role of growth hormone, insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 in development of non-alcoholic fatty liver disease. *Hepatol Int* 2007 **1** 287-294.
11. Barclay JL, Nelson CN, Ishikawa M, Murray LA, Kerr LM, McPhee TR, Powell EE & Waters MJ. GH-dependent STAT5 signaling plays an important role in hepatic lipid metabolism. *Endocrinology* 2011 **152** 181-192.
12. Fan Y, Menon RK, Cohen P, Hwang D, Clemens T, DiGirolamo DJ, Kopchick JJ, Le Roith D, Trucco M & Sperling MA. 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.
13. Zhao JT, Cowley MJ, Lee P, Birzniece V, Kaplan W & Ho KK. 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.
14. Christ ER, Cummings MH, Albany E, Umpleby AM, Lumb PJ, Wierzbicki AS, Naoumova RP, Boroujerdi MA, Sonksen PH & Russell-Jones DL. 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.

15. O'Sullivan AJ, Crampton LJ, Freund J & Ho KK. The route of estrogen replacement therapy confers divergent effects on substrate oxidation and body composition in postmenopausal women. *J Clin Invest* 1998 **102** 1035-1040.
16. Birzniece V, Meinhardt U, Gibney J, Johannsson G, Baxter RC, Seibel MJ & Ho KK. Modulatory Effect of Raloxifene and Estrogen on the Metabolic Action of Growth Hormone in Hypopituitary Women. *J Clin Endocrinol Metab* 2010 **95** 2099-2106.
17. Birzniece V, Sata A, Sutanto S & Ho KK. Paracrine Regulation of Growth Hormone Secretion by Estrogen in Women. *J Clin Endocrinol Metab* 2010 **95** 3771-3776.
18. Lofgren L, Wallberg B, Wilking N, Fornander T, Rutqvist LE, Carlstrom K, von Schoultz B & von Schoultz E. Tamoxifen and megestrol acetate for postmenopausal breast cancer: diverging effects on liver proteins, androgens, and glucocorticoids. *Med Oncol* 2004 **21** 309-318.
19. Riggs BL & Hartmann LC. Selective estrogen-receptor modulators -- mechanisms of action and application to clinical practice. *N Engl J Med* 2003 **348** 618-629.
20. Weissberger AJ, Ho KK & Lazarus L. Contrasting effects of oral and transdermal routes of estrogen replacement therapy on 24-hour growth hormone (GH) secretion, insulin-like growth factor I, and GH-binding protein in postmenopausal women. *J Clin Endocrinol Metab* 1991 **72** 374-381.
21. Gibney J, Johannsson G, Leung KC & Ho KK. Comparison of the metabolic effects of raloxifene and oral estrogen in postmenopausal and growth hormone-deficient women. *J Clin Endocrinol Metab* 2005 **90** 3897-3903.
22. Bellantoni MF, Vittone J, Campfield AT, Bass KM, Harman SM & Blackman MR. Effects of oral versus transdermal estrogen on the growth hormone/insulin-like growth factor I axis in younger and older postmenopausal women: a clinical research center study. *J Clin Endocrinol Metab* 1996 **81** 2848-2853.
23. Sonnet E, Lacut K, Roudaut N, Mottier D, Kerlan V & Oger E. Effects of the route of oestrogen administration on IGF-1 and IGFBP-3 in healthy postmenopausal women: results from a randomized placebo-controlled study. *Clin Endocrinol (Oxf)* 2007 **66** 626-631.
24. Leung KC, Doyle N, Ballesteros M, Sjogren K, Watts CK, Low TH, Leong GM, Ross RJ & Ho KK. Estrogen inhibits GH signaling by suppressing GH-induced JAK2 phosphorylation, an effect mediated by SOCS-2. *Proc Natl Acad Sci U S A* 2003 **100** 1016-1021.
25. Chan DC, Barrett PH & Watts GF. Lipoprotein transport in the metabolic syndrome: methodological aspects of stable isotope kinetic studies. *Clin Sci (Lond)* 2004 **107** 221-232.
26. Chan DC, Gan SK, Wong AT, Barrett PH & Watts GF. Association between skeletal muscle fat content and very-low-density lipoprotein-apolipoprotein B-100 transport in obesity: effect of weight loss. *Diabetes Obes Metab* 2014 **16** 994-1000.
27. Linden D, Sjoberg A, Asp L, Carlsson L & Oscarsson J. Direct effects of growth hormone on production and secretion of apolipoprotein B from rat hepatocytes. *Am J Physiol Endocrinol Metab* 2000 **279** E1335-1346.
28. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V & Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med* 1991 **325** 1196-1204.
29. Campos H, Walsh BW, Judge H & Sacks FM. Effect of estrogen on very low density lipoprotein and low density lipoprotein subclass metabolism in postmenopausal women. *J Clin Endocrinol Metab* 1997 **82** 3955-3963.

30. Sjoberg A, Oscarsson J, Olofsson SO & Eden S. Insulin-like growth factor-I and growth hormone have different effects on serum lipoproteins and secretion of lipoproteins from cultured rat hepatocytes. *Endocrinology* 1994 **135** 1415-1421.
31. Yakar S, Setser J, Zhao H, Stannard B, Haluzik M, Glatt V, Bouxsein ML, Kopchick JJ & LeRoith D. Inhibition of growth hormone action improves insulin sensitivity in liver IGF-1-deficient mice. *J Clin Invest* 2004 **113** 96-105.
32. Olofsson SO, Asp L & Boren J. The assembly and secretion of apolipoprotein B-containing lipoproteins. *Curr Opin Lipidol* 1999 **10** 341-346.
33. Shelness GS & Ledford AS. Evolution and mechanism of apolipoprotein B-containing lipoprotein assembly. *Curr Opin Lipidol* 2005 **16** 325-332.
34. Ameen C & Oscarsson J. Sex difference in hepatic microsomal triglyceride transfer protein expression is determined by the growth hormone secretory pattern in the rat. *Endocrinology* 2003 **144** 3914-3921.
35. Zhao F, Xie P, Jiang J, Zhang L, An W & Zhan Y. The effect and mechanism of tamoxifen-induced hepatocyte steatosis in vitro. *Int J Mol Sci* 2014 **15** 4019-4030.
36. Larosche I, Letteron P, Fromenty B, Vadrot N, Abbey-Toby A, Feldmann G, Pessayre D & Mansouri A. Tamoxifen inhibits topoisomerases, depletes mitochondrial DNA, and triggers steatosis in mouse liver. *J Pharmacol Exp Ther* 2007 **321** 526-535.
37. Cole LK, Jacobs RL & Vance DE. Tamoxifen induces triacylglycerol accumulation in the mouse liver by activation of fatty acid synthesis. *Hepatology* 2010 **52** 1258-1265.
38. Hozumi Y, Kawano M, Hakamata Y, Miyata M & Jordan VC. Tamoxifen inhibits lipoprotein activity: in vivo and in vitro studies. *Horm Res* 2000 **53** 36-39.

384 **Figure legends**

385 **Figure 1:** Changes in VLDL-apoB concentration (A) and VLDL-apoB secretion (B) during
386 tamoxifen (Tam; 20 mg/day) and estradiol valerate (EV; 2 mg/day) treatments. *$p<0.05$
387 compared to baseline. Data are expressed as means ± SEM.

388

389 **Figure 2:** Change from pre-stimulation serum GH levels at 30, 60, 90, and 120 min after
390 arginine infusion measured before (nil; solid line), during oral treatment with estradiol valerate
391 (EV; 2 mg/day; dotted line), and during tamoxifen treatment (20 mg/day; dashed line). Data are
392 expressed as means ± SEM. Conversion factor: 1 mIU/L= 0.33 μg/L.

393 **Table 1**

394 Endpoint measures at baseline and during the treatment with tamoxifen (20 mg/day) and
395 estradiol valerate (2 mg/day).

Outcome measures	Baseline	Tamoxifen	Estradiol
Weight (kg)	64.0 ± 3.0	63.8 ± 3.1	64.3 ± 3.1
Estradiol (pmol/L)	11.7 ± 4.1	10.5 ± 3.7	349.5 ± 71**^
GH max (ng/ml)	17.3 ± 4.0	10.4 ± 3.5**	20.5 ± 10.8
IGF-1 (nmol/L)	14.9 ± 1.0	12.7 ± 1.2*	11.7 ± 1.0**
VLDL-apoB concentration (mg/L)	31.1 ± 5.6	21.8 ± 4.8*	29.0 ± 4.5^
VLDL-apoB FCR (pools/d)	3.8 ± 0.7	3.2 ± 0.8	3.5 ± 0.5
VLDL-apoB secretion (mg/kg/d)	4.5 ± 1.1	3.0 ± 0.8*	4.0 ± 0.6
TG (mmol/L)	0.84 ± 0.1	0.81 ± 0.1	1.0 ± 0.1
VLDL-TG (mmol/L)	1.06 ± 0.1	1.03 ± 0.2	1.29 ± 0.2
VLDL-TG to VLDL-apoB ratio	4.5 ± 0.9	6.2 ± 1.3	4.9 ± 0.9
FFA (μmol/L)	574.5 ± 66	574.2 ± 76	601.1 ± 102

396

397 Data are expressed as means ± SEM. * $p<0.05$ compared with baseline; ** $p<0.01$ compared
398 with baseline; ^ $p<0.05$ compared with tamoxifen. GH max, peak GH response to arginine
399 stimulation, conversion factor: 1 mIU/L= 0.33 μg/L; VLDL, very low density lipoprotein; FCR,
400 fractional catabolic rate; TG, triglycerides; FFA, free fatty acids.

Figure 1

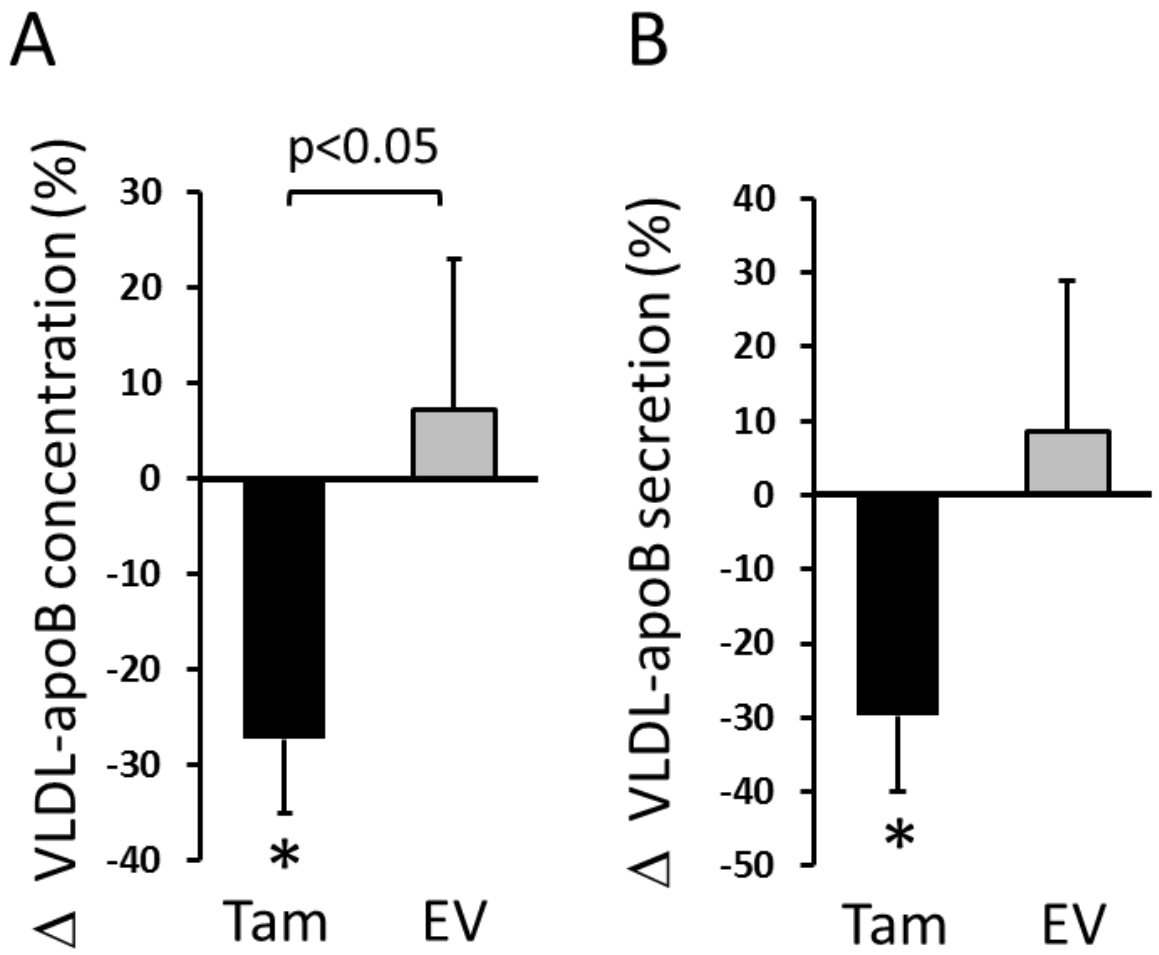
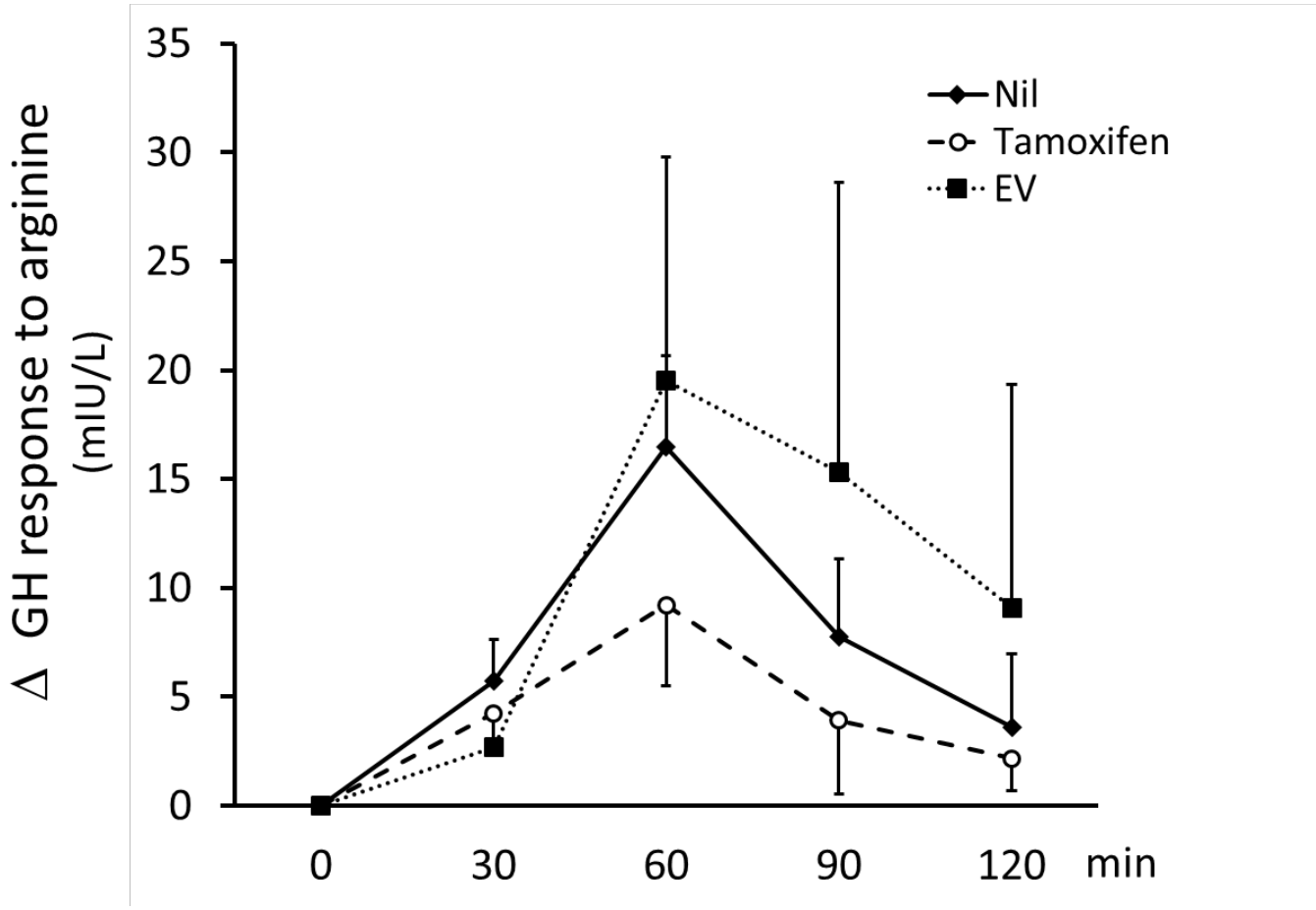

A
p<0.05
Δ VLDL-apoB concentration (%)
30
20
10
0
-10
-20
-30
-40
*
Tam
EV
B
Δ VLDL-apoB secretion (%)
40
30
20
10
0
-10
-20
-30
-40
-50
*
Tam
EV

Figure 2


Δ GH response to arginine (mIU/L)
35
30
25
20
15
10
5
0
0
30
60
90
120
min
Nil
Tamoxifen
EV