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Abnormal Lipoprotein Particles and Cholesterol Efflux Capacity in Patients with Psoriasis

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Abstract

Objectives—Psoriasis is a Th-1/17 mediated inflammatory disease associated with increased risk of cardiovascular disease (CVD). Inflammation may modulate lipoprotein particle number and directly impair HDL functions, in particular reverse cholesterol transport (RCT). We sought to study how chronic in vivo inflammation modulates lipoprotein particle composition using nuclear magnetic resonance spectroscopy (NMR) and HDL efflux in psoriasis.

Methods and Results—We prospectively enrolled a consecutive sample of patients with psoriasis (n=122) and compared lipoprotein and metabolic risk factors to patients without psoriasis (n=134). Fasting lipids, insulin, glucose were measured by standard assays, and lipoprotein concentration and size were measured by NMR. In a random subset (n=100 each group), HDL efflux capacity was quantified using a validated ex vivo system involving the incubation of macrophages with apolipoprotein B-depleted serum from patients. Traditional lipid concentrations were similar in both groups except for HDL concentration which was lower in psoriasis (43 mg/dL (36–58) vs 50 (42–62), p<0.01). However, NMR showed an atherogenic profile in psoriasis similar to that observed in diabetes, with significant increase in LDL particle concentration [1210.5 (1002–1498) vs 1115 (935–1291), p=0.03] with decrease in LDL size [20.6 (20.3–21.1) vs 21.3 (20.6–21.1), p<0.001] beyond CV risk factors and HOMA-IR (p=0.001). Finally, HDL efflux capacity was lower in psoriasis compared to controls in fully adjusted models (beta –0.14, p=0.001).

Conclusions—These data support a more atherogenic lipoprotein profile by NMR and decreased HDL efflux capacity in psoriasis patients compared to controls beyond CVD risk factors. The abnormal lipoprotein particle composition and HDL efflux capacity in psoriasis may provide a link between psoriasis and CVD.

Keywords

inflammation; atherosclerosis; HDL efflux; cholesterol; lipoprotein particles

Introduction

Psoriasis is a helper T 1 (Th1) and Th17 cell, chronic inflammatory disease, which affects 2%–3% of the adult population. Epidemiological data over the past decade demonstrates a consistent association between psoriasis and cardiovascular diseases (CVD) beyond traditional CVD risk factors [1–7]; however, the link between these two diseases remains poorly understood. Increased LDL particle number (LDL-P) and decreased particle size (LDL-Z) as measured by nuclear magnetic resonance (NMR) spectroscopy confers cardiovascular risk beyond LDL cholesterol (LDL-C), particularly when LDL-C and LDL-P are discordant [8]. This pattern is observed in the metabolic syndrome (MetSyn), an inflammatory state which may modulate lipoproteins due to subclinical hepatic inflammation [9]. We recently demonstrated that psoriasis is associated with a systemic inflammatory state, including within the liver [10] and also with the MetSyn [11], and thus hypothesized that psoriasis may increase risk of CVD partly by promoting an atherogenic lipoprotein profile.

Furthermore, our group has demonstrated that HDL dysfunction and impaired reverse cholesterol transport (RCT) occur in human inflammatory syndromes during endotoxemia and diabetes, largely independent of changes in plasma HDL-C and ApoA-I levels [12, 13]. These perturbations in cholesterol efflux capacity from macrophages indeed have a strong inverse association with both carotid intima-media thickness and the likelihood of angiographic coronary artery disease independently of the HDL cholesterol level [14] supporting the notion of lipoprotein dysfunction beyond that detected by lipoprotein concentration. Given these observations, we hypothesized that psoriasis, a chronic inflammatory disease associated with accelerated vascular diseases, would be associated with an abnormal lipoprotein particle profile and impaired cholesterol efflux.

Methods

We consecutively enrolled psoriasis patients (n=122) over a six month period free of clinical CVD defined as no history of cardiovascular symptoms (e.g. chest pain, shortness of breath, syncope), absence of EKG findings consistent with ischemia (primary, regional T-wave changes) or old infarction (Q waves in a regional distribution) and absence of prior diagnosis of positive stress test or revascularization. Patients without psoriasis (n=134) were derived from a random sample of healthy subjects from Philadelphia free of clinical CVD defined as above. Plasma levels of lipids and glucose were measured enzymatically (Cobas Fara II; Roche Diagnostic Systems), plasma insulin levels were measured with the use of a commercial enzyme immunoassay (Linco Research), and plasma CRP levels were assayed with the use of a high-sensitivity latex turbidimetric immunoassay (Wako Ltd). The homeostasis model assessment of insulin resistance ((HOMA-IR) index = fasting glucose (mmol/l) X fasting insulin (μU/ml)/22.5) were used to estimate the degree of insulin resistance. Lipoprotein particle concentration and diameters were measured using an automated NMR [8].

In a random subset of 100 patients with psoriasis and 100 healthy non-psoriasis patients free of clinical CVD, we performed cholesterol efflux capacity per previously published methods [14]. J774 cells, derived from a murine macrophage cell line, were plated and radiolabeled with 2 μCi of 3H-cholesterol per milliliter. ABCA1 was up-regulated by means of a 6-hour incubation with 0.3 mM 8-(4-chlorophenylthio)-cyclic AMP. Subsequently, efflux mediums containing 2.8% apolipoprotein B-depleted serum were added for 4 hours. To prepare apo-B depleted serum, samples were thawed prior to Apo-B precipitation. Briefly, 40 parts polyethylene glycol solution (PEG; 20% PEG 8000MW in 200mM glycine buffer, pH 7.4) was added to 100 parts serum and mixed by pipetting, then incubated at room temperature

for 20 minutes before spinning in a microcentrifuge at 10,000 rpm for 30 minutes at 4°C. ApoB-containing lipoproteins are pelleted by this procedure, and the supernatant, which contains the HDL fraction, is recovered and diluted in 14mM MEM-HEPES (no bicarbonate) + 0.15mM cAMP to 2.8% (equivalent to 2% serum). All steps were performed in the presence of the acyl-coenzyme A:cholesterol acyltransferase inhibitor CP113,818 (2 µg per milliliter).

Liquid scintillation counting was used to quantify the efflux of radioactive cholesterol from the cells. The quantity of radioactive cholesterol incorporated into cellular lipids was calculated by means of isopropanol extraction of control wells not exposed to patient serum. Percent efflux was calculated by the following formula:

$$\left[\frac{\text{microcuries of } 3H\text{-cholesterol in mediums containing 2.8\% apolipoprotein B-depleted serum} - \text{microcuries of } 3H\text{-cholesterol in serum-free mediums}}{\text{microcuries of } 3H\text{-cholesterol in cells extracted before the efflux step}} \right] \times 100$$

All assays were performed in duplicate.

Baseline characteristics were described with medians and interquartile ranges (IQR), and compared between psoriasis and non-psoriasis, using Wilcoxon rank sum and chi square testing for unadjusted analysis. Univariable analysis was performed to evaluate how each NMR variable and HDL efflux was related to traditional risk factors (smoking status, hypertension, type 2 diabetes mellitus and the measured lipid that corresponds with the lipoprotein class analyzed in the model (LDL-C for LDL particles, HDL-C for HDL particles, and triglycerides for VLDL particles), cardiometabolic variables (BMI, waist, triglycerides, HOMA IR) and psoriasis. Multivariable linear regression models were adjusted for age, gender, traditional cardiovascular risk factors, including lipids (including apoA1), and cardiometabolic variables to understand the effect of psoriasis on NMR particles and HDL efflux. Our study (n=122 vs n=134) provided greater than 90% power to detect a difference in NMR variables between psoriasis and controls based on our hypothesis that we would observe differences similar to those between healthy subjects and the metabolic syndrome (10% increase in number and 10% decrease in size)[9], and also for the difference in HDL efflux capacity (n=100 vs 100) between healthy subjects and coronary artery disease subjects [14].

Results

All of our psoriasis patients (n=122) were Caucasian, therefore, we limited our random selection of patients without psoriasis (n=134) to Caucasian to reduce confounding due to racial differences in NMR profiles (Table 1). Psoriasis patients had mild to moderate disease (median body surface area was 2.9%, IQR 1–8.5%) and were mostly on topical therapies. Psoriasis patients were younger [median 44.5 (IQR 34–57) vs 49 (IQR 44–54), p=0.02] and were more obese, had higher blood pressures, more current smoking, more lipid lowering therapy, lower median HDL-C [43 (36–58) vs 49 (42–62), p=0.008] and higher median HOMA-IR [3.47 (2.33–6.61) vs 1.425 (0.940–2.131), p=0.02] reflecting a more adverse CV risk profile. Total cholesterol [186 (164–218) vs 208.5 (189–231)] and LDL-C [106.9 (90–132.5) vs 130 (106.4–147.4)] were lower in psoriasis compared to those without psoriasis (p<0.001) and no difference in triglycerides was observed in this consecutive sample.

Despite having lower mean LDL-C values, NMR spectroscopy revealed that psoriasis patients tended to have higher median LDL particle number (LDL-P) [1210.5 (1002–1498) vs 1129.5 (945–1318), p=0.056] and VLDL-P (p=0.037) compared to controls (Table 1). Both LDL particle size (LDL-Z) (p<0.001) and VLDL-Z (p<0.001) particle size was lower in the psoriasis group. No differences in HDL particle number and size were observed. Psoriasis was associated with a higher LDL-P and smaller LDL-Z after adjusting for age,

gender (LDL-P: $\beta=102.33$, $p=0.02$; LDL-Z: $\beta=-0.49$, $p<0.001$), traditional cardiovascular risk factors (LDL-P: $\beta=206.32$, $p<0.001$; LDL-Z: $\beta=-0.49$, $p<0.001$), and metabolic risk factors (LDL-P: $\beta=166.20$, $p=0.002$; LDL-Z: $\beta=-0.47$, $p<0.001$). When we excluded those on lipid lowering medications and on systemic psoriasis therapies, these differences persisted. No significant association between psoriasis and VLDL particle number and VLDL particle size was observed in fully adjusted models.

As compared with control patients, patients with psoriasis had significantly lower levels of not only HDL cholesterol, but also normalized cholesterol efflux capacity (mean value for psoriasis 0.82; mean value for non-psoriasis patients, 0.98; $P<0.001$) (Table 1). As previously noted [14], HDL cholesterol and apoA1 levels were the strongest predictors of efflux capacity ($P<0.001$ for both). Psoriasis ($P<0.001$) and BMI ($P=0.01$) were associated with decreased HDL efflux capacity. When age, gender, smoking, hypertension, diabetes, BMI and HOMA-IR were adjusted for within the linear regression model, psoriasis remained associated with HDL efflux ($\beta=-0.14$, $p=0.006$). We performed a sensitivity analysis to examine whether lipid lowering medications as well as on systemic psoriasis therapy affected these relationships by excluding those on these medications from primary analyses. When we excluded those on lipid lowering medications and systemic psoriasis therapy, these differences persisted. We finally explored whether there was a relationship between HDL particle sizes and HDL efflux. In psoriasis, there was a positive correlation between large HDL particle size and efflux (Spearman r 0.30; $p=0.008$), a trend toward HDL medium (r 0.20; $p=0.07$) particle size with a trend towards an inverse correlation between HDL small particle size and HDL efflux ($r=-0.20$; $p=0.08$).

Discussion

In this consecutive sample of patients with psoriasis, we demonstrate four novel findings compared to patients without psoriasis: 1) despite normal standard lipid panels, lipoprotein NMR profiles were abnormal; 2) the NMR profiles revealed decreased LDL particle size and increased number; 3) abnormalities in LDL particle size and number persisted after accounting for CVD and metabolic risk factors; 4) decreased capacity of HDL efflux in psoriasis beyond CVD risk factors. These results suggest that psoriasis may confer a unique lipoprotein and cardiometabolic risk not detected by standard risk assessment, further support inflammatory modulation of HDL function [12, 13] and provide novel insight into the heightened CVD risk observed in this disease state.

The pattern we observed of increased LDL-P and decreased LDL-Z is similar to the NMR profile observed in the MetSyn [9], and was recently shown to predict CVD in a prospective study of healthy women [15]. Furthermore, LDL-P appears to be superior than LDL-C in predicting CVD in individuals with discordant levels of LDL-P and LDL-C [8], suggesting that the number and size of cholesterol particles may confer risk beyond simple cholesterol concentration potentially due to increased deposition in the sub-endothelial space, increased uptake by macrophages, increased susceptibility to oxidation and or decreased clearance because of reduced affinity for the LDL receptor.

The NMR profile persisted after adjustment for traditional CV risk, but more interestingly after adjustment for metabolic risk factors, such as BMI and insulin resistance (IR). These findings suggest a unique effect of psoriasis that predisposes to the changes observed on NMR beyond standard cardiometabolic risk factors (ie MetSyn and IR). Chronic hepatic inflammation observed in steatohepatitis or subclinical inflammatory states, such as diabetes, have been shown to increase the hepatic production and secretion of apoB containing particles (ie, LDL and VLDL), an observation reproduced in animal models [16]. Indeed, we have observed that psoriasis is associated with increased hepatic inflammation

[10], potentially via overproduction of TNF-alpha within the skin and serum, which may mimic the milieu observed in these other states.

Previously, we reported the first *in vivo* functional evidence that acute inflammation retards RCT in rodents and provided proof-of-principle for similar effect in humans[13], and recently extended those findings demonstrating reduced HDL capacity to efflux cholesterol from SR-BI and ABCA1, but not ABCG1, model systems[12]. That characterization of lipoprotein particles during human inflammation provided insight into specific changes in HDL efflux however was performed in the setting of evoked inflammation. In this study, we now utilize a chronic *in vivo* inflammatory disease state, psoriasis, and extend these findings which have been associated with increased CVD risk [14, 15]. The reduction in HDL efflux was remarkable because those patients had small differences in HDL-C and apoA-I suggesting that these traditional HDL parameters may be poor markers of important HDL function in humans. Indeed HDL composition and function have been of specific interest in face of recent failure the cholesterol ester transfer protein (CETP) inhibitor torcetrapib and more recently niacin to reduce CVD despite their HDL-C raising effects [17]. Therefore, our work further supports the concept that understanding residual risk of CVD may require more sophisticated phenotyping.

The major implication of these findings for patients with psoriasis is that perhaps traditional lipoprotein markers do not fully capture the metabolic dysfunction in patients with psoriasis and that they may warrant more aggressive control of established CV risk factors. Our study is limited by the correlative nature, not permitting causal inference. Additionally, we did not directly account for psoriasis severity in our models or a family history of CVD among psoriasis patients. We also lack of CVD outcomes, therefore, our studies support the need for the development of larger, prospective studies to assess NMR lipoprotein profiles and HDL efflux capacity in psoriasis with degree of psoriasis severity, treatment effects of psoriasis and CVD outcomes.

References

1. Gelfand JM, Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB. Risk of myocardial infarction in patients with psoriasis. *JAMA*. 2006; 296:1735–1741. [PubMed: 17032986]
2. Gelfand JM, Dommasch ED, Shin DB, Azfar RS, Kurd SK, Wang X, Troxel AB. The risk of stroke in patients with psoriasis. *J Invest Dermatol*. 2009; 129:2411–2418. [PubMed: 19458634]
3. Mehta NN, Azfar RS, Shin DB, Neimann AL, Troxel AB, Gelfand JM. Patients with severe psoriasis are at increased risk of cardiovascular mortality: Cohort study using the general practice research database. *Eur Heart J*. 2010; 31:1000–1006. [PubMed: 20037179]
4. Mehta NN, Yu Y, Pinnelas R, Krishnamoorthy P, Shin DB, Troxel AB, Gelfand JM. Attributable risk estimate of severe psoriasis on major cardiovascular events. *Am J Med*. 2011; 124:775, e771–776. [PubMed: 21787906]
5. Ahlehoff O, Gislasen GH, Charlot M, Jorgensen CH, Lindhardsen J, Olesen JB, Abildstrom SZ, Skov L, Torp-Pedersen C, Hansen PR. Psoriasis is associated with clinically significant cardiovascular risk: A danish nationwide cohort study. *J Intern Med*. 2011; 270:147–157. [PubMed: 21114692]
6. Armstrong AW, Harskamp CT, Ledo L, Rogers JH, Armstrong EJ. Coronary artery disease in patients with psoriasis referred for coronary angiography. *The American journal of cardiology*. 2012
7. Li WQ, Han JL, Manson JE, Rimm EB, Rexrode KM, Curhan GC, Qureshi AA. Psoriasis and risk of nonfatal cardiovascular disease in u.S. Women: A cohort study. *Br J Dermatol*. 2012; 166:811–818. [PubMed: 22175820]
8. Otvos JD, Mora S, Shalaurova I, Greenland P, Mackey RH, Goff DC Jr. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. *J Clin Lipidol*. 2011; 5:105–113. [PubMed: 21392724]

9. Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, Wilson PW, D'Agostino RB, Vasan RS, Robins SJ. Increased small low-density lipoprotein particle number: A prominent feature of the metabolic syndrome in the framingham heart study. *Circulation*. 2006; 113:20–29. [PubMed: 16380547]
10. Mehta NN, Yu Y, Saboury B, Foroughi N, Krishnamoorthy P, Raper A, Baer A, Antigua J, Van Voorhees AS, Torigian DA, Alavi A, Gelfand JM. Systemic and vascular inflammation in patients with moderate to severe psoriasis as measured by [18f]-fluorodeoxyglucose positron emission tomography-computed tomography (fdg-pet/ct): A pilot study. *Arch Dermatol*. 2011; 147:1031–1039. [PubMed: 21576552]
11. Langan SM, Seminara NM, Shin DB, Troxel AB, Kimmel SE, Mehta NN, Margolis DJ, Gelfand JM. Prevalence of metabolic syndrome in patients with psoriasis: A population-based study in the united kingdom. *J Invest Dermatol*. 2012; 132:556–562. [PubMed: 22113483]
12. de la Llera Moya M, McGillicuddy FC, Hinkle CC, Byrne M, Joshi MR, Nguyen V, Tabita-Martinez J, Wolfe ML, Badellino K, Pruscino L, Mehta NN, Asztalos BF, Reilly MP. Inflammation modulates human hdl composition and function in vivo. *Atherosclerosis*. 2012
13. McGillicuddy FC, de la Llera Moya M, Hinkle CC, Joshi MR, Chiquoine EH, Billheimer JT, Rothblat GH, Reilly MP. Inflammation impairs reverse cholesterol transport in vivo. *Circulation*. 2009; 119:1135–1145. [PubMed: 19221221]
14. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips Ja, Mucksavage ML, Wilensky RL, Mohler ER, Rothblat GH, Rader DJ. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *The New England journal of medicine*. 2011; 364:127–135. [PubMed: 21226578]
15. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation*. 2009; 119:931–939. [PubMed: 19204302]
16. Tsai J, Zhang R, Qiu W, Su Q, Naples M, Adeli K. Inflammatory nf-kappab activation promotes hepatic apolipoprotein b100 secretion: Evidence for a link between hepatic inflammation and lipoprotein production. *American journal of physiology. Gastrointestinal and liver physiology*. 2009; 296:G1287–1298. [PubMed: 19342510]
17. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, Shear CL, Revkin JH, Buhr KA, Fisher MR, Tall AR, Brewer B. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med*. 2007; 357:2109–2122. [PubMed: 17984165]

Table 1

Baseline Demographics and Clinical Characteristics Stratified by Psoriasis

	Psoriasis (n=122)	Control (n=134)	p value
Age	44.5 (34–57)	49 (44–54)	0.02
Male	68 (60%)	71 (53%)	0.29
Hypertension	39 (35%)	27 (20%)	0.01
Currently smoking	8 (7%)	0 (0%)	
Waist Size (inches)	40 (35–44)	35.5 (33–39.5)	<0.001
BMI	29.5 (25.9–33.4)	26.9 (24.9–30.1)	<0.001
Diabetes Mellitus	10 (8.8%)	5 (3.7%)	0.10
Hs-CRP	3.3 (.835–9.85)	1.25 (.6–2.4)	0.02
Lipid lowering therapy* (%)	19 (17%)	0 (0%)	<0.001
Psoriasis Severity (%BSA**)	2.9 (1–8.5)	Not applicable	
Psoriasis treatment#			
Topicals only	71 (58%)	Not applicable	
Systemic (methotrexate, anti-TNF)	12 (9%)		
Phototherapy	11 (9%)		
<i>Metabolic Assessment</i>			
Fasting Glucose	88	90	0.9
Insulin	17.65	6.31	<0.001
HOMA-IR	3.472 (2.330–6.611)	1.425 (.940–2.131)	<0.001
<i>Lipid Panel</i>			
Total Cholesterol	186 (164–218)	208.5 (189–231)	<0.001
LDL-C	106.9 (90–132.5)	130 (106.4–147.4)	<0.001
HDL-C	43 (36–58)	49 (42–62)	<0.001
Triglycerides	127 (78–190)	130 (91–159)	0.95
ApoA1	145 (139–152)	150 (142–158)	0.18
<i>NMR Spectroscopy</i>			
LDL particle concentration	1210.5 (1002–1498)	1129.5 (945–1318)	0.056
HDL particle concentration	34.45 (30.5–38.1)	34 (29.7–37.9)	0.50

	Psoriasis (n=122)	Control (n=134)	p value
VLDL particle concentration	61.9 (38.3–95.3)	53.2 (30.6–83.1)	0.04
LDL particle size	20.6 (30.3–21.1)	21.2 (20.6–21.6)	<0.001
HDL particle size	8.8 (8.6–9.4)	8.9 (8.8–9.2)	0.47
VLDL particle size	49.4 (44.2–55.9)	43.45 (40.5–48)	<0.001
<i>HDL Function</i>[^]			
Cholesterol Efflux Capacity	0.83 (0.78–1.00) [^]	0.98 (0.84–1.11) [^]	0.008

Data are expressed as median (interquartile range)

* lipid lowering therapy includes statin, fibrate and/or ezetimibe

** body surface area

may not add up to 100% because some patients not currently on therapy

[^] (n=100)