Effects of Exercise on Plasma High-Density Lipoprotein Cholesteryl Ester Metabolism in Male and Female Miniature Swine

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We studied the effects of exercise on high-density lipoprotein (HDL) cholesteryl ester (CE) metabolism in 6 male and 6 female miniature pigs fed a commercial swine diet supplemented with cholesterol and fat. The diets were fed for a total period of 20 weeks. During the last 12 weeks of the feeding period, the pigs were exercised on a motorized treadmill 5 days per week for 45 min/d at a speed of 9.5 to 10.0 km/h at 0% grade. Homologous HDL preparations were radiolabeled with cholesteryl (1-^{14}C)oleate and intravenously administered to the pigs, followed by blood sampling at the appropriate time points and measurement of radiolabeled HDL CE. This was performed while the animals were sedentary and after the exercise period. Plasma cholesterol increased after the exercise protocol from 7.21 ± 1.90 to 8.50 ± 2.81 mmol/L (mean ± SD, n = 6) in the females and from 8.11 ± 3.61 to 10.07 ± 3.61 in the males. HDL CE transport rates in female pigs were significantly lower (23%) after the exercise protocol (118 ± 14 *v* 91 ± 14 μmol/h/L plasma). HDL CE transport rates in the males were also lower (11%) after exercise (90 ± 20 *v* 80 ± 18 μmol/h/L plasma), but this effect was not statistically significant. Further, the residence time or life span of HDL CE was significantly longer after the exercise protocol in both male and female pigs. Thus, the results of this study suggest that exercise reduces the transport rate of HDL CE and prolongs the life span of HDL CE in hypercholesterolemic pigs.

SEVERAL STUDIES have documented the effects of exercise on plasma lipid concentrations. Exercise has been shown to increase high-density lipoprotein (HDL) cholesterol in humans.[1,2] This increase was associated with an increased lipoprotein lipase activity and a decreased plasma triglyceride concentration.[3] Animal studies have also pointed to a role of exercise in lipid metabolism. Exercise in rats fed a hypercholesterolemic diet increased HDL cholesterol,[4] but such an effect was not found in normolipidemic rats.[5] In pigs, exercise also tended to increase HDL cholesterol levels, although these studies did not produce significant effects.[6-9]

There are also studies that have examined the effects of exercise on the kinetics of the lipoproteins. Herbert et al[10] and Thompson et al[11] reported that exercise training in humans was associated with elevated HDL cholesterol concentrations together with a longer life span of the HDL apoproteins A-I and A-II. Studies in swine also pointed to an effect of exercise on lipoprotein metabolism, although no significant effects on plasma and lipoprotein concentrations were measured. Stucchi et al[8] reported that prolonged training resulted in a longer life span of low-density lipoprotein (LDL) particles without significantly affecting lipid levels. In rats, training increased both the residence time of HDL cholesteryl ester (CE) and the apoproteins together with an increase in HDL cholesterol concentrations.[4]

The pig is widely used as a model for lipoprotein metabolism and also has been used as a model to study the effects of exercise on plasma lipid metabolism.[6-9] In the present study, we further examined the effects of exercise on lipoprotein metabolism in both male and female pigs. We were specifically interested in the effect of exercise on the kinetics of HDL CE. Most of the studies on lipoprotein metabolism have examined the apoprotein particles of HDL, which are considered the vehicles for CE transport. We studied pigs that were fed a hypercholesterolemic diet, since the effects of exercise on lipid metabolism might be more pronounced after feeding an atherogenic diet.[4,5]

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

We used 6 sexually mature 6-month-old female and 6 male miniature Yucatan swine which were fed a hypercholesterolemic diet throughout the study, ie, during both the sedentary period and the exercise period. The hypercholesterolemic diet consisted of a nutritionally complete basal swine diet (Agway Feeds, Syracuse, NY) supplemented with 40% kcal as edible nonhydrogenated coconut oil and 0.7 weight% cholesterol. The animals were individually fed (approximately 1 kg/d) and had free access to water. They were maintained in a temperature- and light-controlled environment in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of New Hampshire.

After a sedentary period of 8 weeks, the miniature swine were trained to run on a motorized belt-type treadmill and the exercise protocol was continued for a period of 12 weeks. The training periods averaged 45 min/d for 5 days per week at a speed of 8.0 to 9.0 km/h and 0% grade. The total distance per week was between 30 and 34 km. Low-voltage shock grids were suspended at the rear of the treadmill and used for the training stimulus. The heart rate of trained animals is reported to be between 168 and 180 bpm at speeds of 9.5 to 10.0 km/h.[8] Although we did not measure the heart rate, our exercise-trained animals ran at a speed of 8.0 to 9.0 km/h, which should have elicited a heart rate of approximately 70% to 80% of their maximum heart rate.

Preparation of Radiolabeled Lipoproteins

Venous blood was collected in heparinized tubes and 2 HDL preparations, 1 from a plasma pool of male pigs and 1 from a plasma

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pool of female pigs, were isolated by a combination of differential and density gradient ultracentrifugation.[12] Cholesteryl (1-^{14}C)oleate (specific activity, 2.07 TBq/mol; Amersham, Buckinghamshire, England) was incorporated into pig HDL as previously described.[13] Briefly, radiolabeled CE was dissolved in 50 µL absolute ethanol and mixed with cynomolgus monkey lipoprotein-deficient serum (LPDS) and pig HDL. Cynomolgus monkey LPDS has high plasma CE transfer protein (CETP) activity, which facilitates the incorporation of radiolabeled CE into lipoproteins. The mixture was incubated in a shaking water bath at 37°C for 24 hours, and the radiolabeled HDLs were reisolated by density gradient ultracentrifugation.[14] This procedure was used before the exercise period and after the exercise period; altogether, 4 radiolabeled preparations were made.

Kinetic Studies

HDL kinetics in both the male and female pigs were determined in the sedentary period and immediately after the exercise period. First, the pigs were fed the hypercholesterolemic diet for a period of 8 weeks without exercise and the kinetics of HDL were determined. Subsequently, the pigs were trained for a period of 12 weeks while consuming the same diet and HDL kinetics were again measured. The pigs were not exercised during the short period of the kinetic studies (up to 70 hours). The 2 HDL preparations isolated from a pool of female pigs and a pool of male pigs were separately labeled. Female radiolabeled HDLs were administered to the female pigs and male radiolabeled HDLs were infused in the male pigs.

The radiolabeled lipoproteins were administered via an indwelling catheter inserted into an ear vein. The radiolabeled lipoproteins were administered with a syringe, and the volume of the administered lipoprotein solution was determined by weighing the syringe full and empty and calculating the difference (grams) assuming that the radiolabeled HDL CE preparation (in saline solution) has a density of 1 kg/L. The radioactivity in 25 µL radiolabeled lipoprotein preparation was also determined, and the radioactivity of the administered dose was calculated. Blood samples were collected from the cranial vena cava into heparinized tubes after 10 minutes and at another 5 or 6 time points for up to 70 hours. Very–low-density lipoprotein (VLDL) and LDL in plasma samples were precipitated with a phosphotungstic acid $MgCl_2$ solution[15] (catalog no. 352-4; Sigma Diagnostics, St Louis, MO) and the supernatant (HDL) was analyzed for radioactivity. Radioactivity in whole serum, HDL, and VLDL + LDL was expressed as a fraction of the radioactivity in the plasma sample obtained 10 minutes after tracer administration. Radioactivity curves for male and female swine were constructed and extrapolated to infinite time. The precursor-product curves could be described by biexponential functions, and the area under the curve was determined by integrating these functions. The area under the VLDL + LDL product curve was calculated as the area under the total plasma die-away curve minus the area under the HDL die-away curve. A noncompartmental model was used to analyze kinetic data, and the various kinetic parameters were calculated.[16,17] Kinetic studies are based on a steady-state situation, and then the production rate of HDL CE is equal to the disposal rate. As a consequence, the transport rate through the HDL system or pool is then also equal to the production or disposal rate. The residence time (hours) or the average life span of HDL CE is calculated as the area under the die-away curve, and the fractional catabolic rate (FCR, per hour) is calculated as the reciprocal of the area under the curve. The fraction of LDL CE derived from HDL is calculated as the area under the LDL product curve divided by the area under the HDL precursor curve. The transport rate is calculated as the FCR × HDL CE concentration.

We also calculated the fraction of total plasma CE derived from the lecithin:cholesterol acyltransferase (LCAT) reaction. Plasma CEs are derived from the esterification of free cholesterol by LCAT and acyl coenzyme A cholesterol acyltransferase (ACAT).[18,19] LCAT acts mainly on HDL, whereas ACAT is active in the liver. ACAT-generated CEs in the liver are secreted into plasma VLDL, which subsequently can be converted to LDL. Pigs do not have plasma CETP activity, and as a consequence, the CE cannot move back and forth between the various lipoprotein fractions. However, as reported previously[20] and confirmed in the present study, there is a unidirectional transfer of HDL CE to LDL (but not to VLDL) in the pig despite the absence of plasma CETP activity. Thus, HDL CE can only be derived from the LCAT reaction. VLDL CE, on the other hand, must be derived from the ACAT reaction, whereas LDL can originate from both the LCAT and ACAT reaction. The fraction of plasma CE generated by LCAT was calculated as [(fraction LDL derived from HDL × LDL CE concentration) + HDL CE concentration]/total plasma CE concentration.

Analytical Methods

Plasma and lipoprotein cholesterol[21] and triglyceride[22] concentrations were measured enzymatically on an autoanalyzer (Cobas Bio; Roche, Basel, Switzerland). Lipoproteins of plasma samples obtained immediately before the kinetic studies were isolated by density gradient ultracentrifugation[14] and analyzed for total cholesterol and CE concentrations.

Statistics

The plasma lipid and kinetic parameters were measured in male and female pigs before and after the exercise protocol. Therefore, we used a 2-way (exercise and gender as factors) repeated-measures ANOVA on 1 variable (measured parameter) to statistically analyze exercise-induced differences in the parameters (lipid concentrations and kinetic parameters before and after the exercise protocol) and differences between genders. The level of significance was preset at P less than .05. Subsequently, multiple comparisons were made (t tests with the Bonferroni adaptation) to determine which groups were significantly different when the ANOVA test indicated a significant exercise or gender effect or an interaction between exercise and gender. The following pairwise comparisons were performed: (1) female sedentary versus female exercised pigs (paired t test), (2) male sedentary versus male exercised pigs (paired t test), (3) sedentary female versus sedentary male pigs (unpaired t test), and (4) exercised female versus exercised male pigs (unpaired t test between postexercise values). Thus, each group was used for 2 comparisons. Therefore, the level of significance for these multiple comparisons was preset at P less than .025 (.05/2) instead of P less than .05 according to the Bonferroni adaptation. Separately from the ANOVA test and the subsequent multiple group comparisons, we also compared the exercise-induced changes in male pigs and female pigs with an unpaired t test. Statistical analyses were performed with the SigmaStat statistical software package (Jandel, San Rafael, CA).

RESULTS

Exercise Effects

The weight of the female and male pigs significantly increased during the exercise period (Table 1). Plasma volume expressed as a percentage of body weight was not affected by the exercise protocol.

Plasma and lipoprotein total cholesterol and CE concentrations are listed in Table 2. Most of the plasma cholesterol was in the LDL fraction, whereas only a very small proportion of total plasma cholesterol was measured in the VLDL fraction. LDL total cholesterol and LDL CE concentrations were significantly higher after the exercise protocol in both the females and the males. HDL cholesterol levels also significantly increased in the male pigs, but not in the female pigs. Plasma triglyceride levels

Table 1. Kinetic Parameters of HDL CE Metabolism in Sedentary and Exercised Hypercholesterolemic Pigs

Parameter	Females		Males		Repeated-Measures ANOVA
	Sedentary	Exercised	Sedentary	Exercised	
Plasma vol (% of body weight)	3.33 ± 0.26	3.41 ± 0.29	4.39 ± 0.80†	4.39 ± 0.60†	G
Body weight (kg)	33.78 ± 4.96	54.73 ± 6.98*	32.35 ± 3.92	50.42 ± 8.31*	E
Dose administered (kBq)	130.28 ± 9.31	159.60 ± 3.46	179.99 ± 18.81	217.07 ± 5.0	
HDL FCR (h^{-1})	0.072 ± 0.017	0.054 ± 0.015*	0.040 ± 0.010†	0.030 ± 0.011*†	E, G
HDL residence time (h)	14.56 ± 3.42	19.40 ± 4.64*	25.59 ± 6.42†	36.19 ± 9.53*†	E, G
HDL transport rate (μmol HDL CE/h/L plasma)	0.118 ± 0.014	0.091 ± 0.014*	0.090 ± 0.020†	0.080 ± 0.018	E, G, ExG
Fraction of LDL CE derived from HDL	0.329 ± 0.096	0.421 ± 0.137	0.202 ± 0.072†	0.143 ± 0.073†	G
Fraction of plasma CE generated by LCAT	0.50 ± 0.13	0.55 ± 0.12	0.54 ± 0.15	0.45 ± 0.12‡	ExG

NOTE. Values are the mean ± SD for 6 pigs. Sedentary pigs were fed the atherogenic diet for a period of 8 weeks; then, the pigs were exercised for a period of 12 weeks. The data were analyzed with a 2-way (gender and exercise as factors) repeated-measures ANOVA on 1 variable (kinetic parameters), and the significance level was preset at $P < .05$. Subsequently, multiple comparisons were made with *t* tests, and the level of significance was preset at $P < .025$ according to the Bonferroni adaptation.

Abbreviations: E, exercise effect; G, gender effect; ExG, interaction between exercise and gender.

*Significant effect of exercise within gender (paired *t* test).

†Significant difference between sedentary males and sedentary females or between postexercise males and postexercise females (unpaired *t* test). Additional comparison of the exercise-induced changes between males and females (unpaired *t* test).

‡$P < .05$.

were lower after the exercise protocol, but this effect was only statistically significant in the females.

Plasma HDL CE kinetic parameters were determined before and after the exercise protocol. Radiolabeled HDLs were intravenously administered and the plasma disappearance rate of the labeled CE was determined. After the period of exercise, the plasma disappearance rate of HDL CE was significantly lower, and this effect was reflected in significantly lower values for the FCR of HDL CE in both the females and the males (Table 1). There was also a transfer of HDL CE to the LDL fraction (Fig 1), similar to a previous study with hypercholesterolemic pigs.[20] However, there was no significant effect of the exercise protocol on this transfer, and the same was true for the fraction of plasma CE generated by the LCAT enzyme.

The exercise protocol resulted in a significantly longer residence time or life span of CE in the HDL fraction (Table 1). Further, the transport rate of HDL CE was lower after the exercise protocol, although this effect did not reach statistical significance in the male pigs (Fig 2).

Gender Effects

The results of this study also indicate gender differences in the various kinetic parameters of HDL CE metabolism. The FCR of HDL CE was significantly lower in male pigs versus female pigs, and the HDL CE residence time was significantly higher in males compared with females (Table 1). The fraction of HDL CE transferred to the LDL fraction was significantly lower in males versus females. These differences were true for the preexercise and postexercise values. Further, the HDL CE transport rate was lower in the males versus the females, but this difference was only statistically significant when the pigs were sedentary. However, there were no statistically significant differences between males and females in either the preexercise or postexercise value for the fraction of plasma CE derived from the LCAT enzyme.

Interaction Between Exercise and Gender

The results of the ANOVA test indicated a significant exercise and gender effect and a significant interaction between these 2 parameters on HDL total cholesterol and HDL CE concentrations (Table 2). An interaction indicates that the effect of 1 factor (exercise or gender) on the measured parameter (HDL CE concentration) depends on the other factor. Subsequent pairwise comparisons with a *t* test with Bonferroni adaptation indicated that there was not a significant exercise effect in the males, and there was a statistically significant effect in the females. Moreover, the exercise-induced changes in HDL total choles-

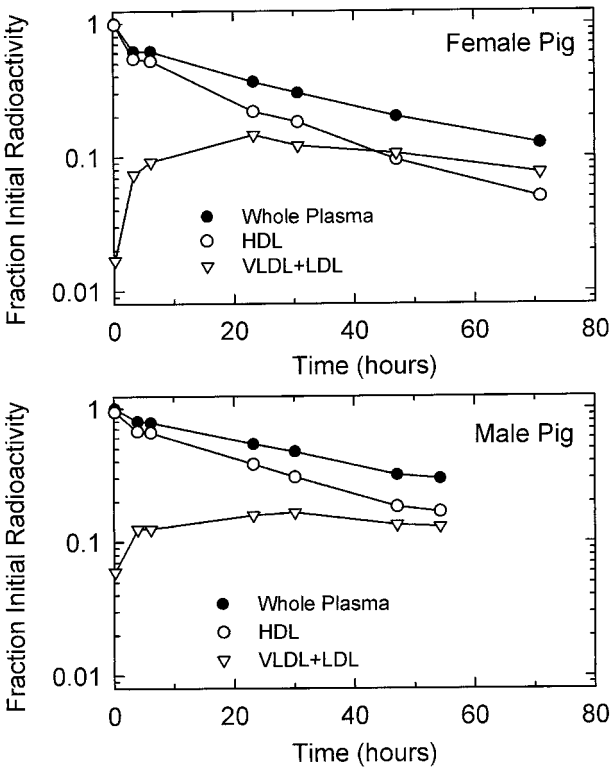

Fig 1. Typical plasma radioactivity curves for cholesteryl (1-^{14}C)oleate in a sedentary male and female hypercholesterolemic pigs after administration of cholesteryl (1-^{14}C)oleate-labeled HDL.

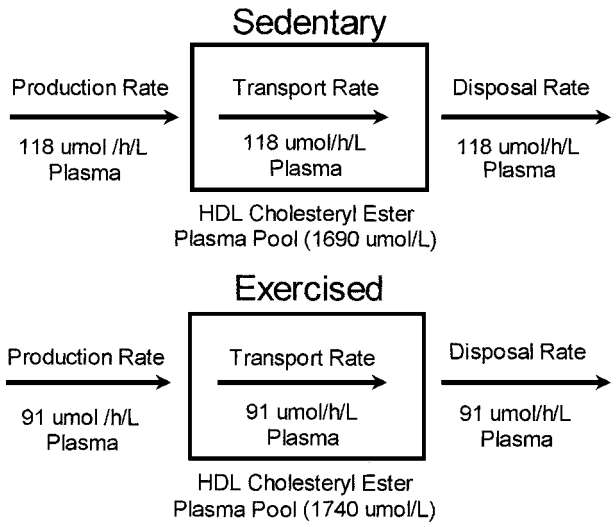

Fig 2. Production, transport, and disposal rates in female pigs before and after exercise. Results are the mean for 6 pigs.

terol and HDL CE were also significantly higher in males versus females. The gender effect, on the other hand, was statistically significant when the pigs were either sedentary or exercised (comparison of male and female posttraining values), but this gender effect was more pronounced in the exercised pigs (Table 2).

Further, the ANOVA test indicated a significant interaction between exercise and gender on the LDL CE level, and exercise-induced changes in the males were also significantly different from those in the females. On the other hand, the ANOVA test indicated no significant interaction between exercise and gender on the triglyceride concentration, whereas the comparison of the exercise-induced changes showed a significantly greater effect of exercise on triglycerides in females versus males.

Further, the ANOVA test indicated a significant exercise and gender effect and a significant interaction between exercise and gender on the HDL CE transport rate. The data in Table 1 show that exercise resulted in a 23% lower HDL CE transport rate in female pigs, whereas this decrease was only 11% in male pigs. Subsequent pairwise comparisons with a *t* test with Bonferroni adaptation indicated that this exercise effect on the HDL CE transport rate was only statistically significant in female pigs; however, a comparison of the exercise-induced changes between males and females did not reach statistical significance. Thus, these results suggest that the effect of exercise on the HDL CE transport rate is dependent on gender, and is more pronounced in female pigs. Similarly, the effect of gender on the HDL CE transport rate was greater in sedentary pigs (24% lower in sedentary males *v* sedentary females) versus exercised pigs (11% lower in males, posttraining values of males compared with posttraining values of females), and this gender effect was only statistically significant when the pigs were sedentary. Thus, these results suggest that the effect of gender on the HDL CE transport rate is dependent on whether the pigs are exercised.

Further, there was a significant interaction between exercise and gender on the fraction of plasma CE derived from the LCAT enzyme, and there was also a significant difference in the exercise-induced changes between males and females.

DISCUSSION

The study objective was to examine the effects of exercise on various parameters of HDL CE metabolism such as the HDL CE transport rate, FCR of HDL CE, and HDL CE residence time. Studies were performed in male and female pigs to determine whether there were also gender effects. We had 2 groups of 6

Table 2. Total Plasma Triglyceride and Cholesterol and CE Concentrations (mmol/L plasma) in Whole Plasma and Lipoprotein Fractions in Sedentary and Exercised Hypercholesterolemic Pigs

Parameter	Females		Males		Repeated-Measures ANOVA
	Sedentary	Exercised	Sedentary	Exercised	
Triglycerides	0.99 ± 0.15	0.39 ± 0.15*	0.59 ± 0.23†	0.29 ± 0.07‡	E, G
Total cholesterol					
VLDL	0.54 ± 0.39	0.47 ± 0.43	0.52 ± 0.50	0.71 ± 0.49	
LDL	5.38 ± 1.92	6.85 ± 2.65*	4.44 ± 2.72	7.39 ± 3.62*	E
HDL	2.15 ± 0.34	2.09 ± 0.40	2.95 ± 0.28†	3.51 ± 0.53*†‡	E, G, ExG
Whole plasma	7.21 ± 1.90	8.50 ± 2.81	8.11 ± 3.61	10.07 ± 3.61*	E
CE					
VLDL	0.34 ± 0.31	0.34 ± 0.32	0.32 ± 0.35	0.51 ± 0.37	
LDL	4.23 ± 1.48	5.19 ± 1.98*	3.15 ± 1.98	5.25 ± 2.55*‡	E, ExG
HDL	1.69 ± 0.28	1.74 ± 0.36	2.29 ± 0.17†	2.78 ± 0.43*†‡	E, G, ExG
Whole plasma	5.42 ± 1.45	6.55 ± 2.09	5.85 ± 2.58	7.46 ± 2.60*	E

NOTE. Values are the mean ± SD for 6 pigs. Sedentary pigs were fed the atherogenic diet for a period of 8 weeks; then, the pigs were exercised for a period of 12 weeks. The data were analyzed with a 2-way (gender and exercise as factors) repeated-measures ANOVA on 1 variable (lipid concentration) and the significance level was preset at $P < .05$. Subsequently, multiple comparisons were made with *t* tests, and the level of significance was preset at $P < .025$ according to the Bonferroni adaptation.

Abbreviations: E, exercise effect; G, gender effect; ExG, interaction between exercise and gender.

*Significant effect of exercise within gender (paired *t* test).

†Significant difference between sedentary males and sedentary females or between postexercise males and postexercise females (unpaired *t* test). Additional comparison of the exercise-induced changes between males and females (unpaired *t* test).

‡$P < .05$.

pigs, and the relatively small number of animals was due to the logistical limitations of the study. Exercising the pigs and studying the kinetics of HDL CE metabolism could only be achieved in a limited number of animals because these studies are cumbersome and time-consuming. These limitations allowed us to perform these studies only in small groups of animals. Nevertheless, the power of the studies appeared sufficient to detect significant effects.

We used an experimental design in which HDL CE kinetics are determined before and after the exercise protocol. However, this design did not control for a time or maturation effect, and during the 12-week period of the exercise protocol, the pigs were still growing. Thus, it is possible that changes in HDL CE kinetics after the exercise protocol have been partly caused by the maturation and growth of the pigs or by a time effect. We are not aware of data in pigs that show how growth and maturation affect lipoprotein metabolism and whether there are changes in lipoprotein metabolism in time. Plasma triglycerides decreased after the 12-week exercise protocol. This decrease is an effect of exercise at least to some extent, since exercise in pigs is associated with decreased plasma triglycerides.[8] Plasma and HDL cholesterol levels in the female pigs significantly increased after the exercise protocol. In a previous study, we found that increased plasma and HDL cholesterol levels in pigs fed a hyperlipidemic diet were associated with increased HDL CE transport rates.[20] However, in the present study, we observed a reduced transport rate after the exercise protocol, which suggests that the changes in the transport rate after the exercise protocol probably were not related to increases in plasma and HDL cholesterol due to the hyperlipidemic diet or a time effect, and that an effect of exercise probably was not overshadowed by the atherogenic diet.

Altogether, we used 4 different labeled HDL CE preparations, 2 preparations (1 for sedentary males and 1 for sedentary females) before the exercise protocol and 2 preparations after the exercise protocol (1 for exercised males and 1 for exercised females). All 4 HDL CE preparations were prepared under identical conditions and with identical procedures. In a previous study, we have shown that 2 HDL CE preparations prepared separately under the same conditions and labeled with either ^{3}H CE or ^{14}C CE had identical plasma disappearance rates in rats.[13] Further, the dose of radioactivity administered does not affect the kinetic results.[18] Even when a high dose of radioactivity is administered, the mass of the radiolabeled CE (tracer) is very small compared with the unlabeled CE in the plasma (tracee). Moreover, in a previous study with rats, we found that a 3-fold increase of the radioactive dose of administered HDL CE did not affect the plasma disappearance rate.[13] Thus, it seems unlikely that differences in the handling of the radiolabeled HDL CE preparations or differences in the dose of radioactivity affected the results of our study.

We fed the miniature swine hypercholesterolemic diets (40 energy% as hydrogenated coconut oil and 0.7 weight% cholesterol) that resulted in considerably higher plasma cholesterol (7 to 10 mmol/L) compared with miniature pigs on a non–cholesterol-supplemented, high-fat diet (approximately 2.5 mmol/L)[6] or Yucatan pigs fed a low-fat, cholesterol-free commercial swine diet (2.14 mmol/L).[20] However, plasma cholesterol was not as high as the values reported on a diet containing 2% cholesterol, 0.7% cholic acid, and 20% coconut oil (about 17 mmol/L).[20]

Total plasma cholesterol and LDL cholesterol concentrations were higher after the exercise protocol in both males and females. HDL did not change in the females after exercise, but significantly increased in the males. Several other studies in pigs failed to demonstrate significant changes in plasma and HDL cholesterol in exercising swine,[6-9] although the effects in these studies were close to statistical significance. It is possible that the changes in plasma and LDL cholesterol observed in this study after the exercise protocol were not related to exercise. Feeding hypercholesterolemic diets for a long period may result in a gradual increase of plasma cholesterol over time, irrespective of whether the pigs are exercised. In addition, the increase in HDL cholesterol observed in male pigs after the exercise protocol may be a function of whole plasma cholesterol levels, since elevated plasma cholesterol as a result of feeding atherogenic diets, in pigs also resulted in elevated HDL cholesterol.[20]

Further, the increase in total and LDL cholesterol may indicate that lipid levels in the pigs had not completely reached a steady-state situation, and kinetic studies can only be performed at steady state. However, the kinetic studies were performed for a period up to 70 hours (3 days), and it seems unlikely that during this relatively short period large changes in cholesterol levels would occur. For example, in the male pigs, there was an increase of 24% in total plasma cholesterol during the 12-week period of exercise. During a 3-day period, the duration of the kinetic studies, this increase would only be less than 1% if one assumes that cholesterol levels increased gradually. Thus, it seems likely that during this short period of the kinetic studies, a situation close to a steady state was reached.

Plasma triglycerides, which are predominantly transported in VLDL, were lower after the training protocol and lower triglyceride concentrations were associated with a longer residence time or life span of HDL CE. There was a significant negative correlation between plasma triglycerides and the HDL CE residence time ($r = -.56$, $P = .005$) when all data for sedentary and exercised males and females were combined. As discussed by Herbert et al[10] and Thompson et al,[11] exercise increases lipoprotein lipase activity, which subsequently reduces the concentration of VLDL and plasma triglycerides. During hydrolysis of VLDL and chylomicrons, lipids are transferred to HDL.[23] This transfer may result in HDL particles with a high core to surface ratio and retard HDL degradation and increase the life span.[10,11,24] Further, the decrease in plasma triglycerides after exercise may also indicate that the pigs were effectively trained.

The present study in hypercholesterolemic pigs indicates that exercise results in a lower transport rate and a longer residence time of HDL CE. Stucchi et al[8] reported similar effects of exercise on LDL apoproteins. They found that exercising Yucatan miniature swine resulted in lower transport rates for LDL_1 and LDL_2 apoproteins. In addition, the FCR of LDL_1 and LDL_2 apoproteins was also lower after training, and as a consequence, the residence times were longer. Pigs do not have CETP activity, and therefore no transfer of LDL CE to HDL can

occur.[20] In rats, which also lack CETP activity, it has been shown that LDL CE and LDL apoproteins have identical turnover rates,[25] and the same is probably true in pigs. Thus, the effects of exercise on LDL apoprotein metabolism in pigs[8] may also apply to LDL CE metabolism, and as a consequence, the transport rates for both HDL and LDL CE appear to be decreased in exercised pigs.

The prolonged residence time after exercise was associated with an increase in HDL CE in the males, but not in the females. It could be expected that a prolonged life span and a lower clearance rate of HDL CE would result in higher HDL CE levels. In female pigs, the increase in residence time after exercise was lower (33%) than in males (41%), and it is possible that this lower increase in the females was too low to produce an increase in HDL cholesterol. Further, it seems that there is no consistent relationship between the clearance of HDL CE and HDL levels. For example, studies in hamsters have shown that changes in HDL cholesterol due to dietary intervention did not effect the clearance rate of HDL CE.[26] In addition, we have previously discussed that there seems to be no relationship between HDL cholesterol concentrations and the production or disposal rate of HDL cholesterol.[19]

Kinetic studies in humans have shown that exercise results in a lower FCR for HDL apoproteins and a consequently longer residence time for HDL apoprotein particles.[10,11] However, the transport rates of HDL apoproteins in these studies were not affected by exercise. Similar results have been reported in studies with exercised rats: exercise in rats was associated with a longer survival time of HDL apoproteins, but there were no changes in the HDL apoprotein transport rate.[4] However, we studied the kinetics of the HDL CE moiety of HDL, and it should be stressed that the apoprotein and CE of HDL are clearly different metabolic entities. The CE moiety is metabolized at a considerably higher rate than the HDL apoprotein both in species with[27] and without[25] CETP activity, and the HDL apoproteins can be considered the vehicles for CE transport. We found in our studies with pigs that not only was the life span of the HDL CE prolonged after exercise but the transport rate of HDL CE was also reduced. Thus, exercise appears to reduce the transport rate of CE through the plasma HDL system but not that of the apoproteins, the vehicles for HDL CE transport. However, results from HDL CE kinetic studies in rats appear conflicting. Pels et al[4] reported that in rats, exercise increased the transport rate of HDL CE, whereas we observed a decrease in pigs. We have no clear explanation for the discrepancy between these results in rats and pigs, but species differences may play a role.

The preexercise and postexercise values for the transfer of HDL CE to LDL were significantly lower in males versus females, which may explain why the males had significantly higher HDL CE concentrations than the females. We found a significant negative correlation between the fraction of LDL CE derived from HDL and the HDL cholesterol level. The fraction of total plasma CE generated by the LCAT enzyme was similar in males and females, but in males, a smaller amount of CEs generated on HDL by the LCAT enzyme were transferred to LDL. Further, the higher HDL cholesterol levels in the males were associated with lower plasma triglycerides as compared with the females (both the pretraining and posttraining values) and there was a significant correlation between plasma triglycerides and HDL cholesterol ($r = -.478$, $P = .018$) when all results of the sedentary and exercised males and females were combined. Other studies have also consistently shown a negative relationship between HDL cholesterol and plasma triglyceride concentrations.[28]

In conclusion, the results of the present study suggest that exercise prolongs the life span of HDL CE in pigs and the transport rate of HDL CE is reduced. Other studies in humans have similarly indicated that the life span of HDL apoproteins, the vehicles for HDL CE transport, is also prolonged but the transport rate of HDL apoproteins is not changed.

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REFERENCES

1. Haskell WL: The influence of exercise on the concentrations of triglycerides and cholesterol in human plasma. Exerc Sport Sci Rev 12:205-244, 1984

2. Wood PD, Haskell WL: The effect of exercise on plasma high density lipoproteins. Lipids 14:417-427, 1979

3. Peltonen P, Marniemi E, Vuori I, et al: Changes in serum lipids, lipoproteins, and heparin releasable lipolytic enzymes during moderate physical training in man: A longitudinal study. Metabolism 30:518-526, 1981

4. Pels AE, Terpstra AHM, White TP: Endurance training increased HDL cholesteryl ester metabolism in rats. J Appl Physiol 70:1743-1747, 1991

5. Pels AE, White TP, Block WD: Effects of exercise training on plasma lipids and lipoproteins in rats. J Appl Physiol 58:612-618, 1985

6. Gross DR, van Oort G, Spiekerman M: Effects of aerobic conditioning on serum lipids in atherosclerotic miniature swine. Fed Proc 42:734, 1983

7. Pedersoli WM: Physical exercise, atherogenic diet, and serum lipids in swine. Curr Ther Res 23:464-473, 1978

8. Stucchi AF, Terpstra AHM, Foxall TL, et al: The effect of exercise on plasma lipids and LDL subclass metabolism in miniature swine. Med Sci Sports Exerc 23:552-561, 1991

9. Van Oort G, Gross DR, Spiekerman AM: Effects of eight weeks of physical conditioning on atherosclerotic plaque in swine. Am J Vet Res 48:51-55, 1987

10. Herbert PN, Bernier DN, Cullinane EM, et al: High-density lipoprotein metabolism in runners and sedentary men. JAMA 252:1034-1037, 1984

11. Thompson PD, Cullinane EM, Sady SP, et al: High density lipoprotein metabolism in endurance athletes and sedentary men. Circulation 84:140-152, 1991

12. Terpstra AHM, Pels AE: Isolation of plasma lipoproteins by a combination of differential and density gradient ultracentrifugation. Fresenius Z Anal Chem 330:149-151, 1988

13. Terpstra AHM, Nicolosi RJ, Herbert PN: In vitro incorporation of radiolabeled cholesteryl esters into high and low density lipoproteins. J Lipid Res 30:1663-1671, 1989

14. Terpstra AHM, Woodward CJH, Sanchez-Muniz FJ: Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifugation: Visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum. Anal Biochem 111:149-157, 1981

15. Assman G, Schriewer H, Schmitz G, et al: Quantification of high-density lipoprotein cholesterol by precipitation with phosphotungstic acid/$MgCl_2$. Clin Chem 29:2026-2030, 1983

16. Shipley RA, Clark RE: Tracer Methods for In Vivo Kinetics. Theory and Application. New York, NY, Academic, 1972, pp 93-110

17. Terpstra AHM: Determination of kinetic parameters of the cholesteryl esters and proteins of plasma lipoproteins. Math Comput Model 120:601-609, 1989

18. Glomset JG: Physiological role of lecithin:cholesterol acyltransferase. Am J Clin Nutr 23:1129-1136, 1970

19. Terpstra AHM, Van Tol A, Beynen AC: Is LCAT activity a determinant of HDL cholesterol concentrations? Nutrition 11:469-470, 1995

20. Terpstra AHM, Stucchi AF, Foxall TL, et al: Unidirectional transfer in vivo of high-density lipoprotein cholesteryl esters to lower-density lipoproteins in the pig, an animal species without plasma cholesteryl ester transfer protein activity. Metabolism 42:1524-1530, 1993

21. Allain CC, Poon LS, Chen CGS, et al: Enzymatic determination of total serum cholesterol. Clin Chem 20:476-482, 1974

22. Bucolo G, David H: Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 19:476-482, 1973

23. Eisenberg S: High density lipoprotein metabolism. J Lipid Res 25:1017-1058, 1984

24. Brinton EA, Eisenberg S, Breslow JL: Elevated high density lipoprotein cholesteryl levels correlate with decreased apolipoprotein A-I and A-II fractional catabolic rate in women. J Clin Invest 84:262-269, 1989

25. Terpstra AHM: Comparison of the intravascular metabolism of cholesteryl esters and apoproteins of plasma low- and high-density lipoproteins in the rat (*Rattus norvegicus*), an animal species without plasma cholesteryl ester transfer activity. Comp Biochem Physiol B Biochem Mol Biol 106:837-843, 1993

26. Woollett LA, Kearny DM, Spady DK: Diet modification alters plasma HDL cholesterol concentrations but not the transport of HDL cholesteryl esters to the liver in the hamster. J Lipid Res 38:2289-2302, 1997

27. Ha YC, Calvert GD, McIntosh G, et al: A physiological role for the esterified cholesterol transfer protein: In vivo studies in rabbits and pigs. Metabolism 30:380-383, 1981

28. Davis CE, Gordon D, LaRosa J, et al: Correlations of plasma high-density lipoprotein cholesterol levels with other plasma lipid and lipoprotein concentrations. Circulation 62:IV24-IV30, 1980 (suppl 4)