Single session exercise stimulates formation of preβ$_1$-HDL in leg muscle

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Abstract **Physical activity can raise the level of circulating HDL cholesterol. Preβ$_1$-HDL is thought to be either the initial acceptor of cellular cholesterol or virtually the first particle in the pathway of the formation of HDL from apolipoprotein A-I and cellular lipids. We have therefore sought to identify preβ$_1$-HDL in arterial and venous circulations of exercising legs in healthy individuals and in subjects with stable Type 2 diabetes mellitus. Blood samples were taken simultaneously from the femoral artery and vein before and after 25 min cycling exercise. The major findings were, first, that exercise significantly increased plasma concentration of preβ$_1$-HDL (20% increase, $P < 0.05$) and second, that the preβ$_1$-HDL concentration was significantly higher in the venous compared with the arterial blood both before and after exercise in both diabetics and controls. In the combined population, formation of preβ$_1$-HDL at rest was 9.9 ± 5.2 mg/min and exercise enhanced preβ$_1$-HDL formation 6.6-fold in both groups.**—Sviridov, D., B. Kingwell, A. Hoang, A. Dart, and P. Nestel. **Single session exercise stimulates formation of preβ$_1$-HDL in leg muscle.** ***J. Lipid Res.*** **2003.** 44: **522–526.**

Supplementary key words reverse cholesterol transport • high density lipoprotein • cholesterol • atherosclerosis

Physically active people appear to be at reduced risk of cardiovascular disease (1, 2) although the required amount of exercise is uncertain (1, 3). The protective effect has been partially attributed to the increased concentration of HDL, which is inducible with regular, moderately intensive exercise (3, 4). The effect of exercise on HDL concentration is the most consistent and by far the most pronounced effect of exercise on lipoprotein metabolism (4–6). An increase in HDL cholesterol (HDL-C) was reported even after a single bout of intensive exercise (4). The mechanisms responsible for the effect of physical activity on HDL concentration are likely to be multiple. Lipids, mainly nonesterified cholesterol and phospholipid, are transferred to HDL during the catabolism of triglyceride-rich lipoproteins (TRL), which increases with exercise though the activation of lipoprotein lipase (LPL) (7, 8). Several other components of reverse cholesterol transport (RCT) that may affect HDL concentration, such as the activity of lecithin cholesterol acyltransferase (LCAT) and cholesteryl ester transport protein (CETP), are affected by exercise (9, 10). Additional sources of HDL-C might also be derived during acute exercise from cellular cholesterol especially from exercising muscle as other lipids become utilized for fuel. Muscle triglyceride becomes depleted with prolonged endurance exercise (8). It is possible that when cells become depleted of triglyceride, cellular cholesterol is also mobilized and released to its primary acceptor, HDL. In physically fit people, the HDL-C concentration correlates strongly with lean body mass (11).

The possibility that HDL-C might be generated in an exercising muscle has been investigated by Kiens and Lithell (12), who compared a pretrained leg muscle mass with its untrained pair during an acute period of exercise. In six healthy individuals, LPL activity and the uptake of triglycerides from TRL were greater in trained muscle. Importantly, there was a significantly higher venous-arterial difference in the HDL-C concentration across the leg in the trained muscle that correlated significantly with the arterial-venous difference in VLDL triglyceride. Thus, the production of HDL-C increased in the trained leg muscle and was ascribed to degradation of VLDL. A similar conclusion was drawn by Ruys et al. (13), who observed increased production of HDL in the exercising forearm of individuals who had eaten a fat meal.

Evidence for a contribution to circulating HDL of adaptive changes in the metabolism of exercising muscle would be strengthened by demonstrating net production of the earliest and smallest HDL particle, preβ$_1$-HDL, from such muscle. Preβ$_1$-HDL is considered as the initial acceptor of cellular cholesterol during RCT, or virtually

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Abbreviations: CETP, cholesteryl ester transfer protein; LBF, leg blood flow; LPL, lipoprotein lipase; PLTP, phospholipid transfer protein; RCT, reverse cholesterol transport; TRL, triglyceride-rich lipoproteins.

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the first particle in the pathway of HDL formation (14, 15). We have therefore sought to identify preβ$_1$-HDL in arterial and venous circulations of exercising legs in healthy individuals and in subjects with stable Type 2 diabetes mellitus. We chose to include diabetic patients in whom HDL metabolism is perturbed and because dyslipidemic patients have been shown to have raised concentrations of circulating preβ$_1$-HDL (16).

MATERIALS AND METHODS

Subjects

After providing written informed consent, nine type 2, noninsulin dependent diabetic males aged 48 ± 4 (mean ± SD) and seven controls (46 ± 5 years) participated in the study which was approved by the Alfred Hospital Ethics Committee, and conducted in accordance with the Declaration of Helsinki of the World Medical Association. All subjects were nonsmokers, free of overt coronary disease (stress ECG) with a body mass index of 25.9 ± 1.0 kg · m^{-2} for controls and 28.1 ± 1.4 kg · m^{-2} for diabetics ($P = 0.25$). Control subjects did not take any medication. Of the type 2 diabetics, seven were controlled by diet and two were medicated with metformin (half-life 3.5 h). Of those medicated, one was also taking gliclazide (half-life 12 h). Medication was not taken the night before or on the morning of the studies. All were normally active but were not specifically exercise trained.

Experimental procedures

The exact experimental procedures have been described previously (17). Briefly, after an overnight fast, subjects attended the Alfred Hospital at 0800 h. Teflon catheters were placed in the right femoral artery and right femoral vein under local anesthetic (1% lignocaine, Astra, Sydney, Australia) using strict aseptic conditions. A thermistor probe was inserted though the venous catheter and advanced ~8 cm beyond the catheter tip. The catheters were used for simultaneous arterial and venous blood sampling, arterial blood pressure measurement, and for venous blood flow measurement.

After resting for ~30 min, leg blood flow (LBF) was measured, heart rate and blood pressure were recorded, and blood samples were simultaneously obtained from the two catheters. Subjects then cycled supine at a predetermined workload eliciting 60 ± 2% pulmonary oxygen uptake (VO_2) peak for 25 min. After 25 min of exercise, LBF was measured using continuous infusion thermodilution, as described previously (17).

Blood sampling and analysis

Simultaneous blood samples were drawn into EDTA tubes from the femoral artery and vein before and after 25 min of exercise. Blood was immediately placed on ice, then centrifuged at 1,500 *g* with the plasma frozen at −80°C for later analysis. It was demonstrated in preliminary experiments that under these conditions the concentration of preβ$_1$-HDL remains the same as in fresh samples and does not change for at least 1 year. These strict conditions were essential because keeping plasma at +4°C for more than 30 min as well as prolonged storage at −20°C or slow freezing lead to sharp elevation of preβ$_1$-HDL levels, probably due to decay of mature HDL particles. Plasma total cholesterol, triglycerides (TG), HDL-C, and apolipoprotein A-I (apoA-I) were measured using enzymatic spectrophotometric techniques with a Cobas-BIO centrifugal analyzer (Roche Diagnostic Systems, Basel, Switzerland). Preβ$_1$-HDL concentration was measured by ELISA (18) (Daiichi Pure Chemicals, Tokyo, Japan) (Inquiries about preβ$_1$-HDL ELISA assay and anti-preβ$_1$-HDL monoclonal antibody should be forwarded to Dr. Osamu Miyazaki, Diagnostics Research Laboratories, Daiichi Pure Chemicals Co., Ltd., 2117 Muramatsu Tokai Ibaraki 319-1182, Japan; Fax: +81-29-282-0402; e-mail: miyazaki-o@daiichichem.co.jp.)

Statistics

All results are expressed as mean ± SD. Group characteristics were compared by One Way ANOVA. Differences between parameters in venous and arterial blood and before and after exercise were analyzed by one way repeated measurements ANOVA with Bonferoni adjustment. Differences in preβ$_1$-HDL production before and after exercise were analyzed by Wilcoxon Signed Rank Test. Production of preβ$_1$-HDL was calculated by multiplying venous-arterial difference (μg/ml) by LBF (ml/min) to give a value in μg/min.

RESULTS

Measurements of preβ$_1$-HDL

Preβ$_1$-HDL concentration was expressed as apoA-I content of this subfraction, which was measured by ELISA utilizing specific anti-preβ$_1$-HDL monoclonal antibody (18). The preβ$_1$-HDL assay, as well as the specific anti-preβ$_1$-HDL antibody, has been characterized previously (18, 19). It was demonstrated that the monoclonal antibody reacts exclusively with preβ$_1$-HDL (18). Moreover, when used for the isolation of preβ$_1$-HDL the antibody completely removed preβ$_1$-HDL from human plasma and presented isolated preβ$_1$-HDL as a pure individual fraction (19). When several samples were analyzed both by ELISA and by nondenaturing two-dimensional electrophoresis, the relative abundance of preβ$_1$-HDL in plasma samples as well as differences between the samples were similar for both techniques (not shown).

The average concentration of preβ$_1$-HDL at rest found in this study was 130 ± 51 μg/ml (mean ± SD; n = 16). Although this is higher than average preβ$_1$-HDL concentration found in other healthy Australian individuals [82 ± 43 μg/ml (mean ± SD; n = 70)], it is within the range of previous measurements (13–207 μg/ml) and not dissimilar from that observed by others (20, 21). The proportion of apoA-I in the preβ$_1$-HDL subfraction (10.8%) is also similar to that found in our previous studies, when the relative concentration of preβ$_1$-HDL was measured using nondenaturing two-dimensional electrophoresis (22, 23). The reasons for higher average preβ$_1$-HDL concentration in the plasma of individuals examined in this study are not known, though they may fortuitous or related to the invasive nature of the procedure (see Materials and Methods).

The average plasma preβ$_1$-HDL concentration found in this laboratory both by ELISA and by nondenaturing two-dimensional electrophoresis as well as that reported from another laboratory using only the latter method (20, 21) is 4–5-fold higher than that reported by Miyazaki et al., using the ELISA method (18). The method was therefore cross standardized between the two laboratories (ours and that in Japan) by measuring the same plasma samples in a

TABLE 1. Concentration of HDL-C, apoA-I, TG, and preβ$_1$-HDL in arterial and venous blood at rest and after single session exercise

	Artery				Vein			
	TG	HDL-C	ApoA-I	Preβ$_1$-HDL	TG	HDL-C	ApoA-I	Preβ$_1$-HDL
	mmol/l		*mg/dl*	*μg/ml*	*mmol/l*		*mg/dl*	*μg/ml*
Rest								
control	1.0 ± 0.1	1.11 ± 0.28	131 ± 10	131 ± 51	1.0 ± 0.1	1.17 ± 0.17	128 ± 12	150 ± 33
diabetics	1.4 ± 0.4[a]	0.89 ± 0.13	115 ± 8[a]	129 ± 53	1.4 ± 0.4[a]	0.87 ± 0.19[a]	117 ± 13	162 ± 64
Exercise								
control	1.1 ± 0.1	1.18 ± 0.12	132 ± 13	150 ± 29	1.1 ± 0.1	1.17 ± 0.21	134 ± 12	161 ± 58
diabetics	1.5 ± 0.5[a]	0.95 ± 0.22	119 ± 8[a]	163 ± 51	1.5 ± 0.5	0.95 ± 0.10[a]	120 ± 8[a]	193 ± 71

Blood samples were collected from femoral artery and vein of seven healthy controls and nine diabetic subjects at rest and after 25 min exercise and concentrations of TG, HDL-C, apoA-I, and preβ$_1$-HDL were determined as described in Materials and Methods. Means ± SD are given.

[a] $P < 0.03$; versus healthy controls (Bonferroni One Way ANOVA).

blinded study. Similar values were reported in both laboratories, excluding methodological variations and confirming good reproducibility. We hypothesize that the differences observed partly reflect ethnic differences between Australian and Japanese populations.

Formation of preβ$_1$-HDL in leg muscle

Plasma concentrations of total cholesterol, triglycerides, HDL-C, apoA-I, and preβ$_1$-HDL in blood taken from the femoral artery and vein before and after exercise were compared. Plasma total cholesterol concentration was 4.6 ± 0.7 mmol/l for controls and 4.1 ± 0.7 mmol/l for diabetics ($P = 0.22$). Compared with controls, the concentrations of apoA-I and HDL-C in both venous and arterial blood were lower in the diabetics whereas the concentration of triglycerides was higher; there was no statistically significant difference in preβ$_1$-HDL concentrations (**Table 1**). To study the response of lipid parameters to exercise, data from control and diabetic patients were combined. This was justified by the finding that both groups exercised at the same absolute and relative workload and changes in lipid parameters in response to exercise were similar in both groups.

The concentration of preβ$_1$-HDL on the venous side was significantly higher than on the arterial side at rest ($P < 0.05$) (**Table 2**, **Fig. 1**), showing that preβ$_1$-HDL is formed during passage of blood from artery to vein. The magnitude of preβ$_1$-HDL formation at rest was calculated from the rate of blood flow and the venous-arterial difference in preβ$_1$-HDL concentration. Leg blood flow (LBF) was 403 ± 41 ml/min at rest and 3,053 ± 210 ml/min after exercise (mean ± SEM; n = 16). The formation of preβ$_1$-HDL during passage from artery to vein at rest was estimated as 9.9 ± 5.2 mg/min.

Effect of exercise on preβ$_1$-HDL formation and its plasma levels

Acute exercise stimulated the formation of preβ$_1$-HDL by 6.6-fold when the increase in flows is considered (**Fig. 2**). There was no difference between nondiabetic and diabetic patients in the ability to generate preβ$_1$-HDL ($P = 0.2$). No statistically significant difference between arterial and venous concentrations of triglycerides, HDL-C, and apoA-I was observed (Tables 1, 2).

A single bout of moderate exercise raised the concentration of preβ$_1$-HDL in both arterial and venous blood both in absolute terms and as a proportion of total apoA-I ($P < 0.04$) (Tables 1, 2). A small increase of apoA-I concentration (3–4%) was also observed, which reached statistical significance in venous ($P < 0.01$), but not in arterial blood (Table 2). Exercise did not have a statistically significant effect on the levels of HDL-C or triglycerides in either venous or arterial blood, although there was a tendency to higher HDL-C levels after exercise (Tables 1, 2).

TABLE 2. Effect of acute exercise on concentration of HDL-C, apoA-I, and preβ$_1$-HDL in arterial and venous blood

	Artery				Vein			
	HDL-C	ApoA-I	Preβ$_1$-HDL		HDL-C	ApoA-I	Preβ$_1$-HDL	
	mmol/l	*mg/dl*	*μg/ml*	*% of apoA-I*	*mmol/l*	*mg/dl*	*μg/ml*	*% of apoA-I*
Rest	0.99 ± 0.24	122 ± 13	130 ± 51	10.8 ± 4.7	1.00 ± 0.23	122 ± 14	154 ± 51[b]	12.9 ± 4.8[b]
Exercise	1.05 ± 0.35	125 ± 16	160 ± 48[a]	12.8 ± 4.3[a]	1.04 ± 0.30	126 ± 15[a]	179 ± 66[a,b]	14.3 ± 5.7[a,b]

Blood samples were collected from femoral artery and vein of 16 subjects before and after 25 min exercise and concentrations of HDL-C, apoA-I, and preβ$_1$-HDL were determined as described in Materials and Methods. Mean ± SD are given.

[a] $P < 0.04$, rest versus exercise.

[b] $P < 0.05$, vein versus artery (Bonferroni One Way Repeat Measurements ANOVA).

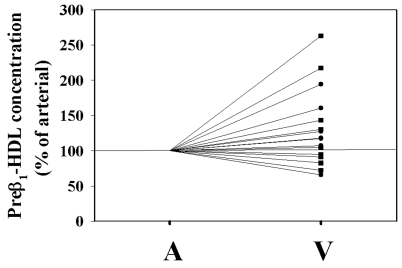

Fig. 1. Arterio-venous difference in preβ$_1$-HDL concentration at rest. Preβ$_1$-HDL concentration in venous blood (V) was calculated as a percentage of that in arterial blood (A) for seven healthy controls (circle) and nine diabetic subjects (square).

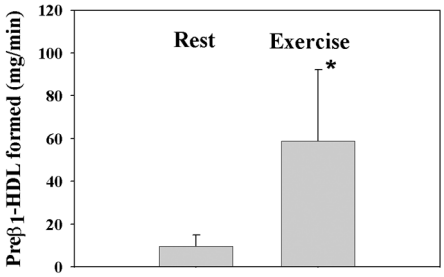

Fig. 2. Formation of preβ$_1$-HDL during passage from artery to vein at rest and after a 25 min bout of exercise at 60% VO_2 peak. Preβ$_1$-HDL formation was calculated as preβ$_1$-HDL concentration in the vein minus preβ$_1$-HDL concentration in artery multiplied by leg blood flow. *$P < 0.03$ (Wilcoxon Signed Rank test, n = 16). Mean ± SEM are shown.

DISCUSSION

The major finding of this paper is that a substantial amount of preβ$_1$-HDL was generated during passage from the femoral artery to the femoral vein. The formation of preβ$_1$-HDL increased dramatically after a single bout of exercise. This suggests that a significant proportion of preβ$_1$-HDL, and consequently of mature HDL, might be formed extra-hepatically and its formation responds to a physiological stimulus such as exercise. The amount of preβ$_1$-HDL synthesized at rest was 9.9 mg/min, which would translate into 14.2 g of preβ$_1$-HDL a day. This may be an underestimate to the extent that some preβ$_1$-HDL may leave the tissue by way of lymph. Nanjee et al. (24) have found particles resembling preβ$_1$-HDL in the leg lymph, suggesting that it was formed extrahepatically. The rate of apoA-I synthesis in humans has been estimated to be about 700 mg/day (25, 26). Thus, if preβ$_1$-HDL is the sole precursor of HDL, and one leg represents about one-third of the total body muscle mass, then it follows that there is substantial recycling of apoA-I between nascent and mature HDL particles. This would be consistent with the recognized cycle of nascent to mature to nascent HDL mediated by CETP, PLTP, and hepatic lipase on the catabolic side and cholesterol efflux and LCAT on the anabolic side (16). Our data identify a major contributor to the anabolic phase, to which are added nascent HDL from liver and possibly other tissues. Muscat et al. (27) have recently suggested that muscle is a potential site for reverse cholesterol transport and may contribute to the control of HDL levels. The high rate of cycling appears to exceed significantly the net turnover of apoA-I and may be analogous to the higher rate of turnover of esterified cholesterol in plasma that reflects the activity of LCAT and exceeds that of total cholesterol net turnover (28). Contributing to this high flux of preβ$_1$-HDL may be a possible defect of removal of this particle from plasma. Chetiveaux et al. (29) have also demonstrated the existence of a separate pool of preβ-HDL with kinetic parameters different from α-HDL. If formation of preβ$_1$-HDL and remodeling of preβ$_1$-HDL to α-HDL is accompanied by cholesterol efflux (16), that may represent a significant contribution to reverse cholesterol transport. However, it cannot be excluded that HDL may also be formed directly without the conversion from intermediate preβ$_1$-HDL.

The importance of preβ$_1$-HDL is that it is a metabolically active particle in the initial process of removal of cholesterol from cells (reverse cholesterol transport) (14, 16). It was suggested that preβ$_1$-HDL may be an initial acceptor of cellular cholesterol during cholesterol efflux (14, 30) and/or a first product of lipidation of lipid-free apoA-I by ABCA1-dependent formation of HDL (16). The role of preβ$_1$-HDL as a precursor of mature HDL has been strengthened by a recent finding of a greater rate of incorporation of newly synthesized apoA-I into preβ-HDL (29). Its concentration increases with dyslipidemia (31) and with overweight (22). Whether these conditions are associated with increased efflux of cholesterol from cells, increased catabolism of triglyceride-rich lipoproteins (both of which generate more HDL-C) or through inefficient conversion of preβ$_1$-HDL particles to mature HDL is not known. The enhanced formation of preβ$_1$-HDL after a single bout of exercise may be related to metabolic events in highly active muscle and/or increased flow of blood though the muscles. Our data are generally consistent with those reported by Kiens and Lithel (12) and Ruys et al. (13) who demonstrated formation of HDL during passage of blood though muscle. However, in those papers HDL formation was accompanied by a rise in HDL-C and was apparently linked to increased lipolysis of triglyceride-rich lipoproteins that supply fatty acids to exercising muscle. In our studies, neither triglycerides nor HDL-C levels changed significantly. It must be noted, however, that even if all preβ$_1$-HDL was formed as a result of remodeling of HDL and/or triglyceride-rich lipoproteins, that would account for less than 1.5% change in the concentration of triglyceride and HDL-C; the change would not necessarily be detected by the methods employed.

In conclusion, we have demonstrated that preβ$_1$-HDL is formed during passage of blood though muscle and this process is stimulated by exercise.

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