Testosterone Regulates Metabolism of Plasma Chylomicrons in Rats

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Previously we demonstrated a marked sex difference in the metabolism of chylomicrons in adult rats. In males, radiolabeled chylomicrons displayed a longer dwell time on endothelial surfaces, which resulted in a decreased chylomycin uptake by the liver. The increased rate of chylomycin metabolism in females was associated with increased postheparin lipoprotein lipase activity. In the present study, we have investigated the effects of physiological doses of sex steroid hormones on the metabolism of chylomicrons and postheparin lipoprotein lipase activity. No sex differences were found in prepubertal animals. We also found no difference in chylomycin metabolism in control female, castrated female, estrogen-treated female, castrated male, and estrogen-treated male rats. However, control male, testosterone-treated female and testosterone-treated male rats showed increased endothelial binding of chylomicrons and decreased chylomycin uptake by the liver. Postheparin lipoprotein lipase activity also was decreased by testosterone administration. In parallel with the alterations in chylomycin metabolism, serum high density lipoprotein levels in male rats decreased with testosterone administration. These results indicate that the differences in chylomycin metabolism, postheparin lipoprotein lipase activities, and serum high density lipoprotein levels observed between male and female rats are due to testosterone.

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The incidence of atherosclerotic vascular disease is lower in women than in men; however, in women the risk of atherosclerosis increases after menopause. Major differences in lipid metabolism have been observed between the sexes. In men there are significant increases in the serum levels of cholesterol, triglycerides, and low density lipoproteins (LDL) and therefore a relative decrease in high density lipoproteins (HDL) compared to premenopausal women. After menopause, the serum cholesterol, triglycerides, and LDL levels increase. These observations suggest that differences in the hormonal milieu affect serum lipoprotein levels and in men result in a lipoprotein pattern that is more atherogenic than that observed in women. Thus, the difference in the incidence of coronary heart disease between men and premenopausal women is probably, at least in part, due to sex-specific differences in lipoprotein metabolism.

Previously, we have shown that there is a quantitative difference in the metabolism of plasma chylomicrons (CM) between adult male and female rats. At 10 and 20 minutes after the injection of CM, male rat hearts contained significantly more label than female rat hearts, indicating that CM particles remained attached longer on the endothelial surfaces. Consequently CM particles were taken up by the liver at a slower rate. We have defined the time that CM remains attached on endothelial surfaces as the "dwell time" of the particle. This is an operative term for the measurement of the amount of CM label bound on endothelial surfaces. The more rapid processing of CM in female rats was associated with higher postheparin lipoprotein lipase (LPL) activity.

To further investigate the mechanism of these sex differences in CM metabolism, the present study was designed to determine whether sex steroid hormones affect CM metabolism in rats. We studied the effects of estrogen and testosterone on postheparin LPL activity, dwell time, and liver uptake of CM. We also examined the effect of testosterone administration on serum HDL cholesterol levels.

Methods

Materials

Radiochemicals (125I-albumin, 4-14C-cholesterol, and 11,12-3H-retinol) were purchased from DuPont NEN Research Products, Boston, MA. Retinol, testosterone propionate, and estradiol benzoate were purchased from Sigma Chemical, St. Louis, MO. Heparin (173 U/mg) was obtained from Evans Medical, Liverpool, UK.

Animals

Mature (180 to 250 g) and immature, age-matched (60 g) male and female Sprague-Dawley rats were
The 10-minute interval was chosen on the basis of our flotation in an SW-40 rotor for 5 × 10⁶ g/minute at 10°C.

Lever and Heart Recovery of Chylomicron Label by

were allowed to drink water, and each donor rat was restrained, and chyle was collected on ice for 14C-cholesterol-labeled CM (10 mg triglyceride, 75 µg 123I-cholesterol, 0.10 mCi of 14C-cholesterol, 0.05 mCi of 3H-retinol, and 20 µg of retinol carrier. The rats purchased from Bantin and Kingman (Fremont, CA) and were maintained on Purina rat chow #5012 and water ad libitum. In all experiments comparing males and females, the rats were matched for weight. For hormone replacement studies, the rat weights were matched at the time of castration. Where indicated, castration was performed surgically 2 weeks before hormone injections. For hormone replacement studies, mature animals were injected every other day for 3 weeks with testosterone propionate in corn oil (300 µg in 0.1 ml per 100 g body weight), estradiol benzoate in corn oil (25 µg in 0.1 ml per 100 g body weight), or the oil vehicle alone. These quantities of estrogen and testosterone have been shown to represent physiological replacement doses in rats. Before the experiments, the rats were fasted overnight, but drinking water was available. All animal studies were performed in accordance with institutional policies, and all procedures were approved by the Subcommittee on Animal Studies.

CM were prepared from mesenteric lymph duct by using a procedure described previously. To stimulate CM production, rats were given via the stomach a 2 ml mixture of a corn oil–milk emulsion sonicated together with 0.05 mCi of 3H-retinol, 0.10 mCi of 11,12-3H-retinol, and 20 µg of retinol carrier. The rats were restrained, and chyle was collected on ice for periods of up to 10 hours. During this period, the rats were allowed to drink water, and each donor rat was injected subcutaneously with 10 ml of 0.15 M NaCl solution to maintain hydration. CM were isolated by flotation in an SW-40 rotor for 5 × 10⁶ g/minute at 10°C.

Recovery of Chylomicron Label by Liver and Heart

The tissue distribution of radiolabels was determined 10 minutes after the injection of 3H-retinol- and 14C-cholesterol-labeled CM (10 mg triglyceride, 75 µg total cholesterol) via a tail vein as described previously. The 10-minute interval was chosen on the basis of our previous time study. At 30 minutes, there was no detectable label found in the hearts, and 95% of the injected CM had been transported to the liver. The livers were perfused via the portal vein and the hearts via the aorta with 20 ml of 0.15 M NaCl to remove blood. The livers and hearts were weighed for isotope analysis. The average weight of the livers was 12 g, and the weight of the hearts averaged 1 g. There was no significant variation with changing hormonal status, and the weights of the hearts and livers in the castrated males did not increase more than 10% with increasing testosterone administration. Duplicate 1 g portions of livers and whole hearts were then homogenized in 1 ml 0.15 M NaCl and were extracted with 40 ml of chloroform/methanol (2:1, vol/vol). An aliquot (5 ml) of the clear extract was evaporated to dryness and then analyzed for 14C and 3H in a Tri-Carb liquid scintillation spectrometer (model 2450; Packard Instruments, Downers Grove, IL). Label incorporation was calculated for whole livers and for 1 g heart weight. 125I-albumin was used to verify the absence of blood in tissue homogenates as described previously.

Postheparin Lipoprotein Lipase Activity

Heparin (10 U/kg) was injected into the inferior vena cava in control male and female rats, castrated rats, and castrated rats which had undergone hormone replacement therapy. Exactly 5 minutes after the heparin injection, blood was taken from the abdominal aorta and was allowed to clot; then serum was obtained. The LPL activity measurements were performed according to the method of Whayne and Felts by using Intralipid as a substrate. Free fatty acids were titrated at 0 time and after 30 minutes of incubation at 37°C. One unit of free fatty acids was defined as 1 µmole of free fatty acids released per hour per 1 ml of serum. When the reaction was carried out in 1 M NaCl, no net increase in free fatty acids was detected, indicating that this method carried out at pH 8.4 specifically determined the LPL activity and not the hepatic triacylglycerol lipase activity.
**Analytic Procedures**

The triglycerides in the CM preparations were determined by the method of Fletcher, and total cholesterol was estimated as described. Serum HDL concentrations were determined by using Sigma Kit No. 352-3. The statistical differences were determined by using a two-tailed Student's t test.

**Results**

**Immature Animals**

We initially examined CM metabolism in immature male and female rats. Recovery of 14C-cholesterol and 3H-retinol in liver and heart tissue was determined 10 minutes after the injection of labeled CM into immature and mature control animals (Figures 1 and 2). In agreement with our previous observations, there was a significant (p<0.01) decrease in the uptake of both 14C-cholesterol and 3H-retinol in the livers of mature male rats when compared to those of mature female rats (Figure 1). However, no differences were detected between the sexes when the liver uptake of CM was compared in immature male and female animals (Figure 1). As in our previous studies in the heart, the uptake of 14C-cholesterol- and 3H-labeled CM was markedly greater in adult male rats (Figure 2). However, in immature animals (Figure 2), there was no sex difference in the uptake of either label in the heart. The delayed liver uptake and the increased binding by the heart (dwell time) of CM in male rats was observed only after the maturation of the animal. These results show that the sex differences in CM metabolism between male and female rats are related to maturity and are probably due to differences in sex hormones.

**Hormonal Manipulation**

To test the effects of hormonal manipulation on CM metabolism, we next studied control (mature) male and female rats, castrated male and female rats, and castrated male and female rats which had undergone either testosterone or estrogen replacement therapy. Figures 3 and 4 show the uptake of 14C-cholesterol and 3H-retinol-labeled CM with both labels. No significant difference in the CM uptake by the liver (Figure 3) was found between control female, castrated female, estrogen-treated female, castrated male, and estrogen-treated castrated male rats. However, control male rats, testosterone-treated castrated male rats, and testosterone-treated castrated female rats showed a decreased...
liver uptake of \(^{14}\text{C}\)-cholesterol and \(^{3}\text{H}\)-retinol-labeled CM. These results indicate that testosterone regulates CM metabolism and that the previously observed decrease in CM uptake by the liver in male rats is due to testosterone.

The effect of hormonal manipulation on CM uptake by the heart in control (mature) male and female rats, castrated male and female rats, and castrated male and female rats which had undergone either testosterone or estrogen replacement therapy is shown in Figure 4. No significant differences were observed between control female, castrated female, estrogen-treated female, castrated male, and estrogen-treated castrated male rats. However, there was a marked increase in the \(^{14}\text{C}\)-cholesterol and \(^{3}\text{H}\)-retinol uptake in the hearts of male rats and castrated male and female rats which had undergone testosterone replacement. These results further indicate that CM metabolism is influenced by sex hormones and that testosterone is responsible for the previously observed sex difference in CM dwell time. Castration itself affected only male animals, and estrogen replacement had no effect in castrated animals of either sex.

The effect of various doses of testosterone on \(^{3}\text{H}\)-retinol-labeled CM uptake by livers of castrated male rats is shown in Figure 5. In male rats, castration increased the CM liver uptake from 31.35±6.38% (control value) to 49.4±3.60%. At doses of 0.15 \text{mg} of testosterone/100 \text{g} rat weight, which is considered to be in the physiological range,\(^4\),\(^5\) the liver uptake of CM was suppressed to that of a control male rat. Testosterone affected CM uptake at low doses (below physiological), and higher replacement doses had no further effect. In the hearts (Figure 6), there was a large increase in the dwell time of CM at very low doses of testosterone; however, at physiological doses of testosterone, the dwell time leveled off and was not affected by further increases in testosterone administration. These results demonstrate that small doses of testosterone have a marked effect on CM metabolism and suggest that these alterations are of physiological significance.

**High Density Lipoprotein Cholesterol**

In male rats, castration increased serum HDL from 30.3±2.0 to 46.6±6.5 \text{mg/dl}. HDL levels in serum of castrated male rats were reduced by subphysiological testosterone doses (0.05 to 0.10 \text{mg} of testosterone/100 \text{g} rats weight), and there was no further change observed with concentrations of testosterone above the physiological level (0.15 \text{mg} of testosterone/100 \text{g} rats weight). The response of serum HDL cholesterol concentrations to testosterone administration (Figure 7) paralleled that of CM metabolism. The correlation coefficient between serum HDL levels and CM metabolism (uptake by livers, Figure 5) was found to be 0.86 (p<0.05).

**Lipoprotein Lipase Activity**

Table 1 shows the effects of castration and sex hormone replacement on plasma postheparin LPL activity. In agreement with our previous findings, there was a significant difference in the plasma postheparin LPL activity.
Between male and female control rats. Moreover, this sex difference was obliterated by castration, with the activity increasing in male rats to a level similar to that observed in females. The increase in the enzyme activity in castrated male rats can be prevented by the administration of physiological doses of testosterone. Similarly, the enzyme activity can also be suppressed in castrated female rats by testosterone treatment. These results indicate that postheparin LPL is susceptible to hormonal manipulations and that activity is suppressed by testosterone. A highly significant correlation was found between postheparin LPL activities in hormonally manipulated animals and CM metabolism. The correlation coefficient between LPL activities (Table 1) and CM uptake by the liver (Figure 3) and heart (Figure 4) was 0.89 (p<0.005) and −0.90 (p<0.005), respectively.

**Discussion**

Nearly all dietary lipids and cholesterol enter the bloodstream via CM.14 The metabolism of CM proceeds in two steps: extrahepatic and hepatic.14,15 In the extrahepatic step, CM are attached to LPL located on the endothelial surfaces of tissues such as heart, muscle, and adipose tissue. After partial hydrolysis of CM by LPL, at some critical point, the CM detaches from the endothelium, enters the circulation as a CM remnant, and then is rapidly taken up by the liver. There is evidence indicating the importance of CM metabolism in the atherogenic process. In type III hyperlipemia, the accumulation of postprandial CM remnants due to delayed clearance is associated with premature atherosclerosis.16 Zilverstreelt17 has suggested that CM remnants may be particularly atherogenic, since CM are also hydrolyzed by LPL on the endothelial surfaces of large arteries, and their cholesterol may become incorporated into the intima of the artery wall, stimulating the formation of atherosclerotic lesions.17

In this study, we have again demonstrated that there are significant differences in CM metabolism between adult male and female rats. In adult male rats, there was a significantly longer dwell time and a decreased rate of liver uptake of CM. Since no differences in CM metabolism were observed between prepubertal male and female animals, our data indicate that the observed sex differences are dependent on changes associated with maturation. In adult rats, we found that in female rats castration per se had no effect on the CM metabolism; however, castration in male rats reversed the observed sex differences and resulted in CM metabolism resembling that of female rats. Estrogen replacement had no effect on castrated animals of either sex, but testosterone replacement affected CM metabolism in both castrated male and female rats. Control male rats and testosterone-treated castrated male and female rats showed an increased dwell time and decreased rate of liver uptake of CM. Thus, clearly testosterone accounts for the sex difference in CM metabolism.

The exact mechanism for the testosterone effect is still not completely elucidated; however, our results suggest that the testosterone effect may be mediated by alterations in the activity of LPL, an important determinant of CM and very low density lipoprotein (VLDL) metabolism. Previously, we have shown that female rats contain significantly more plasma postheparin LPL activity than male rats. In this study, we have demonstrated that testosterone suppresses postheparin LPL activity. Specifically, in castrated rats of both sexes, replacement with testosterone resulted in a marked decrease in postheparin LPL activity. The highly significant correlation between LPL activities of hormonally manipulated rats and CM uptake by livers (r=0.89) and hearts (r=−0.90) suggests that testosterone alters CM metabolism by modulating the LPL activity in rats. Studies by others have shown that androgen administration inhibits rat adipose tissue LPL.18 Estrogen also has been shown to reduce adipose tissue LPL activity in both control and castrated animals.19,20,21 However, we did not observe this effect on postheparin LPL activity at physiological replacement doses of estrogen.

Furthermore, our data suggest that testosterone may also modulate serum HDL levels by affecting LPL activity. The testosterone dose-response curves show a highly significant correlation between the metabolism of CM (liver uptake) and serum HDL levels (r=0.86). Changes in LPL activity can affect HDL levels, since HDL are formed after the hydrolysis of triglyceride-rich CM and VLDL by LPL.22,23 Thus, a reduction in HDL concentration could be a consequence of the slower catabolism of triglyceride-rich CM due to the decreased rate of LPL activity on the endothelial surfaces.24,25

Other investigators have also shown that sex hormones have an effect on circulating plasma lipoproteins. Patsch et al.8 studied the effects of sex hormones on the amount and composition of all plasma lipoproteins. They found that, in Sprague-Dawley rats, the levels of HDL were higher in female than in male rats. However, there were no significant sex-related differences in the levels of total plasma lipids, apolipoproteins, and the levels of.
centrifuged VLDL and LDL fractions. They also demonstrated that serum lipid concentrations were not significantly affected by castration or sex hormone administration in either sex, but HDL levels were decreased by testosterone and increased by estrogen. Takeuchi et al. demonstrated that testosterone reduced plasma HDL levels in rats, and they proposed that this reduction was probably secondary to the reduction of VLDL catabolism.

In summary, we have shown that, in rats, sex-specific differences in CM metabolism are induced by testosterone and that the influence of testosterone on CM metabolism and serum HDL levels may be mediated through testosterone effects on LPL. Testosterone decreases postheparin LPL activity and reduces the hydrolysis rate of CM. These changes in lipid metabolism, if applicable to humans, may play an important role in the increased risk of atherosclerosis observed in men.

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