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Estrogen effects on triglyceride metabolism in analbuminemic rats

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Background. Triglyceride (TG) levels are normally lower in female rats, while the opposite is the case in the Nagase analbuminemic rats (NAR). Increased TG levels in normal males are caused by a testosterone-mediated decrease in postheparin (PH) lipoprotein lipase (LpL). Castration of males reduces TG, while castration of females is without effect. TG levels are reduced by castration of the female NAR, suggesting that estrogen rather than testosterone causes hypertriglyceridemia in this strain. The mechanism for this increase is unknown.

Methods. We measured secretion of very-low density lipoprotein (VLDL) TG using Triton WR 1339 clearance as the disappearance from blood of ^{3}H-trioleate and ^{14}C-cholesterol–labeled chylomicrons (CM), and the activity of the PH lipases: LpL and hepatic lipase (HL). All were determined in Sprague-Dawley (SD) and NAR female, male, and ovariectomized (OVX) rats.

Results. TG levels were significantly greater in female NAR in comparison to all other groups. Ovariectomy of NAR significantly ameliorated hypertriglyceridemia. VLDL TG secretion was significantly greater in intact female NAR compared with all other groups. There were no other differences in VLDL TG secretion among the other groups. The clearance of CM was greatest in female SD rats, and OVX had no effect. NAR cleared CM less well than did SD rats ($P < 0.001$), but among NAR, clearance was greatest in OVX NAR and male NAR ($P < 0.002$). Both PH LpL activity and HL activity were lowest in female NAR ($P < 0.05$). Ovariectomy partially corrected the defect in HL ($P < 0.05$).

Conclusion. TG levels in female NAR are in part a result of increased VLDL-TG secretion, an effect mediated by estrogen. The presence of an estrogen-mediated catabolic defect that was alleviated by OVX was also observed. This catabolic defect is likely a result of an estrogen-mediated decrease both in LpL and HL expressed only in the presence of analbuminemia.

Key words: lipoprotein lipase, hepatic lipase, very low density lipoprotein, analbuminemia, catabolism.

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Plasma triglyceride (TG) levels are usually lower in female than in male Sprague-Dawley (SD) rats [1], while the opposite is true in Nagase analbuminemic rats (NAR) [2, 3]. Lipoprotein lipase (LpL) is the enzyme responsible for the initial delipidation of TG-rich lipoproteins such as chylomicrons (CM) and very-low density lipoprotein (VLDL). The biologically active LpL pool is tethered to the vascular endothelial cells by electrostatic interaction with heparan sulfate. This pool is heparin releasable and is called the postheparin LpL pool (PH-LpL) [4]. The measurement of LpL and hepatic lipase (HL) activity in plasma before and after heparin treatment has been used to estimate the pool size of biologically active lipase and is referred to as PH-LpL or PH-HL. Plasma PH-lipase values vary with hormonal status. The activity of PH-LpL is greater in female SD rats than in males [5]. Castration of male rats increases PH-LpL activity, and testosterone administration to males, castrated males, and females decreases PH-LpL activity [1], but neither ovariectomy nor estrogen administration to castrated male or female has an effect on PH-LpL activity [1]. Conversely, estrogen reduces PH-HL activity in vivo [6, 7]. These observations suggest that one mechanism for greater TG levels in normal males is a testosterone-mediated decrease in PH-LpL activity.

In contrast to SD rats, TG levels are increased in NAR, especially female NAR [8, 9], as well as rats with experimentally induced nephrotic syndrome and Zucker obese rats [10]. The reversal of the normal pattern of lower TG levels in females may be a consequence of increased TG synthesis in these altered metabolic states. Women given unopposed estrogens typically show a mild increase in plasma TG [11], but some develop severe hyperlipidemia [12, 13]. Increases in plasma TG under these conditions are mediated by increased hepatic TG secretion in both humans and rats without a corresponding increase in fractional catabolic rate [14, 15]. It is possible that under conditions when the mechanisms for

removal of TG rich lipoproteins are impaired, the estrogenic effects on TG secretion become unmasked, leading to marked increases in plasma TG levels.

Hypertriglyceridemia caused by defective catabolism is a risk factor for the development of atherosclerosis in women [16] and for renal disease [17, 18]. Obese Zucker rats are a genetically hypertriglyceridemic breed that spontaneously develops renal failure. Within this strain, the onset of proteinuria and renal failure is accelerated in females [10]. Ovariectomy reduces TG levels in female Zucker-obese rats compared with that found in males and delays the onset of renal failure [10]. Aging female NAR, but not male NAR or OVX NAR, also develop proteinuria and glomerulosclerosis [9]. Renal injury in NAR appears to be estrogen dependent and is unique in that hypertriglyceridemia is the only known risk factor to present before proteinuria develops [19].

We previously demonstrated that TG secretion was increased in female NAR compared with female SD rats [8]. Moreover, as noted earlier, plasma TG is higher in female NAR than in male NAR. Increased TG levels must be a consequence of either increased synthesis, decreased catabolism, or a combination of both. It is not known how the catabolism of TG-rich lipoproteins is affected by gender in NAR and whether the effects, if any, are mediated by estrogen. We measured both secretion and catabolism of TG-rich lipoproteins in OVX SD animals and NAR and compared them with those of intact male and females. We also measured the known parameters affecting TG-rich lipoprotein metabolism, such as the levels of HL and LpL.

METHODS

Animals

Sprague-Dawley rats were purchased from Bantin Kingman ($N = 33$; Fremont, CA, USA) for the experiments in Davis (CA, USA), and from Harlan-Orlac ($N = 22$; UK) for the experiments in Utrecht. NAR ($N = 51$) were from our own pathogen-free colonies in Utrecht and Davis. The Davis colony was founded with animals obtained from the Utrecht colony in 1993 and were bred in Davis.

Seventeen SD rats and 17 NAR were bilaterally ovariectomized (OVX) at five weeks under pentobarbital anesthesia and sterile conditions. The remaining females were sham operated without excision of the ovaries. The rats were housed behind barriers in temperature regulated, 12-hour light/dark cycled rooms. The rats were provided with free access to standard rat chow until the evening before the experiments. Seven weeks after ovariectomy, the vascular clearance of CM and heparin-releasable lipase was measured in the group at Davis, and VLDL-TG secretion rates were measured in the group at Utrecht. The protocols were approved by the University of California, Davis, and the Utrecht University animal study boards.

Chemical analysis

Enzymatic kits were used for the determination of plasma TG concentrations (kit #148-270; Boehringer GmbH, Mannheim, Germany; and kit #339-10; Sigma, St. Louis, MO, USA).

Triglyceride secretion rate

Triton WR-1339 is a nonionic detergent that blocks the removal of TG from the circulation, causing a linear increase in the plasma TG concentration. The rate of this increase is a measure of total TG secretion rate. The TG secretion rate was determined in 22 SD rats (6 female SD, 6 OVX SD, and 10 male SD rats) and 20 NAR (7 female NAR, 7 OVX NAR, and 6 male NAR) after an overnight fast. Six hundred mg/kg Triton WR-1339 in 0.9% saline were injected into the tail vein. Plasma samples were taken just prior to injection and at 30-, 60-, and 90-minutes postinjection. The time points were subjected to linear regression analysis, and the secretion rate was determined as the predicted hourly accumulation of plasma TG.

Heparin releasable LpL and HL activity

Postheparin lipase was determined in 16 SD rats (4 female SD, 6 OVX SD, and 6 male SD rats) and 13 NAR (6 female NAR, 4 OVX NAR, and 3 male NAR) after an overnight fast. Rats were anesthetized with an intraperitoneal injection of 0.75 g/kg of sodium pentobarbital. Fifty U/kg heparin were injected intravenously, and five minutes later, blood was collected by aortic puncture into a tube containing ethylenediaminetetraacetic acid (EDTA). The plasma was separated and stored at −70°C until assayed.

Lipoprotein lipase activity was assessed using a protocol adapted from Kraus, Levy, and Fredrickson [20]. Ten μCi of glycerol [9,10-^{3}H] trioleate, 1.5 mg phosphatidyl choline, and 37.5 mg triolein in chloroform solution were combined in a 50 × 16 mm tube and then dried completely under N_2. The test substrate was formed by combining 2.8 mL substrate buffer (0.2 mol/L Tris, 0.150 mol/L NaCl, pH to 8.6), 1.2 mL 10% fatty acid-free bovine serum albumin (BSA) in substrate buffer, 0.25 mL 1% Triton X-100 in substrate buffer, and 0.25 mL fasting heat-inactivated human plasma in a tube. The mixture was then sonicated on ice using a Branson Sonifier 250 for five minutes at power output 5 and 50% duty.

Heparin releases both LpL and HL, but LpL is inhibited by 1.0 mol/L NaCl. Thus, plasma LpL was measured as the difference between total lipase activity and lipase activity in the presence of 1.0 mol/L NaCl. HL activity was measured directly as the lipase activity not inhibited by 1 mol/L NaCl. Tests and their respective blanks were

prepared by the addition of 0.1 mL of the labeled emulsion to 0.4 mL of sample diluted in phosphate-buffered saline (PBS) + 0.5% BSA or 0.5% BSA alone. Both tests and blanks were incubated for 30 minutes at 37°C. The reaction was stopped by the addition of 1.62 mL chloroform/MeOH/heptane (1.25:1.41:1.00) to each tube. The tubes were vortexed, and 0.50 mL of 0.4 mol/L boric acid/0.4 mol/L K_2CO_3, pH 10.0, was added. The samples were vortexed again for 15 seconds and then centrifuged for 10 minutes at 1000 × g to separate phases. One milliliter of the top phase was counted in liquid scintillation fluid on a Beckman Scintillation counter. Total emulsion radioactivity was determined by counting 0.1 mL emulsion. A standard curve relating LpL activity to LpL mass was created by measuring the radioactivity released from 0.1 mL aliquots of emulsion by increasing the amounts of purified LpL. The curve was linear with respect to LpL mass (r^2 = 0.950).

Preparation of chylomicrons

Radiolabeled CMs were obtained by the method of Bollman, Cain, and Grindlay [21]. Rats were gavage fed a 2 mL emulsified prep of corn oil/milk (1:2) sonicated with glycerol [9,10-^{3}H] trioleate (0.15 mCi) and [^{14}C] cholesterol (0.05 mCi). The thoracic duct was cannulated, and chyle was collected on ice for 18 hours. CMs were isolated by layering the chyle under 0.15 mol/L NaCl and flotation on a SW-40ti rotor (Beckman Instruments, Palo Alto, CA, USA) at 5 × 10^6 g + min at 16°C for 18 hours.

Plasma chylomicron clearance

Seventeen SD rats (6 female SD, 5 SD OVX, and 6 male SD rats) and 18 NAR (6 female NAR, 6 OVX NAR, and 6 male NAR) were fasted overnight, and blood was obtained by tail bleed prior to injection to determine fasting TG levels. Labeled CMs were injected into a tail vein, and blood samples were subsequently sampled in duplicate at 2-, 4-, 6-, 8-, 10-, 12-, and 15-minutes postinjection. Lipids were extracted by the method of Dole, as modified by Trout, Estes, and Freidberg [22]. The extracts were counted in a liquid scintillation counter for 10 minutes. CM clearance curves were calculated by linear regression using the method of least squares on the natural log of percentage remaining counts versus elapsed time. The half-life of disappearance ($t^{1/2}$) was determined as ln (2)/β, where β is the determined rate constant. Unlike $t^{1/2}$, β is normally distributed and was used for statistical analysis. Results are reported in $t^{1/2}$ since this value is more intuitive. To determine whether significant differences existed between clearance of TG (^{3}H) and cholesterol (^{14}C), we used Δβ for each animal.

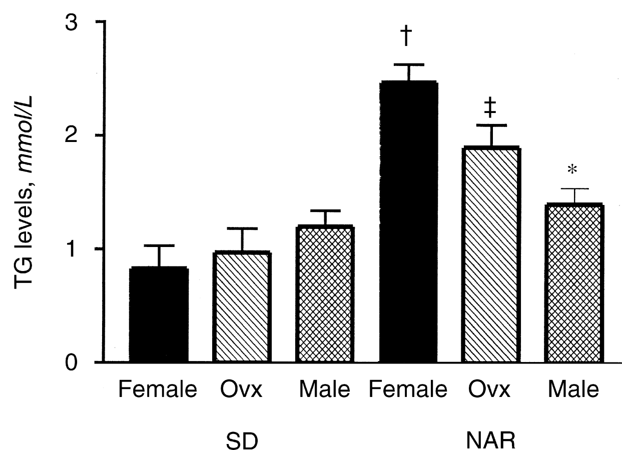

Fig. 1. Plasma triglyceride levels in male, female, and ovariectomized (OVX) Sprague-Dawley (SD) rats and Nagase analbuminemic rats (NAR). Mean plasma triglycerides levels were measured from tail blood of rats fasted for 16 hours. Intact females (Fem), OVX females, and males are represented from each breed. Error bars represent SEM. †$P < 0.05$ vs. female SD; ‡$P < 0.05$ vs. female NAR, female SD; $P <$ 0.05 vs. female NAR, OVX NAR, female SD.

Statistical analysis

Differences between experimental groups were determined by one-way analysis of variance (ANOVA), except for analysis of CM and TG clearance in which two-way ANOVA was used. If significant differences were determined ($P < 0.05$), the differences of the means were analyzed by the posthoc Student-Newman-Keuls test. Results are expressed as arithmetic means ± SEM.

RESULTS

Plasma composition

Plasma TGs were significantly greater in female NAR than in OVX NAR ($P < 0.05$). While ovariectomy reduced TG levels, this was insufficient to reach the levels found in male NAR ($P < 0.05$). Plasma TG levels in male NAR were not significantly different from that in male SD rats. TG levels in female NAR were significantly greater than in female SD rats ($P < 0.001$). In contrast to the pattern observed in NAR, TG levels were 50% greater in SD males than in SD females, and OVX caused a 25% increase in TG levels in females. However, these changes were not statistically significant (Fig. 1).

VLDL-TG secretion

Very-low density lipoprotein-TG secretion was greatest in female NAR and differed significantly from all of the other groups ($P < 0.05$; Fig. 2). VLDL-TG secretion was the same in OVX NAR, male NAR, and all three groups of SD rats.

Heparin releasable LpL and HL activity

Postheparin LpL activity in SD rats was unaffected by gender, but OVX resulted in a 1.4-fold increase in

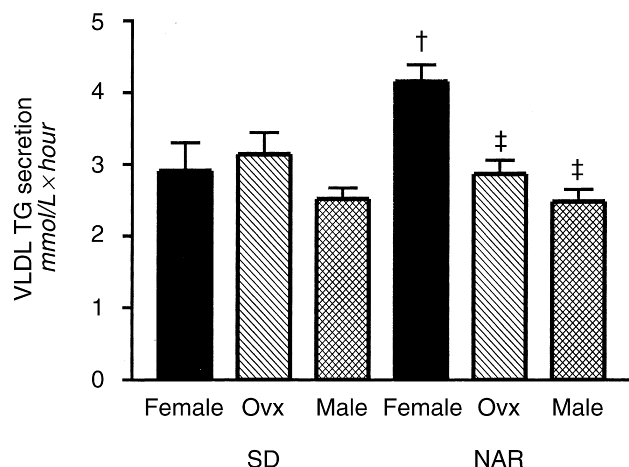

Fig. 2. Plasma very-low density lipoprotein (VLDL) secretion rates in male, female, and ovariectomized SD rats and NAR. The mean VLDL TG secretion rates were measured from TG concentration in serum from tail blood of fasted rats following an injection of 600 mg/kg Triton WR 1339. Intact females (Fem), ovariectomized females (OVX), and males are represented from each breed. Error bars represent SEM. †$P < 0.05$ vs. female SD; ‡$P < 0.05$ vs. female NAR.

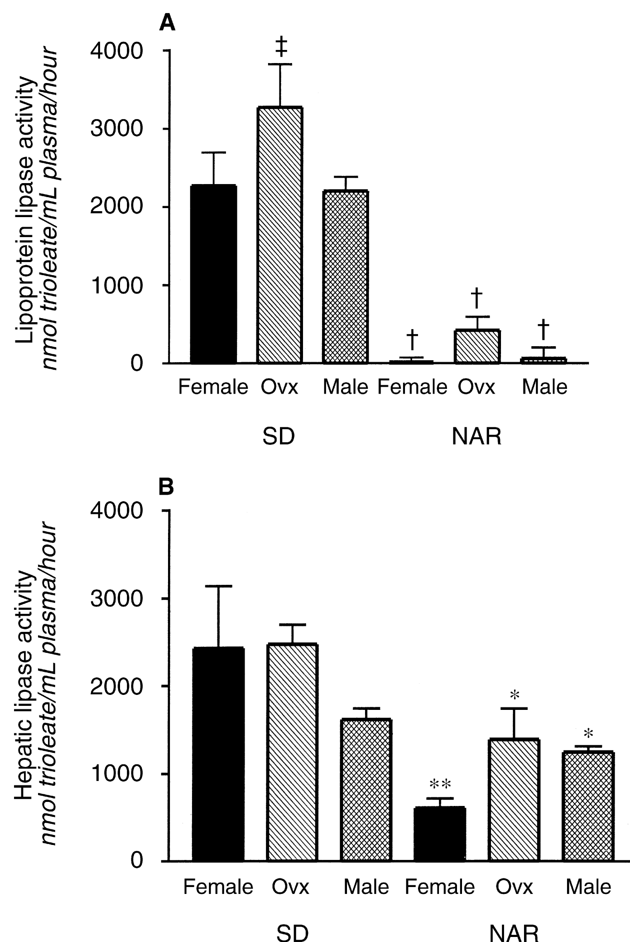

Fig. 3. Postheparin lipase activity. Plasma lipase activity at five minutes following injection of 50 U heparin/kg. (*A*) Lipase activity inhibited by 1 mol/L NaCl. (*B*) Lipase activity not inhibited by 1 mol/L NaCl. Intact females, ovariectomized females (OVX), and males are represented from each breed. Error bars represent SEM. †$P < 0.001$ vs. all SD; ‡$P < 0.05$ vs. female SD; *$P < 0.05$ vs. female, OVX SD; **$P < 0.05$ vs. SD male.

PH-LpL activity ($P < 0.05$; Fig. 3). PH-LpL activity was greatly reduced in all NAR ($P < 0.0005$). In NAR, PH-LpL activity did not exceed 10% of the activity found in their SD counterparts. As with SD rats, PH-LpL activity in OVX NAR tended to be greater than in female NAR (P = ~0.03, uncorrected). This increase approached but did not reach significance. Female NAR had virtually no PH-LpL activity (Fig. 3A).

In NAR, PH-HL activity was also reduced versus SD rats ($P < 0.001$). In female NAR, PH-HL activity was reduced compared with all SD rats ($P < 0.05$), but OVX NAR and male NAR were only less than female SD and OVX SD rats ($P < 0.05$) and were not different from male SD (Fig. 3B).

Chylomicron clearance

Clearance of CM-TG and CM-cholesterol (CM-chol) were analyzed for the effects of treatment group versus lipid using two-way ANOVA. CM-TG clearance was significantly greater in female SD rats than in male SD rats ($P < 0.005$), and OVX had no effect (Fig. 4). CM-TG clearance was significantly lower in all NARs ($P < 0.05$). CM-TG clearance was not significantly greater in female NAR than in male NAR, but CM-TG clearance in NAR OVX was significantly greater than either male or female NAR ($P < 0.05$; Fig. 4).

Chylomicron-cholesterol (CM-chol) clearance was significantly reduced in all NAR versus female and OVX SD rats ($P < 0.05$), but only female NAR CM-chol clearance was reduced compared with SD males ($P < 0.05$; Fig. 5). To determine the relationship between TG and cholesterol clearance, we first analyzed whether $\Delta\beta$ was significantly different from zero for each treatment group. We found that $\Delta\beta$ was not different from zero for each NAR group (female NAR = -0.009 ± 0.005, male NAR = -0.018 ± 0.005, OVX NAR = -0.016 ± 0.006), but it was less than zero for all SD rats (female SD = -0.126 ± 0.011, SD male = -0.049 ± 0.005, SD OVX = -0.107 ± 0.019, $P < 0.05$). Among SD, $\Delta\beta$ was less in female SD than in male, and ovariectomy had no effect ($P < 0.05$).

When the effect of both CM-TG and CM-chol was combined, the effect of CM-lipid clearance was even more pronounced. Clearance CM-lipids were greatest in female SD rats, and ovariectomy had no effect ($P < 0.05$). Clearance was significantly reduced in SD male rats ($P < 0.05$). Among NAR, CM lipid clearance was greatest in male NAR ($P < 0.05$) but was significantly less than SD male rats ($P < 0.05$), while female NAR

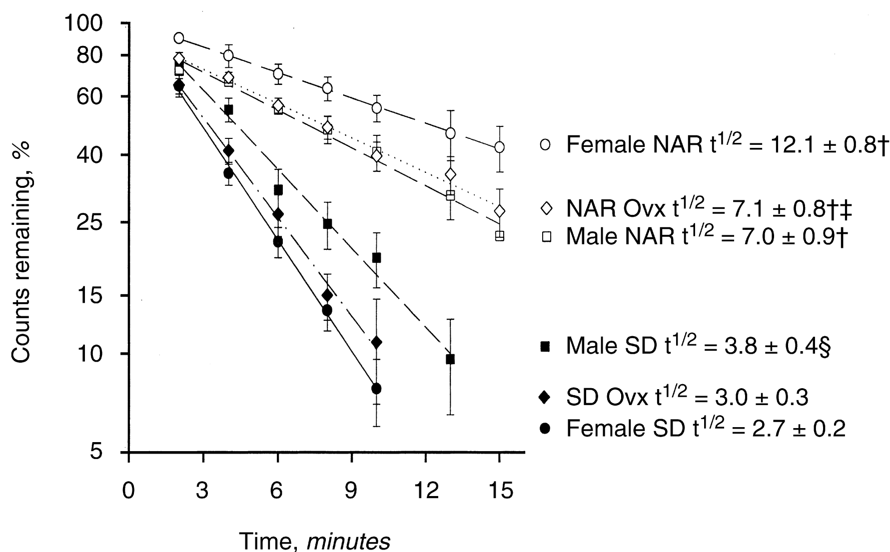

Fig. 4. Chylomicron triglyceride (TG) catabolism. Disappearance of label from rat blood after tail vein injection of labeled chylomicrons as measured by $\log_{10}$ (counts remaining). The $t^{1/2}$ is in minutes. Error bars represent SEM for each time point. †$P < 0.001$ vs. all SD; §$P < 0.05$ vs. female and OVX SD; ‡$P < 0.05$ vs. female and male NAR.

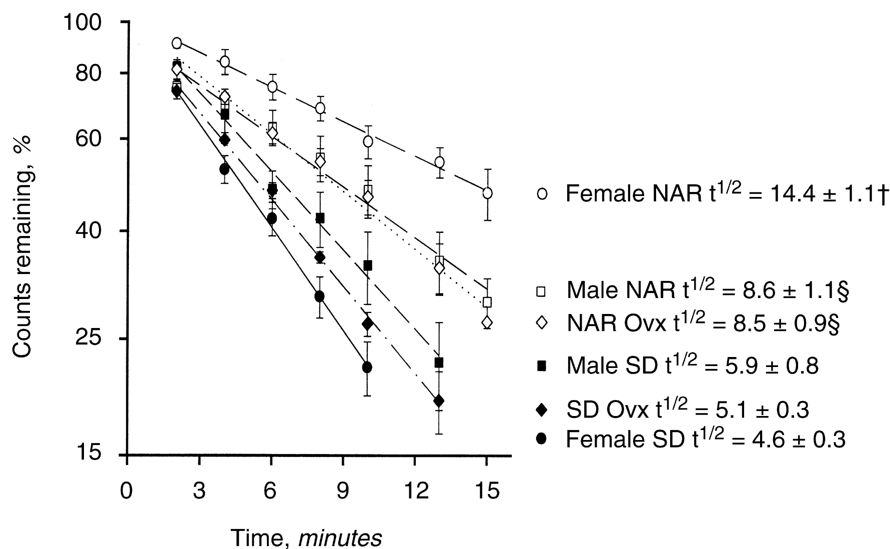

Fig. 5. Chylomicron cholesterol catabolism. Disappearance of glycerol [9,10-^{3}H] trioleate from rat blood after tail vein injection of labeled chylomicrons as measured by $\log_{10}$ (counts remaining). The $t^{1/2}$ is in minutes. Error bars represent SEM for each time point. †$P < 0.001$ vs. all SD; §$P < 0.05$ vs. female and OVX SD.

clearance was the least of all treatment groups ($P < 0.05$). Among NAR, the effect of ovariectomy on total CM-lipid clearance was to significantly increase it to male NAR levels ($P < 0.05$).

DISCUSSION

The NAR is characterized by an almost complete lack of plasma albumin, which is replaced by a number of mostly high molecular weight proteins [23]. Consequently, NAR also exhibit reduced plasma oncotic pressure (π). Included in the abnormalities in plasma protein composition is an increase in lipoprotein levels, but unlike the case of other proteins, a decrease in the fractional catabolic rate of lipoproteins, including high-density lipoprotein (HDL) [24], contributes to their increased level in plasma. The decrease in clearance of CM (and by extension of VLDL) is quite mild when compared with what occurs in the nephrotic syndrome [25], and the degree of hyperlipidemia, especially in the males, is correspondingly less than seen in males with nephrotic syndrome [2].

Female NAR have greater lipid levels than do male NAR [2, 3] This pattern is the opposite of what occurs in normal SD rats. Our findings confirm this reversed pattern of TG levels in NAR, and they confirm that estrogen mediates this reversal. In female NAR, lipid levels increase with age, and this is corrected by OVX [9]. We found that ovariectomy did not completely abolish hypertriglyceridemia in female NAR, suggesting an important but not exclusive role of estrogen in mediating their increased TG levels.

There are two possible causes of increased TG levels

in female NAR: increased hepatic TG secretion or decreased catabolism of TG-containing lipoproteins. In normal rats, estrogen causes increased hepatic production of VLDL [14, 26], and this clearly also occurs in NAR. TG secretion is increased only in female NAR, not in OVX, suggesting that increased TG levels in these animals are at least in part the result of increased TG secretion mediated by estrogen. Estrogen-mediated increased TG synthesis in normal women is responsible for the slight increase in TG levels of about 15 mg/dL. However, when there are other factors that also predispose to hyperlipidemia, the effects of estrogen can be far greater [12, 27] and can be life threatening [28, 29]. The effect of estrogen on TG secretion exacerbates serum TG levels in NAR, which are also predisposed to hyperlipidemia.

This predisposition in all NAR is a result of reduced TG catabolism. In this study, we found greatly reduced levels of PH lipase in NAR, especially of LpL. Despite a trend of increased PH-LpL in OVX NAR, the LpL depletion was an effect of analbuminemia. Thus, the large lipase pool in SD arts allows for the normoalbuminemic female animals to offset increased hepatic secretion by increase catabolism. Increased secretion in female NAR cannot be offset in this way.

Estrogen protects postmenopausal women from cardiovascular disease by increasing HDL and lowering LDL levels, despite mildly increasing plasma TG levels. Both increases in HDL and decreases in LDL are thought to derive in part from decreased HL activity [30]. These potentially salutary changes in lipoprotein profile do not occur in NAR. Despite an almost complete PH-LpL depletion in NAR in the absence of estrogen, PH-HL activity is not reduced, and even when estrogen is present, it is not reduced to the same extent as is LpL, suggesting that estrogen's effects on HL are similar in NAR as in normal animals. HL represents the greatest lipolytic potential for clearance of TG in NAR. Estrogen then would reduce HL and be expected to reduce CM rather than increase CM clearance as it does in normal animals, where estrogen also increases LpL. This latter mechanism does not occur to any biologically relevant extent in analbuminemia.

In female SD rats, the lower serum TGs are a result of increased clearance, which is most likely a consequence of increased PH-LpL [1]. Testosterone in SD rats mediates a decrease in both PH-LpL activity [1] and LpL gene expression [31], resulting in higher serum TG levels. However, in NAR, testosterone levels are markedly reduced [32]; thus, its negative effect may not be a factor in this strain.

In animals with normal lipolytic capacity, the clearance of CM-TG is distinct from and precedes CM-chol clearance. CM-TG is lipolyzed by LpL in peripheral tissues, and the remnant cholesterol containing core is cleared by receptors in the liver. HL mediates this process by tethering the remnant to heparan sulfate proteoglycans, aiding uptake of the remnant by hepatic apoE receptors [33]. The delay in clearance of CM-TG (^{3}H) in female, male, and OVX NAR is not different from the delay in CM-chol (^{14}C) clearance. This finding along with the severe PH-LpL depletion suggests that minimally lipolyzed particles are cleared intact in NAR, or fully lipolyzed remnants are cleared immediately. Indeed, there is a greater delay between delipidation (^{3}H clearance) and remnant uptake (^{14}C clearance) in SD rats than in NAR, suggesting that at the very least there is no additional impedance to the uptake of remnant particles in NAR. The lack of peripheral lipase increases the importance of HL, presumably both in its role as a TG lipase and as a mediator of remnant clearance, the presence of HL is pivotal in maintenance of serum TG in NAR. Estrogen worsened levels of HL and, similarly, the clearance of CM lipids, providing evidence for this hypothesis.

In the case of NAR total lipid clearance, the effect of estrogen exacerbated already defective clearance, leading to greatly prolonged lipid clearance as the catabolic defect was corrected by ovariectomy. Thus, estrogen worsens the already deficient clearance, while the presence of estrogen in SD did not adversely affect lipid clearance; however, SD animals do not lack lipolytic capacity, as evidenced by high peripheral LpL levels and the presence of a discrete mechanism for the removal of CM-TG and CM-chol.

While estrogen hormone replacement clearly is beneficial to women, in certain instances, this may not be so. Hypertriglyceridemia is emerging as a risk factor for glomerulosclerosis [17, 18], and hyperlipidemia contributes to renal injury in female NAR [19] and in the Obese Zucker rat [10]. Estrogen causes increased lipid levels, and the hyperlipidemia causes profound renal injury in these models [19, 34]. These results contrast with findings in normal animals in which the progression of renal disease is less severe in females [35]. It may be worthwhile to also examine whether estrogen administration to individuals having reduced plasma albumin concentration affords any benefit to blood lipid profiles.

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REFERENCES

1. Staprans I, Rapp JH, Pan XM, Ong DL, Feingold KR: Testosterone regulates metabolism of plasma chylomicrons in rats. *Arteriosclerosis* 10:591–596, 1990
2. Joles JA, Willekes-Koolschijn N, van Tol A, Geelhoed-Mieras MM, Danse LH, van Garderen E, Kortlandt W, Erkelens DW, Koomans HA: Hyperlipoproteinemia in one-year-old analbuminemic rats. *Atherosclerosis* 88:35–47, 1991
3. Takahashi M, Kusumi K, Shumiya S, Nagase S: Plasma lipid concentrations and enzyme activities in Nagase analbuminemia rats (NAR). *Jikken Dobutsu* 32:39–46, 1983
4. Jonasson L, Hansson GK, Bondjers G, Bengtsson G, Olivecrona T: Immunohistochemical localization of lipoprotein lipase in human adipose tissue. *Atherosclerosis* 51:313–326, 1984
5. Staprans I, Felts JM: A possible mechanism for accelerated atherogenesis in male versus female rats. *Arteriosclerosis* 9:224–229, 1989
6. Basdevant A: Steroids and lipid metabolism: Mechanism of action. *Int J Fertil* 37(Suppl 2):93–97, 1992
7. Applebaum DM, Goldberg AP, Pykealisteo OJ, Brunzell JD, Hazzard WR: Effect of estrogen on post-heparin lipolytic activity: Selective decline in hepatic triglyceride lipase. *J Clin Invest* 59:601–608, 1977
8. Joles JA, Bijleveld C, van Tol A, Geelen MJ, Koomans HA: Ovariectomy decreases plasma triglyceride levels in analbuminaemic rats by lowering hepatic triglyceride secretion. *Atherosclerosis* 117:51–59, 1995
9. Joles JA, van Goor H, van der Horst ML, van Tol A, Elema JD, Koomans HA: High lipid levels in very low density lipoprotein and intermediate density lipoprotein may cause proteinuria and glomerulosclerosis in aging female analbuminemic rats. *Lab Invest* 73:912–921, 1995
10. Gades MD, Stern JS, van Goor H, Nguyen D, Johnson PR, Kaysen GA: Estrogen accelerates the development of renal disease in female obese Zucker rats. *Kidney Int* 53:130–135, 1998
11. Nabulsi AA, Folsom AR, White A, Patsch W, Heiss G, Wu KK, Szklo M: Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women: The Atherosclerosis Risk in Communities Study Investigators. (comments) *N Engl J Med* 328:1069–1075, 1993
12. Glueck CJ, Lang J, Hamer T, Tracy T: Severe hypertriglyceridemia and pancreatitis when estrogen replacement therapy is given to hypertriglyceridemic women. (comments) *J Lab Clin Med* 123:59–64, 1994
13. Molitch ME, Oill P, Odell WD: Massive hyperlipemia during estrogen therapy. *JAMA* 227:522–525, 1974
14. 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. (comments) *N Engl J Med* 325:1196–1204, 1991
15. Walsh BW, Sacks FM: Effects of low dose oral contraceptives on very low density and low density lipoprotein metabolism. *J Clin Invest* 91:2126–2132, 1993
16. Austin MA, Hokanson JE: Epidemiology of triglycerides, small dense low-density lipoprotein, and lipoprotein a as risk factors for coronary heart disease. *Med Clin North Am* 78:99–115, 1994
17. Grone EF, Walli AK, Grone HJ, Miller B, Seidel D: The role of lipids in nephrosclerosis and glomerulosclerosis. *Atherosclerosis* 107:1–13, 1994
18. Keane WF: Lipids and the kidney. *Kidney Int* 46:910–920, 1994
19. Joles JA, van Goor H, Braam B, Willekes-Koolschijn N, Jansen EH, van Tol A, Koomans HA: Proteinuria, lipoproteins and renal apolipoprotein deposits in uninephrectomized female analbuminemic rats. *Kidney Int* 47:442–453, 1995
20. Krauss RM, Levy RI, Fredrickson DS: Selective measurement of two lipase activities in postheparin plasma from normal subjects and patients with hyperlipoproteinemia. *J Clin Invest* 54:1107–1124, 1974
21. Bollman JL, Cain JL, Grindlay JA: Techniques for the collection of lymph from the liver, small intestine or thoracic duct of the rat. *J Lab Clin Med* 33:1349–1352, 1948
22. Trout DL, Estes EH, Freidberg SJ: Titration of free fatty acids of plasma: A study of current methods and a new modification. *J Lipid Res* 1:199–202, 1960
23. Emori T, Takahashi M, Sugiyama K, Shumiya S, Nagase S: Age-related changes in plasma proteins of analbuminemic rats. *Jikken Dobutsu* 32:123–132, 1983
24. Kaysen GA, Hoye E, Jones H Jr, van Tol A, Joles JA: Effect of oncotic pressure on apolipoprotein A-I metabolism in the rat. *Am J Kidney Dis* 26:178–186, 1995
25. Davies RW, Staprans I, Hutchison FN, Kaysen GA: Proteinuria, not altered albumin metabolism, affects hyperlipidemia in the nephrotic rat. *J Clin Invest* 86:600–605, 1990
26. Haffner SM, Valdez RA: Endogenous sex hormones: Impact on lipids, lipoproteins, and insulin. *Am J Med* 98(Suppl 1A):40S–47S, 1995
27. Couch SC, Philipson EH, Bendel RB, Wijendran V, Lammi-Keefe CJ: Maternal and cord plasma lipid and lipoprotein concentrations in women with and without gestational diabetes mellitus: Predictors of birth weight? *J Reprod Med* 43:816–822, 1998
28. Suga S, Tamasawa N, Kinpara I, Murakami H, Kasai N, Onuma T, Ikeda Y, Takagi A, Suda T: Identification of homozygous lipoprotein lipase gene mutation in a woman with recurrent aggravation of hypertriglyceridaemia induced by pregnancy. *J Intern Med* 243:317–321, 1998
29. Henderson H, Leisegang F, Hassan F, Hayden M, Marais D: A novel glu421lys substitution in the lipoprotein lipase gene in pregnancy-induced hypertriglyceridemic pancreatitis. *Clin Chim Acta* 269:1–12, 1998
30. Brinton EA: Oral estrogen replacement therapy in postmenopausal women selectively raises levels and production rates of lipoprotein a-i and lowers hepatic lipase activity without lowering the fractional catabolic rate. *Arterioscler Thromb Vasc Biol* 16:431–440, 1996
31. Bjeorntorp P: Growth hormone, insulin-like growth factor-I and lipid metabolism: Interactions with sex steroids. *Horm Res* 46:188–191, 1996
32. Nagase S, Takahashi M: Research related to hormones in nagase analbuminemic rats. *Gan To Kagaku Ryoho* 14:763–770, 1987
33. Krapp A, Ahle S, Kersting S, Hua Y, Kneser K, Nielsen M, Gliemann J, Beisiegel U: Hepatic lipase mediates the uptake of chylomicrons and beta-VLDL into cells via the LDL receptor-related protein (LRP). *J Lipid Res* 37:926–936, 1996
34. Joles JA, van Goor H, Koomans HA: Estrogen induces glomerulosclerosis in analbuminemic rats. *Kidney Int* 53:862–868, 1998
35. Silbiger SR, Neugarten J: The impact of gender on the progression of chronic renal disease. *Am J Kidney Dis* 25:515–533, 1995