Adipose Tissue ATP Binding Cassette Transporter A1 Contributes to High-Density Lipoprotein Biogenesis In Vivo

Soonkyu Chung, PhD; Janet K. Sawyer, MS; Abraham K. Gebre, MS; Nobuyu Maeda, PhD; John S. Parks, PhD

Background—Adipose tissue (AT) is the body’s largest free cholesterol reservoir and abundantly expresses ATP binding cassette transporter A1 (ABCA1), a key cholesterol transporter for high-density lipoprotein (HDL) biogenesis. However, the extent to which AT ABCA1 expression contributes to HDL biogenesis in vivo is unknown.

Methods and Results—Adipocyte-specific ABCA1 knockout mice (ABCA1⁻/⁻) were generated by crossing ABCA1floxed mice with aP2 Cre transgenic mice. AT from ABCA1⁻/⁻ mice had <10% of wild-type ABCA1 protein expression but normal hepatic and intestinal expression. Deletion of adipocyte ABCA1 resulted in a significant decrease in plasma HDL cholesterol (∼15%) and apolipoprotein A-I (∼13%) concentrations. AT from ABCA1⁻/⁻ mice had a 2-fold increase in free cholesterol content compared with wild-type mice and failed to efflux cholesterol to apolipoprotein A-I. However, cholesterol efflux from AT to plasma HDL was similar for both genotypes of mice. Incubation of wild-type AT explants with apolipoprotein A-I resulted in the formation of multiple discrete-sized nascent HDL particles ranging in diameter from 7.1 to 12 nm; similar incubations with ABCA1⁻/⁻ AT explants resulted in nascent HDL <8 nm. Plasma decay and tissue uptake of wild-type ¹²⁵I-HDL tracer were similar in both genotypes of recipient mice, suggesting that adipocyte ABCA1 deficiency reduces plasma HDL concentrations solely by reducing nascent HDL particle formation.

Conclusions—We provide in vivo evidence that AT ABCA1-dependent cholesterol efflux and nascent HDL particle formation contribute to systemic HDL biogenesis and that AT ABCA1 expression plays an important role in adipocyte cholesterol homeostasis. (Circulation. 2011;124:1663-1672.)

Key Words: adipose tissue ▪ adipocytes ▪ apolipoproteins ▪ cholesterol ▪ lipids ▪ lipoproteins
vivo to plasma HDL formation. For example, macrophage ABCA1 expression is abundant, but it has no significant impact on plasma HDL levels. On the other hand, hepatic and intestinal ABCA1 expression is estimated to contribute 70% to 80% and 15% to 20% of the plasma HDL pool, respectively. Intriguingly, deletion of both hepatic and intestinal ABCA1 resulted in ~90% reduction in plasma HDL, whereas global ABCA1 knockout (KO) mice have <5% of normal levels of HDL cholesterol, similar to subjects with Tangier disease. These results suggest that ABCA1 expression in cells other than hepatocytes and intestinal epithelial cells would minimally affect plasma HDL formation. However, formal testing of the contribution of a cell type to the plasma HDL pool requires specific deletion of ABCA1 in that cell.

AT is a major storage pool of FC that expands during obesity and abundantly expresses ABCA1, but the role of adipocyte ABCA1 expression in FC efflux and HDL formation in vivo is unknown. AT has been proposed as being important in plasma HDL production on the basis of indirect evidence such as an inverse association between adipose cholesterol content and plasma cholesterol concentrations. A recent study provided in vivo evidence that adipocytes support the transfer of FC to plasma HDL. In that study, mouse embryonic fibroblasts (from wild-type [WT], ABCA1 KO, and scavenger receptor class B type I [SR-BI] KO mice) were differentiated into adipocytes, radiolabeled with 3H-cholesterol, and injected into the peritoneal cavity of mice. Reverse cholesterol transport of 3H-cholesterol from adipocytes to the feces depended on adipocyte expression of ABCA1 and SR-B1. However, direct in vivo evidence for the role of adipocyte ABCA1 in plasma HDL formation remains lacking, and the extent to which adipose ABCA1 expression contributes to plasma HDL, particularly in the obese state, has not been investigated.

The purpose of this study was to directly test the role of adipocyte ABCA1 in the formation of nascent HDL particles and its contribution to the plasma HDL pool in vivo. To accomplish this, we generated adipocyte-specific ABCA1 KO (ABCA1-AT−/−) mice and investigated their plasma lipid and lipoprotein phenotypes during chow and high-fat (HF) diet feeding. We also determined the role of ABCA1 in adipocyte cholesterol homeostasis and nascent HDL particle formation using AT explants from these mice. Our results suggest a significant role for AT ABCA1 expression in nascent HDL particle formation and AT cholesterol homeostasis.

Methods

Animals

Adipocyte-specific ABCA1 KO mice (ABCA1-AT−/−) were generated by crossing ABCA1floxed mice (backcrossed into the >99% C57BL/6 background) with fatty acid binding protein 4 (aP2) Cre transgenic mice (Jackson Laboratories). For all experiments, 10- to 16-week-old male mice were used. To expand AT mass, mice were switched from a commercial chow diet at 8 weeks of age to a commercial HF diet (42% calories from fat, 0.2% cholesterol; TD 88137, Harlan) for an additional 8 to 16 weeks, depending on the experiment. The mice were housed in the Wake Forest University Health Sciences animal care facilities with a 12-hour light/12-hour dark cycle. All protocols and procedures were approved by the Wake Forest University Health Sciences Animal Care and Use Committee.

Plasma Cholesterol Determination and Fast Protein Liquid Chromatography

Plasma was collected by tail bleeding of mice fasted for 4 hours. Plasma from 4 mice was pooled and size fractionation by fast protein liquid chromatography with a Superose 6 TM column (Amersham Biosciences). Plasma cholesterol was determined with an enzymatic colorimetric assay kit (Roche Applied Science).

Quantitative Polymerase Chain Reaction and Western Blot Analysis

Total RNA was extracted by Trizol (Invitrogen) according to the manufacturer’s protocol. cDNA preparation and quantitative polymerase chain reaction were conducted as described previously. To prepare tissue lysate, ~0.1 g snap-frozen tissue was homogenized in radioimmunoprecipitation assay buffer. For AT, the lysate was incubated on ice for 10 minutes to remove the solidified fat cake on top. Immunoblotting of ABCA1 was conducted as previously described with 4% to 16% SDS-PAGE. Monoclonal antibodies directed against PPARγ and GAPDH were purchased from Sigma. Polyclonal antibodies against apoE, apoA-I (Biodesign), and anti-SR-B-I (Novartis) were also used for Western blotting.

Adipose Cholesterol Determination

AT cholesterol was determined by gas-liquid chromatography after extraction of total lipid from 0.1 g AT with chloroform:methanol (2:1). Data were normalized to tissue wet weight or protein, measured by the Lowry assay.

Preparation of Ex Vivo Cultures of AT

To establish the ex vivo cultures of AT, freshly isolated epididymal fat (0.1–0.2 g) was minced into ~2-mm pieces, moved into 10-cm cell culture dishes, and incubated overnight in 1.5 mL serum-free Dulbecco Modified Eagles Medium F12 Ham (Santa Cruz). The next day, AT cultures were washed thoroughly with caution to remove floating cells without aspirating the minced tissue explants.

Cholesterol Efflux From AT

Measurement of cholesterol efflux was conducted according to standard methods, except ex vivo AT cultures were used. Briefly, ex vivo cultures of AT (pooled from n=4 mice per genotype; n=4 replicates) were incubated with 5 μCi of [3H]-cholesterol (complexed to bovine serum albumin) for 24 hours. AT explants were then washed carefully, and 1 mL fresh serum-free medium was added. Cholesterol efflux was initiated by adding either 20 μg protein/mL of human apoA-I (ABCA1-dependent efflux) or 50 μg protein/mL mouse HDL (ABCA1-independent efflux) as acceptors. After 4 hours of additional incubation, cholesterol efflux was quantified by measuring [3H]-cholesterol in 500 μL medium and in the cells after isopropanol extraction. Percentage efflux was calculated as follows: dpm [3H]-cholesterol in medium/dpm [3H]-cholesterol (medium + cells) × 100%.

Nascent HDL Formation

All the procedures for nascent HDL formation were similar to our previous study. Briefly, 10 μg/mL lipid-free [125I]-apoA-I (105 cpm/μg) was incubated with AT explants for 24 hours. Conditioned medium (1 mL) was then fractionated by high-resolution fast protein liquid chromatography size–exclusion chromatography with 3 Superdex 200 HR columns in series. 125I radioactivity in each fraction was quantified with a gamma counter (Beckman). Electrophoretic mobility of adipose-derived nascent HDLs was analyzed with a Paragon lipoprotein agarose gel electrophoresis system (Beckman) according to the manufacturer’s instructions. Electrophoretic mobilities (α, β, pre-β) were determined after lipid staining of plasma from low-density lipoprotein receptor KO mice. The size distribution of nascent HDL particles in conditioned medium was also deter-
HDLCatabolismandTissue-SpecificUptake

125I-radiolabeledtyraminecellobiose(TC)-HDL ([125I]-TC-HDL) waspreparedaspreviouslydescribed,exceptHDLandisolatedfrom
WTmouseplasma.15,21Recipientmice(n=4pergenotype)were
anesthetizedwithisoflurane,andalso-[125I]-TC-HDL(500 000cpm/
animal)wasinjectedviaajugularvenocatheter.Bloodsamples(~30
μL)weredrawnat5minutes,30minutes,and1,3,6and24hours
aftertracerejectionbyretro-orbitalbleeding,and15μLplasmaswase
usedforquantificationof[125I]radioactivity.Datawereexpressedasa
percentage of the 5-minute plasma radioactivity remaining in
plasmaafterinjectionofthetracer.Thedescriptionofdiseases
weregeneratedwithabiexponentialcurve-fittingprogram(Graph-
Pad Prism 5; SanDiego,CA).Forthedeterminationoftissue-
specificuptakeofHDL,micewereeuthanized24hoursafter
[125I]-TC-HDLinjection,andalivers,kidney,andeipidymalfatpads
wereremovetoreally125Iradioactivity.

Statistics

Resultsarepresentedasmean±SEM.Datawereanalyzedstatisti-
callybythemeanofmultipletests.Agenerallinearmodel
wasusedtoassessrelationshipsbetweenFCandABCA1within
acrossfatdepotwhilecontrollingforintra-animalcorrelations.A
Student t test was used to test for differences in AT ABCA1
expressionandradiolabeledHDLtrajectoriespecificuptakebetweenWT
andABCA1−/−mice.Forcollectionsofvariables(ietotal
plasmacholesterol,epididymalfatcholesterolcontent)amongall3
genotypesofmiceorto2treatments(cholesterolefflux),1-way
ANOVA,followedbytheTukeymultiplecomparisontesttoidentify
individualgroupdifferences,wasused.Two-wayANOVAwith
repeatmeasures(timescourse,0–16weeks)wasusedtocompare
totalplasmacholesterolconcentrationsduringHFdietfeeding
betweenWTandABCA1−/−mice.Statisticaltestswerper-
formedwithGraphPadsoftwareorSASsoftware.

Results

ABCA1ExpressionVariesAmongFatDepots

ATexhibitsregionalheterogeneitywithregardtoacellular
composition,responsetoendocrinehormonalsignals,ametabolicfunc-
tion.22Togaininsightintotherolesoffatdepositspecific
cholesterolregulationbyABCA1,C57BL/6mice(n=3)were
fedanHF(HF/high-cholesterol)diet(42%fatand0.2%chole-
terol)toincreasemass.Afterthemicedidconsumethediet
for8weeks,wcmeasuredcholesterolcontentbygas-liquid
chromatographyandrelativetissueABCA1expressionbyWestern
blotindifferentfatdepots.Therewasafoldvariationsinfat
content nocholesteryl ester)amongfat depots (epididymal>mesenteric>pericardial=inguinalepiscrotuse)
brinat).ABCA1expressionwascorrelativelyvariableamong
depots,withaslightlypositivecorrelation(2=0.34,P=0.01)
betweendepotcholesterolcontentandABCA1expression,not
SR-BIorapoEexpression(Figure1Aand1B).

Next,weneatGATABCA1expressionusingepidid-
ymal fat depots from C57BL/6 mice. There are conflicting
results in the literature on the extent to which 3T3-L1
fibroblasts express ABCA1 before they are differentiated into
adipocytes.17,19TomindeterminewhetherABCA1isexpressed
innonadipocytecellsrresidinginAT,stromalvascularcellswere
fractionatedfromfloatingmatureadipocyteswiththe
useofpreviouslydescribedmethods.23ABCA1expression
wasalmostexclusivelyconfinedtoadipocytes,withlittle
expressioninstromalvascularcellsonline-onlyDatasup-
plemen).Toestablishaprimadipocyteculturemensystem
frommouseAT,isolatedstromalvascularcellswereincu-
batedwithadifferentiationcocktail.23After10days,~60%
of
wasconvertedtolipiddistributedadipocytesbyOilRed
staining(Figure1Aintheonline-onlyDataSupplement).
ABCA1expressionlevelswerealsoincreasedduringadipo-
gensis(Figure1D).Todeterminecellularlocationof
ABCA1,adipocytecultureswereimmunostained
withABCA1monoclonalantibody.ABCA1stainingwasobserved
intheplasmamembraneandcytosolofadipocytes,
suggestingthatABCA1maytrafficbetweentheplasma
membraneandcytosol(Figure1bintheonline-onlyData
Supplement).

ATGeneratesNascentHDLParticlesof
MultipleSizes

ABCAdependentcholesterolmigrationanditsimpactonHDL
plasmaproductivitywasstudiedbyin vivoisolatingAC
ATplasmachangesbymonitoringapoA-Iintheplasma
(Figure1E).Moreimportant,thesizetopositionof
nascent HDL species from AT followed a pattern similar
thatfromHEK293-ABCA1−/−miceexainingheterogenouslysized
nascent HDL particles that migrated in the pre-β
positiononagarose
(Figure1E).Beyond,thesizedistribution
ofnascentHDLspeciesfromATfollowedapatternsimilar
tothatfromHEK293-ABCA1−/−miceexpressing
(Figure1F)andmousetongdo
cellsandMcArdle7777hepatoma
cells,11with5distinctsubfractions
inthevantosegment
1665
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DeletionofAdipocyteABCA1DecreasesPlasma
HDLLevels

ToinvestigatetherolesofadipocyteABCA1inATchole-
terolmobilizationanditsimpactonHDLplasmalevel
in vivo,wegeneratedadipocyte-specificABCA1KO
mice(ABCA1−/−).Adipocyte-specificABCA1inactivation
wasachievedbycrossingC57BL/6ABCA1flox/floxxenics
withap2(fattyacidbindingprotein4)-Cre
transgenicscavengedbackcrossed
theC57BL/6background(JacksonLaboratories).
ABCA1−/−mice were fertile, and pups were born at
theexpectedmendelianfrequency.Recombinationofthe
floxedABCA1allelincreasedinade>90%decreaseinABCA1
proteinexpressioninABCA1−/−miceepididymalfat
and87%loweringofABCA1mRNAinepididymal
Fat(Figure2A).InactivationofadipocyteABCA1hadlofeffect
onABCA1expressioninaliverandintestine,whicharethe
mainorganstobeknowntobeimportantforHDL
biogenesis(Figure2B).However,ABCA1expressionwasnoticeably
reducedinresidentperitonealmacrophages(Figure2C),
thioglycolate-elicitedmacrophages(Figure2D),and,toa
lesserextent,inbone marrow-derivedmacrophages(Figure
2C).ABCA1expressioninWCATwasabundantandcomparable
tothatinliverand

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macrophages, but its expression was markedly blunted in ABCA1−/−A mouse epididymal, brown, and mesenteric fat (Figure 2D). Thus, deletion of ABCA1 expression was highly efficient in AT and somewhat variable in macrophages. However, macrophage ABCA1 expression has minimal impact on plasma HDL levels,14,25–27 so we proceeded with studies on the role of adipocyte ABCA1 expression on HDL metabolism and adipocyte cholesterol balance.

Next, we evaluated the contribution of adipose ABCA1 expression on plasma cholesterol concentrations and distribution using WT and ABCA1−/−A mice consuming chow or an HF diet. There was a stepwise reduction in total plasma cholesterol with successive deletion of ABCA1 alleles (WT, 83±3 mg/dL; heterozygotes, 70±6 mg/dL; homozygotes, 62±4 mg/dL; Figure 3A) that reached statistical significance for male WT versus ABCA1−/−A mice (P<0.0084 by ANOVA with the Tukey post hoc test). In addition, Western blot analysis of plasma suggested a reduction in apoA-I (KO/WT ratio 0.72) and apoE (KO/WT ratio 0.86) concentration but not lecithin-cholesterol acyltransferase (KO/WT ratio = 1.03) (Figure 3B). The lower plasma cholesterol levels for ABCA1−/−A mice persisted throughout the 16 weeks of HF diet (2-way ANOVA with repeated mea-
Deletion of Adipocyte ABCA1 Blunted ABCA1-Dependent Cholesterol Efflux and Large Nascent HDL Formation in AT

To determine whether deletion of ABCA1 in adipocytes resulted in increased FC content, similar to what we observed in macrophages from macrophage-specific ABCA1 KO mice, we measured TC and FC in epididymal fat from WT and ABCA1 KO mice. We found a gene dose-related increase in FC content in ABCA1 KO mice compared with WT mice. However, HDL-mediated FC efflux (ABCA1 independent) was unaffected by deletion of adipose ABCA1 expression (Figure 5B). To determine whether the ablation of ABCA1-dependent efflux in ABCA1 KO mice was accompanied by loss of nascent HDL formation, we measured the relative expression of ABCA1, aP2, and scavenger receptor class B type I (SR-BI) in different tissues (ie, liver, thioglycolate-elicited peritoneal macrophages, and 3 fat depots) of WT and ABCA1 KO mice. Male mice were used in all assays, and each lane represents an individual mouse. GAPDH was used as a load control in each blot.
nascent HDL formation, we incubated [125I]-apoA-I with ex vivo cultures of WT or ABCA1−/− mice epididymal fat and monitored nascent HDL formation by non-denaturing gel electrophoresis and high-resolution fast protein liquid chromatography (Figure 5C). WT epididymal fat generated multiple, heterogeneous-sized HDL subfractions that ranged in size from 7 to 12 nm in diameter, which we have designated pre-β 1 to 5 in order of increasing particle size. We have also observed similar nascent HDL particle size heterogeneity in cultures of HEK293 cells stably expressing ABCA1, mouse peritoneal macrophages, and rat McArdle 7777 hepatoma cells.11,20 In contrast, epididymal fat from ABCA1−/− mice generated only particles <10 nm in diameter (ie, pre-β 2).

**Discussion**

This study was designed to elucidate the role of adipocyte ABCA1 expression in nascent HDL particle formation and its contribution to the plasma HDL pool in vivo. To address this issue, we generated ABCA1−/− mice and studied them while they were consuming a chow or HF diet. Several novel observations were made in this study. First, AT ABCA1 expression varied over a 5-fold range among different ATs, suggesting an important role for ABCA1 expression in AT sterol homeostasis. Second, ABCA1−/− mice had <10% expression of ABCA1 protein in AT compared with WT mice, with a concomitant 2-fold increase in AT cholesterol content. Although FC efflux from AT explants to HDL was similar between WT and ABCA1−/− mice, FC efflux to apoA-I was eliminated in ABCA1−/− AT, suggesting an important and specific role for ABCA1 in AT FC homeostasis. Third, WT AT explants were capable of generating multiple, discrete-sized nascent HDL subfractions from apoA-I, ranging in diameter from 7 to 12 nm, similar to other cell types. However, ABCA1−/− AT generated nascent HDL particles <10 nm, suggesting a considerable loss of function in the ability to generate nascent HDL. The decreased ability to generate nascent HDL was reflected in vivo as a 15% reduction in plasma HDL concentration in ABCA1−/− versus WT mice that was attributed to HDL production because HDL catabolism was similar between the 2 genotypes of mice. Collectively, these results establish a novel role for adipocyte ABCA1 in AT cholesterol mobilization and cholesterol balance and in vivo HDL production.

AT, the main depot for triglycerides, is used to meet systemic energy needs.3 It is also the body’s largest cholesterol storage depot; uniquely, >95% of AT cholesterol is FC as a result of low esterification activity by acyl-coenzyme A: cholesterol acyltransferase.3 As adipocytes expand in size during obesity, FC and lipid droplet FC increase,3 but plasma membrane FC decreases.24 This redistribution of FC increases plasma membrane fluidity, decreases insulin signaling, and increases adipocyte cholesterol biosynthesis.28 Thus, maintaining the optimal amount and intracellular distribution of FC in adipocytes appears functionally important in AT. Because cholesterol biosynthesis is relatively low in AT,29 FC efflux may be an important regulator of sterol homeostasis. Recent studies indicate that ABCA1 and SR-BI are important
regulators of cholesterol efflux from adipocytes. Initially, adipocytes were thought to have low ABCA1 expression, but subsequent studies using 3T3L1 adipocytes and AT and our data in isolated adipocytes (Figure 1C and 1D) demonstrate that ABCA1 is abundantly expressed. Furthermore, we observed that ABCA1 expression varies over a 5-fold range among AT depots and that this variation is highly correlated with AT FC content (Figure 1A and 1B). These data suggest that as metabolically active visceral fat stores enlarge during TG accretion, FC also increases; this in turn upregulates expression of ABCA1 to rebalance cellular stores.

To determine the role of AT ABCA1 expression in plasma HDL concentrations, we generated ABCA1 knockout mice by crossing ABCA1 knockout mice with aP2 Cre recombinase. ABCA1 protein expression in epididymal fat was decreased in proportion to the number of inactivated ABCA1 alleles (Figure 1A). Recombination of the ABCA1 gene was efficient in all AT depots examined, with >90% elimination of ABCA1 protein expression in ABCA1−/− mice.

Expression of SR-BI, another protein important in adipocyte FC efflux, was unaffected (Figure 1D). ABCA1−/− mice had lower plasma total and HDL cholesterol concentrations compared with WT mice, whether they were fed chow or an HF diet (Figure 3C and 3D). To determine whether this phenotype was due directly to deletion of AT ABCA1 or indirectly to changes in ABCA1 expression in other tissues, the specificity of the recombination was investigated. ABCA1 expression in liver and intestine, 2 tissues previously shown to be important in determining plasma HDL concentrations, was similar in WT and ABCA1−/− mice and could not account for the differences in plasma HDL concentrations. aP2 Cre recombinase has been reported to be active in macrophages, so we also examined ABCA1 expression in resident, elicited peritoneal, and bone marrow–derived macrophages. ABCA1 expression was variably reduced in macrophages from ABCA1−/− mice, confirming previous results that aP2 Cre recombinase was active in macrophages. However, evidence from our laboratory and others using transplantation of total ABCA1 KO bone marrow has demonstrated that macrophage ABCA1 expression has no significant impact on plasma HDL concentrations. Taken together, these data suggest that AT ABCA1 expression plays a significant role in determining plasma HDL concentrations.

Although our data suggested that HDL production by AT was reduced in the absence of adipocyte ABCA1 expression, deletion of adipocyte ABCA1 may have indirectly increased HDL catabolism, resulting in reduced plasma HDL levels. Previous studies in humans suggest that HDL catabolism plays an important role in determining plasma HDL concentrations. To formally examine this possibility, we performed HDL turnover studies using radiolabeled WT HDL because HDL composition was similar between WT and ABCA1−/− mice (data not shown). Plasma decay of the HDL tracer and uptake of radiolabel by the liver and kidney, the 2 most important tissues in HDL protein uptake and catabolism, were similar for both genotypes of mice (Figure 4). From these data, we conclude that decreased plasma HDL concentrations in ABCA1−/− mice were due to decreased HDL production by AT relative to WT mice and not to differences in HDL catabolism.

Although our study and others implic ate AT in HDL production, direct evidence for the assembly of nascent HDL particles by AT has not been demonstrated. In this study, we show that epididymal fat from ABCA1−/− mice had a doubling of FC content, was defective in FC efflux to apoA-I, and was defective in the production of nascent HDL particles >10 nm in diameter (Figure 5). Zhang et al have shown that both ABCA1 and SR-BI, but not ABCG1, are important for FC efflux from adipocytes, differentiated from mouse embryonic fibroblasts. Our study confirms and extends their results by showing that ABCA1 is critical for maintaining FC content and efflux to ABCA1 in primary AT explants. Furthermore, the 2-fold increase in AT FC content suggests that SR-BI cannot compensate for the loss of ABCA1 expression. This was confirmed by the similar expression of SR-BI among AT depots in WT and ABCA1−/− mice (Figure 2D). Previous studies have shown that ectopic expression of ABCA1 in cell lines is necessary and sufficient to...
generate several discrete-sized nascent HDL particles. We have designated these particles pre-HDL1 to 5 on the basis of increasing particle size on nondenaturing gradient gels and fast protein liquid chromatography columns and mobility on agarose or 2-dimensional gels. These nascent pre-HDL particles appear to be formed simultaneously in cell culture systems, with no evidence for a precursor-product dependency (ie, small particles larger particles). Similar discrete, nascent HDL particle sizes have previously been observed for McArdle 7777 hepatoma cells, mouse peritoneal macrophages, and primary hepatocytes (unpublished data), in addition to transfected cell lines, suggesting that ABCA1 expression is the necessary and sufficient condition for nascent HDL size heterogeneity among these varied cell types. Epididymal fat explants from WT mice also generated nascent HDL particles with apoA-I that were strikingly similar in size to those reported for other cell culture systems (Figure 1F). However, epididymal fat explants from ABCA1−/− mice did not generate nascent HDL >10 nm (Figure 5C). We speculate that the formation of pre-β 2 HDL resulted from the residual (<10%) ABCA1 protein expression in AT of ABCA1−/− mice (Figure 2D). Support for this idea comes from a study in which siRNA silencing of ABCA1 in McArdle 7777 hepatoma cells resulted in >70% to 80% reduction of ABCA1 protein expression and decreased formation of nascent HDL >10 nm. Overall, our studies provide evidence that AT ABCA1 expression directly contributes to the formation of multiple, discrete-sized nascent HDL particles.

Obesity is associated with chronic low-grade inflammation, adipocyte dysfunction (ie, adipokine secretion, impaired lipolysis, insulin resistance), and low plasma HDL. Adipocyte ABCA1 expression may represent a compensatory mechanism to limit inflammation and adipocyte dysfunction that accompanies obesity and metabolic syndrome. Because adipocyte FC content increases with obesity and adipocyte hypertrophy, we speculate that ABCA1 is upregulated in adipocytes to maintain optimal FC balance between the lipid droplet and plasma membrane and adipocyte function. With development of obesity, accretion of FC during adipocyte expansion may sequester FC on lipid droplets, shunting it away from ABCA1-mediated HDL particle formation, resulting in decreased plasma HDL concentrations. In contrast, rapid weight loss may result in FC mobilization to the

Figure 5. Adipocyte ATP binding cassette transporter A1 (ABCA1) generates heterogeneous-sized nascent high-density lipoprotein (HDL) particles. A, Free cholesterol accumulation in epididymal adipose tissue (AT) with progressive inactivation of adipocyte ABCA1 alleles. Wild-type (WT; +/+), heterozygote (−/−/−), and homozygote (−/−/−/−) of adipocyte-specific ABCA1 knockout mice. B, Cholesterol efflux to apolipoprotein (apo) A-I (ABCA1 dependent) and HDL (ABCA1 independent). Ex vivo cultures of epididymal fat from +/+ and −/−/− mice were radiolabeled with 3H-cholesterol for 24 hours, washed, and then incubated with no addition (no Trt), apoA-I (hAI; 20 μg/mL), or mouse HDL (mHDL; 50 μg/mL) for 4 hours to measure 3H-FC efflux into the medium. For A and B, values are mean ± SEM; n=4. *P<0.05, **P<0.01, ***P<0.001; not significant (ns) at P=0.05. C, Nascent HDL formation in the presence of 125I-apoA-I in ex vivo cultures of epididymal AT from +/+ and −/−/− mice. After a 24-hour incubation, conditioned medium was fractionated by high-resolution fast protein liquid chromatography, and radiolabeled apoA-I in each fraction was quantified with a gamma counter. Elution regions for different-sized nascent pre-β HDL particles are denoted by the vertical dashed lines. Inset shows the size of AT–derived nascent HDLs separated by nondenaturing gradient gel electrophoresis (4% to 30%). Migration positions of the radiolabeled nascent HDL particles were visualized with a Phosphorimager.
adipocyte plasma membrane, resulting in increased ABCA1-mediated HDL particle formation and increased plasma HDL concentrations. Thus, adipocyte ABCA1 may be a critical negative regulator of obesity and metabolic syndrome, preventing adipocyte dysfunction and abnormal plasma membrane FC accumulation by facilitating FC efflux and nascent HDL formation.

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Disclosures

None.

References

CLINICAL PERSPECTIVE

Adipose tissue is a major pool of whole-body free cholesterol (FC) and abundantly expresses ATP binding cassette transporter A1 (ABCA1), a membrane protein necessary for high-density lipoprotein (HDL) particle formation. Although adipocytes abundantly express ABCA1, their contribution to HDL production in vivo is unknown. Using adipocyte-specific ABCA1 knockout mice, we demonstrate for the first time that adipocytes make nascent, discrete-sized HDL particles that represent a significant (≈15%) source of plasma HDL. Furthermore, deletion of ABCA1 in adipocytes results in a 2-fold increase in FC content, suggesting that other FC export mechanisms cannot compensate for loss of ABCA1. Adipose tissue ABCA1 expression varies over a 5-fold range among different adipose depots in wild-type mice and is significantly correlated with adipose tissue FC content, suggesting an important role for ABCA1 in adipose tissue FC homeostasis. Because adipocyte FC content increases with obesity and adipocyte hypertrophy, we speculate that ABCA1 is upregulated in adipocytes to maintain optimal FC balance between the lipid droplet and plasma membrane and adipocyte function. Accretion of FC during adipocyte expansion may sequester FC on lipid droplets, shunting it away from ABCA1-mediated HDL particle formation, resulting in decreased plasma HDL concentrations with development of obesity. In contrast, rapid weight loss may result in FC mobilization to the adipocyte plasma membrane, resulting in increased ABCA1-mediated HDL particle formation and increased plasma HDL concentrations. Thus, adipocyte ABCA1 quantitatively contributes to plasma HDL levels and may be critical as a negative regulator of obesity and metabolic syndrome by preventing adipocytes from abnormal FC accumulation and dysfunction.
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Supplemental Material

Adipose tissue ABCA1 contributes to HDL biogenesis in vivo

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Supplement Figure 1. ABCA1 is exclusively expressed in adipocytes in primary cultures of adipocytes. A. Oil red O staining of primary stromal vascular cells and differentiated adipocytes (at d10). B. Localization of adipocyte ABCA1, immunostained with monoclonal ABCA1 antibody (red) in differentiated cultures of adipocytes. Nuclei were counter-stained with DAPI (blue).
Supplement Figure 1

A

Stromal Vascular

Differentiated Adipocytes

B