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The N-terminal structural domain of apolipoprotein A-V forms a helix-bundle

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Superphospholipidation of Human Plasma High-Density Lipoproteins Enhances Multiple Steps in Reverse Cholesterol Transport

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Introduction: HDL-phosphatidylcholines (PC) comprise the essential cholesterol-binding component of lipoproteins and are the acyl donor in the esterification of cholesterol by lecithin:cholesterol acyltransferase. Importantly, cholesterol efflux from macrophages to human sera is a positive function of HDL-PL. Methods and Results: We developed and tested a new method, HDL superphospholipidation (SPLn) using a modified detergent removal method that incorporates 1-palmitoyl-2-oleoyl PC into HDL thereby increasing HDL-PC by 1000%; this occurs with no loss of lipid-free apo A-I. According to size exclusion chromatography and native gradient gel electrophoresis, SPLn increases the HDL particle weight in a dose dependent way, from ~120 kDa to ~300 kDa. Kinetic analysis of cellular cholesterol efflux to the SPLn HDL shows that K_m and V_{max} for SPLn HDL are lower and higher respectively than for native HDL; the catalytic efficiency, K_m/V_{max} , increases linearly by more than 400%. Relative to HDL, SPLn increases the rate of cholesteryl ester formation by LCAT by 100 and 900% in the absence and presence of exogenous free cholesterol. Conclusion: Given the clinically important observation that small increases in serum HDL-PL are associated with profound increases in cholesterol efflux to serum and that SPLn of small amounts of plasma improve at least two RCT steps-efflux and esterification, SPLn is a potential new therapeutic modality for enhancing RCT and providing cardioprotection.

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Cyclic Phosphatidate Is a Second Messenger that Negatively Regulates the Nuclear Hormone Receptor PPARγ

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The current hypothesis for PPARγ regulation suggests that endogenous agonists activate this transcription factor to control gene transcription. Here we show that activation of phospholipase D2 (PLD2) generates 1-acyl-2,3-cyclic-glycerophosphate (CPA), a novel lipid second messenger and a high-affinity antagonist of PPARγ. Stimulation of PLD2 *de novo* generates CPA, which in turn attenuates PPARγ-dependent gene expression and lipid accumulation in the RAW 264.7 macrophage cell line and prevents neointima formation in a model of arterial wall remodeling. CPA represents the first identified physiological inhibitor of PPARγ, suggesting that PPARγ's activity is under the control of not only endogenous agonists but antagonists as well. The present data challenge this view by providing evidence that CPA, a novel lipid second messenger, is a negative regulator of PPARγ activity and modulates cellular responses controlled by the nuclear hormone receptor involved in diseases including neointima formation and lipid uptake. Individual variations in the activation of the PLD2-CPA axis are likely also modulate the therapeutic responses to the widely used class of TZD drugs targeting PPARγ. ACKNOWLEDGMENTS This work was supported by United States Public Health Service Grants CA92160 (to G.T.), HL61469 (to G. T.), HL79004 (to G. T.) and the American Heart Association Grants 50006N, 355199B (to A. P.), and 0525489B (to T. T.), and National Science Foundation Grant CHE0353885 (to A. P.).

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Triglyceride Reduces Lysosomal Sterol Accumulation and Restores Lysosome Function in Cholesteryl Ester-Rich Macrophage Foam Cells

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Atherosclerosis is characterized by progressive thickening of the artery wall due, in part, to the presence of cholesterol-engorged macrophage foam cells. We have shown that in late-stage disease, much of the foam cell sterol accumulates within large, swollen lysosomes. Human macrophages incubated in culture with modified LDLs mimic the lysosomal lipid accumulation and possess large concentrations of both free (FC) and esterified (CE) cholesterol. The sterol in lysosomes is trapped and can disrupt lysosome and overall macrophage function. In atherosclerosis, however, foam cells are also exposed to triglyceride (TG)-rich particles (TRPs). Little is known about how TRPs affect foam cell cholesterol metabolism. We have incubated sterol-engorged macrophages with TRP and find a dose dependent increase in sterol clearance from lysosomes and increased cellular cholesterol efflux. This suggests that TG enhances CE-particle degradation and stimulates mobilization of sterol from lysosomes. We show that cholesterol accumulation inhibits lysosomal function by increasing the lysosome pH and that TG-enrichment returns the lysosome to more normal pH resulting in enhanced CE degradation and clearance. Previously, we have shown that sterol-induced lysosomal neutralization was due, in part, to a FC-dependent loss of lysosomal v-ATPase activity. We suggest that increased lysosomal membrane FC induced an increase in membrane order resulting in inactivation of v-ATPases and this leads to the increased pH. Here we show that TG can reverse this. We analyzed changes in membrane order (via electron paramagnetic resonance) and v-ATPase activity when lysosomal sterol is increased. There was an increase in membrane order with sterol accumulation. Our results indicate that lysosomal FC-engorgement disrupts lysosome function, in part, by altering v-ATPase activity through changes in membrane order and that TG can restore lysosome function and cellular sterol clearance by altering lysosome properties. Thus, TRP are potential modulators of foam cell sterol accumulation and atherogenesis.

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ApoA-IV Expression Alters the Proximal-Distal Gradient of Intestinal Triglyceride Absorption Efficiency

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Background: Studies in IPEC1 intestinal cells have established that apoA-IV expression increases transcellular triglyceride (TG) transport by enabling secretion of larger TG-rich lipoproteins; yet post-prandial plasma TG curves and fat balance studies have found that TG absorption is normal in apoA-IV knockout (A4KO) mice. However, because cellular TG absorption is integrated longitudinally along the small intestine, an effect on segmental TG absorption efficiency could mask expression of a phenotype in A4KO mice. **Hypothesis**: Intestinal apoA-IV expression alters the proximal-distal gradient of TG absorption efficiency. **Methods**: Segmental lipid transport in A4KO and C57BL6 mice was examined using everted gut sacs. Animals were maintained on chow, fasted overnight, and sacrificed. The small intestine was dissected free and divided into four equal segments which were everted, filled with buffer, and incubated in an oxygenated solution of mixed micelles containing [3H] oleic acid. At timed intervals the sacs were removed, flushed, and homogenized. [3H] in the luminal washes and tissue homogenates was assayed; tissue fatty acid uptake and transport were calculated as nmole/g tissue. **Results:** Segmental tissue fatty acid uptake in C57BL6 and A4KO mice was the same at all times. Transmucosal fatty acid transport into serosal fluid is given in the table below. **Conclusions:** In A4KO mice fatty acid transport efficiency was reduced in the proximal jejunum at 90 min, but was paradoxically increased at 60 min in the distal jejunum. This early distal shift in the normal proximal>>distal absorption efficiency gradient in A4KO mice could explain why whole animal studies have failed to observe a malabsorption phenotype. The ability of apoA-IV to shift lipid absorption proximally could provide a reserve absorptive capacity that becomes critical under conditions of high lipid loads (e.g., in nursing neonates) or when mucosal function is compromised (e.g., disease or infection).

	Segment 1 (duodenum)	Segment 2 (proximal jejunum)	Segment 3 (distal jejunum)	Segment 4 (ileum)
A4KO (60 min)	94±16	151± 24	137±27[A]	23±4
C57BL6 (60 min)	61± 6	150± 36	74± 13	22±2
A4KO (90 min)	149±39	230±29[B]	146± 29	43±4
C57BL6 (90 min)	150±46	385± 62	148± 12	30±4

Means ± SE; N = 4 to 12; A, p=0.041 A4KO v C57 segment 3 @ 60 min; B, p=0.046 A4KO v C57 segment 2 @ 90 min

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The N-Terminal Structural Domain of Apolipoprotein A-V Forms a Helix Bundle

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Apolipoprotein (apo) A-V has been shown to be a potent regulator of plasma triacylglycerol (TG) levels. Recent findings indicate that, similar to apoA-I and apoE, apoA-V is comprised of two independently folded structural domains. The boundary between these two structural domains was elucidated using limited proteolysis, mass spectrometry analysis, and N-terminal sequencing. In the present study, we hypothesize that apoA-V possesses an N-terminal four-helix bundle structural motif. Based on primary structure analysis, a recombinant C-terminal truncated apoA-V variant was constructed, expressed, and purified. Unlike full-length apoA-V, this variant, apoA-V (1–146) is soluble at neutral pH and is resistant to limited proteolysis. Far UV circular dichroism (CD) spectroscopy analysis revealed that lipid-free apoA-V (1–146) contains ~70% α-helix secondary structure. Inclusion of the helix-inducing cosolvent, trifluoroethanol, did not significantly change the far UV CD spectrum of lipid-free apoA-V (1–146). Guanidine-HCl denaturation studies conducted as a function of solution pH, indicate apoA-V (1–146) undergoes a one step, native to unfolded transition, with a midpoint at ~ 2.0 M guanidine-HCl (pH 3.0) or ~1.0 M guanidine-HCl (pH 7.4). Fluorescent dye binding experiments showed that lipid-free apoA-V (1–146) has less exposed hydrophobic surface compared to lipid-free full-length apoA-V and, at neutral pH, has fewer exposed hydrophobic sites than at pH 3.0. Single tryptophan variants of apoA-V (1–146) with Trp residues strategically engineered in putative α-helices, or putative linker-regions between helices, were constructed for fluorescence quenching studies. ApoA-V (1–146) W@5 was highly susceptible to quenching by both KI and acrylamide. On the other hand, two other single Trp variants (W@73 and W@97) were less accessible, suggesting these Trp residues are on non-polar faces of amphipathic helices. In conclusion, our data supports that the N-terminal domain of apoA-V forms a helix bundle. The concept that apoA-V contains a helix bundle motif suggests conformational changes occur upon lipid interaction that may affect interactions with cell surface molecules related to its function in TG metabolism.

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Lp-PLA2 Promotes the Uptake of LDL in HepG2 Cells

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Lipoprotein-associated phospholipase A2 (Lp-PLA2) has been recognized in many population studies as an independent risk factor for cardiovascular and cerebrovascular disease. However,