Impact of Mifepristone, a Glucocorticoid/Progesterone Antagonist, on HDL Cholesterol, HDL Particle Concentration, and HDL Function

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Context: Mifepristone is a glucocorticoid and progestin antagonist under investigation for the treatment of Cushing's syndrome. Mifepristone decreases high-density lipoprotein (HDL) cholesterol (HDL-C) levels in treated patients, but the clinical significance of this is unclear because recent studies suggest that functional properties of HDL predict cardiovascular disease status better than does HDL-C concentration.

Objective: The aim of the study was to characterize the impact of mifepristone administration on HDL particle concentration and function.

Design and Setting: We conducted a double-blind, randomized, placebo-controlled trial at a single-site, clinical research center.

Participants: Thirty healthy postmenopausal female volunteers participated in the study.

Intervention: Individuals were randomized to receive daily oral mifepristone (600 mg) or placebo for 6 wk.

Main Outcome Measures: We measured HDL-C, serum HDL particle concentration, and HDL-mediated cholesterol efflux by treatment group.

Results: As expected, ACTH, cortisol, estradiol, and testosterone levels increased in the mifepristone group. Mifepristone treatment decreased HDL-C and HDL particle concentration by 26 and 25%, respectively, but did not alter pre-β HDL concentration. In contrast, the serum HDL-mediated cholesterol efflux decreased with mifepristone treatment by only 12%, resulting in an effective increase of the efflux capacity per HDL particle. No changes were observed in cholesterol ester transfer protein or lecithin:cholesterol acyltransferase activity.

Conclusions: Treatment with mifepristone reduced HDL-C, HDL particle concentration, and serum HDL cholesterol efflux in postmenopausal women. However, on a per particle basis, the efflux capacity of serum HDL increased. These observations support the concept that a decrease in HDL-C may not represent proportional impairment of HDL function. **(*J Clin Endocrinol Metab* 97: 1598–1605, 2012)**

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Abbreviations: apo, Apolipoprotein; BMI, body mass index; CETP, cholesterol ester transfer protein; CVD, cardiovascular disease; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LCAT, lecithin:cholesterol acyltransferase; LDL, low-density lipoprotein; UC, unesterified cholesterol.

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Chronic elevations in corticosteroids lead to central obesity, insulin resistance, and type 2 diabetes, hypertension, and an increased risk of atherosclerotic vascular disease in patients with Cushing's syndrome (1). Current therapies for Cushing's syndrome often result in poor control of these complications. Mifepristone is a potent glucocorticoid receptor antagonist and is under investigation for the treatment of Cushing's disease in patients who fail to respond to conventional therapy (2).

Central obesity and insulin resistance are typically associated with dyslipidemia characterized by elevated levels of triglyceride and small, dense low-density lipoprotein (LDL), and low levels of high-density lipoprotein (HDL) cholesterol (HDL-C) (3). Low levels of HDL-C are strongly associated with an increased risk of cardiovascular disease (CVD). Patients with Cushing's syndrome have elevated triglyceride levels and very low-density lipoprotein production rates consistent with their insulin-resistant state, but paradoxically also have elevated HDL-C compared with controls (4). In non-Cushing's subjects, a 1-month burst and taper of the glucocorticoid prednisone raised total cholesterol and HDL-C but not triglyceride levels (5). Taken together, these studies suggest an independent effect of glucocorticoids on HDL metabolism that is dissociated from longer-term effects of central weight gain and insulin resistance.

HDL is a complex of cholesterol, phospholipids, triglycerides, and proteins with apolipoprotein (apo) A-I (apoA-I), a single major protein constituting about 70% of the total HDL protein (6). HDL is thought to exert its cardioprotective effects primarily by promoting cholesterol efflux from macrophages in the artery wall (7, 8). The concentration of HDL in blood is monitored clinically as HDL-C. However, HDL is composed of a heterogeneous mixture of particles that carry a wide range of proteins (6, 9–12), and the relationship between HDL-C levels, HDL function, and specific populations of HDL particles is poorly understood. Several lines of evidence support the proposal that the cardioprotective effects of HDL can be dissociated from blood levels of HDL-C (12–15). Consistent with this hypothesis, recent studies indicate that the ability of serum HDL (serum depleted of apoB-containing particles) to promote cholesterol efflux from macrophages is independent of HDL-C and apoA-I levels (15). Moreover, the efflux capacity of serum HDL is a better predictor of CVD status than either HDL-C or apoA-I (14). These observations suggest that the function of HDL in cholesterol efflux may better predict CVD risk than does HDL-C concentration.

Recent studies suggest that mifepristone improves glycemic control but lowers HDL-C in patients with Cushing's disease (16), consistent with its glucocorticoid antagonist mechanism, but effects of mifepristone on HDL function have not been previously reported. To investigate the effects of glucocorticoid antagonism on HDL metabolism, healthy postmenopausal women were randomized to treatment with mifepristone or placebo. Our observations indicate that mifepristone lowers HDL-C and HDL particle concentration, while at the same time improving the specific efflux capacity of serum HDL per particle.

Subjects and Methods

Study population

Healthy postmenopausal women (absence of menses for 12 months and FSH >35 IU/ml), ages 45–65, euthyroid, and with a body mass index (BMI) of 18–30 kg/m^2 were recruited by advertisement to a single study site (Diablo Clinical Research, Walnut Creek, CA). Serum HDL-C above 40 mg/dl and triglycerides below 200 mg/dl were required for inclusion. Major exclusion criteria were: acute or chronic disease state, significantly abnormal clinical laboratory test, concomitant or recent use of lipid-reducing drugs, drugs known to interfere with lipid metabolism, estrogen and/or progesterone replacement, smoking, consumption of more than one alcoholic beverage daily, signs and/or symptoms of adrenal insufficiency (*e.g.* orthostatic hypotension, fatigue, anorexia, nausea, abdominal pain, joint and muscle pain), endometrial thickness of more than 5 mm on transvaginal ultrasound, history of unexplained vaginal bleeding or cancer, recent or planned diet or exercise modification or use of diet mediations, diabetes mellitus, fasting blood glucose above 100 mg/dl and/or treatment with an antidiabetic medication, or renal insufficiency. Informed consent was obtained in all cases before any study procedures. The protocol was approved by the Aspire Institutional Review Board (La Mesa, CA).

Study design

Forty-three subjects were screened, of whom 30 enrolled and received at least one dose of study medication. Six participants in the mifepristone group withdrew from the study before d 43; in four of these cases, withdrawal was due to the development of a rash. One participant withdrew consent, and a sixth withdrew due to a constellation of four mild adverse events (abdominal cramping, fatigue, muscle/body aches, and fluid retention). Of all adverse events observed, only the abdominal cramping required treatment. All complaints resolved without long-term sequelae. There were no serious adverse events. One participant was noted to have a small uterine fluid collection on d 43 that was stable at d 84 and did not require further evaluation.

After screening, participants were randomized in a 2:1 fashion to receive either mifepristone (two 300 mg once daily; Corcept Therapeutics) or placebo for 6 wk. The study drug was administered on d 1, 8, 15, and 29 in the clinic and was self-administered on other days. In addition to these dates, participants were evaluated at the clinical study site on d 43 and 84 (off treatment follow-up). Safety laboratories and a brief physical examination were performed at all study visits. Fasting (overnight, minimum 10 h) blood was obtained on d 1 (baseline), 8, 15, 43, and 84.

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Safety laboratory measures

Standard lipid quantification and safety laboratory tests including serum electrolytes, creatinine, liver function tests, complete blood counts, and hormones were performed by a central clinical laboratory, and serum estradiol and total testosterone were measured by liquid chromatography tandem mass spectrometry (Quest Diagnostics, San Juan Capistrano, CA). Normal ranges for postmenopausal women are less than 10 pg/ml and 2–40 ng/dl for estradiol and testosterone, respectively.

Lipoprotein and apolipoprotein analyses

HDL subfraction measurements and apolipoprotein analyses were performed at Children's Hospital Oakland Research Institute on fasting serum samples obtained on d 1, 15, and 43. Concentrations of HDL particles were directly measured as a function of their size by ion mobility, a technique based on gas-phase differential electric mobility (9, 14). For the present analyses, two HDL subfractions were determined: large HDL_{2b} (10.5–14.5 nm) and small HDL_{3+2a} (7.6–10.4 nm). Total HDL particle concentration was calculated as the sum of HDL_{3+2a} and HDL_{2b}. Serum apoA-I, apoA-II, and apoB were measured by sandwich-style ELISA using primary antibodies (Biodesign International, Saco, ME). Assay controls were validated by Northwest Lipid Laboratory (Seattle, WA). Assays were performed in triplicate with an interassay variation of less than 10%.

Measurement of HDL pre-β particles was performed at the University of California, San Francisco. Sample plasma was electrophoresed in agarose, immunofixed by monospecific antihuman apoA-I antiserum and gels stained with Coomassie blue. The pre-β regions were quantified by densitometry. Pre-β-1 HDL concentration was estimated from a five-point calibration curve (log mg/dl *vs.* peak area, r = 0.98) run in the same gel. Within-run variation was controlled by normalizing test values to control plasma of known pre-β-1 HDL concentration (coefficient of variation = 10%). Samples were run in triplicate, paired by subject but blinded by treatment.

HDL-mediated cholesterol efflux

Cholesterol efflux capacity of serum HDL from cultured macrophages was measured using serum collected on d 1 and 43 at the University of Washington using the method described by Rader and colleagues (14, 15). J774 macrophages were labeled with [^{3}H]cholesterol (1 μCi/ml; Perkin-Elmer, Waltham, MA) in DMEM containing 1 mg/ml fatty acid-free BSA and the acyl CoA; cholesterol acyltransferase inhibitor Sandoz 58-035 (5 μg/ml; Sigma, St. Louis, MO) overnight, and ATP-binding cassette transporter A1 was induced with cAMP (0.5 mM) overnight. The cells were then incubated with DMEM/fatty acid-free BSA with or without 2.8% apoB-depleted serum (serum HDL) for 4 h at 37 C. The apoB was depleted by precipitation with polyethylene glycol (14, 15). The [^{3}H]cholesterol content of medium and cells was quantified, and serum HDL cholesterol efflux capacity was calculated as a fraction of total [^{3}H]cholesterol released into the medium after subtraction of values obtained in the absence of serum.

LCAT and CETP enzyme activity

Lecithin:cholesterol acyltransferase (LCAT) and cholesterol ester transfer protein (CETP) activities were measured at the University of California, San Francisco, using a microplate method employing colorimetric cholesterol assays for total cholesterol and unesterified cholesterol (UC). LCAT activity was quantified in plasma from decrease of UC concentration after 4-h incubation at 37 C. CETP activity was measured as the change in HDL cholesterol ester content during a 6-h, 37 C incubation of plasma in the presence of LCAT inhibitor. The HDL plasma fraction was prepared by polyethylene glycol precipitation of LDL/very low-density lipoproteins, and cholesterol ester was calculated as the difference of total cholesterol and UC.

Statistical analyses

The primary endpoint of the study was the change in serum HDL-C concentration between groups at the end of treatment. Enrollment of 30 subjects (randomized 2:1, mifepristone:placebo) allowed for 90% power to detect a 10% change in HDL-C (variance = 25%; $\alpha = 0.05$), but 80% power was maintained if 24 subjects completed the study. The primary analyses were performed on those subjects who completed the entire study (n = 14 for mifepristone, n = 10 placebo). Serum from one individual in the placebo group was not available for the efflux analyses. All variables were tested for normality using the method of Shapiro-Wilk. Because the majority of variables were not normally distributed at baseline, nonparametric methods were used as follows: Friedman's two-way ANOVA, for ranks for within-group comparison between baseline (d 1) and end-of-treatment (d 43); and a Wilcoxon rank sum test, applied to analyze between-group differences at baseline and changes over time. Correlation analysis was performed using Spearman's method to examine baseline variables (nonnormal distribution), and Pearson's method to examine relationships between longitudinal changes (because relative changes from baseline were normally distributed). All analyses were performed using either STATA version 10 (StataCorp, College Park, TX) or SPSS version 19 (IBM, Armonk, NY). In all cases, an α of less than 0.05 was considered significant.

Results

Study participants

There were no significant differences in baseline characteristics between the mifepristone and placebo-treated groups, including baseline hormone and lipid profiles (Table 1). The average ($\pm$SD) ages in the treatment and placebo groups were 59 $\pm$ 4 and 59 $\pm$ 5 yr, respectively. No significant differences in weight or BMI were observed in the two groups. Complete blood counts and serum chemistries, other than a small decrease in potassium (d 43 mean change = -0.54 mEq), were not affected by treatment and remained in the normal range in both groups throughout the study.

Serum hormone concentrations

As expected, treatment with the glucocorticoid receptor antagonist mifepristone significantly increased morning serum ACTH and cortisol concentrations (Table 1), due to the loss of negative feedback at the pituitary. Similarly, serum sex steroid concentrations were significantly increased in the treatment group, likely driven by in-

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TABLE 1. Effects of mifepristone treatment on body weight and serum glucose, hormones, and lipids levels

	Mifepristone (n = 14)		Placebo (n = 10)	
	Day 0	Day 43	Day 0	Day 43
Weight (kg)	70.0 (65, 81)	69.7 (65, 82)	62.5 (55, 71)	63.6 (56, 71)
BMI (kg/m^2)	25.6 (23, 28)	25.8 (24, 29)	24.3 (21, 26)	24.8 (21, 26)
ACTH (pg/ml)	14.5 (11, 24)	99 (70, 171)[a,b]	13 (10, 18)	13 (11, 17)
Cortisol (μg/dl)	14.8 (13, 16)	41 (39, 53)[a,b]	14.6 (12, 20)	12.1 (10, 14)
Estradiol (pg/ml)	8.0 (0, 21)	26.5 (10, 44)[a,b]	8.0 (0, 13)	8.5 (0, 24)
Total testosterone (ng/dl)	15.0 (11, 21)	51.5 (45, 71)[a,b]	12.5 (7, 14)	13.0 (7, 16)
Glucose (mg/dl)	92.0 (85, 99)	88.5 (85, 92)	92.0 (85, 99)	91.0 (86, 97)
Insulin (μU/ml)	12.0 (8, 21)	10.0 (7, 11)[a]	11.0 (10, 14)	10.5 (8, 18)
HOMA-IR	2.5 (1.9, 5.2)	2.2 (1.4, 2.4)[a]	2.4 (2.2, 2.9)	2.2 (1.7, 4.4)
Total cholesterol (mg/dl)	226 (204, 238)	180 (149, 222)[a,b]	231 (206, 238)	234 (213, 251)
Triglyceride (mg/dl)	96 (78, 121)	111 (82, 146)	104 (72, 188)	84 (69, 170)
LDL-C (mg/dl)	119 (83, 129)	100 (83, 129)[b]	123 (112, 147)	127 (111, 165)
HDL-C (mg/dl)	70 (49, 59)	53 (49, 59)[a,b]	76 (46, 87)	75 (52, 91)

All data are presented as median (25th, 75th percentiles).

[a] $P < 0.05$ *vs.* baseline (Friedman two-way ANOVA for ranks).

[b] $P < 0.05$ for differences *vs.* placebo in change from baseline (Wilcoxon rank sum test).

creased steroidogenesis in the setting of elevated ACTH (2, 17). TSH was slightly increased with mifepristone treatment (baseline = 2.0 ± 1.3 mIU/liter; d 43 = 4.9 ± 3.0 mIU/liter; $P < 0.05$), but free T_4 was unchanged and both measures of thyroid function remained in the normal range throughout the study in both groups (data not shown).

Glucose metabolism

Fasting glucose concentrations were not different between treatment groups and were not affected by mifepristone administration. Fasting insulin concentrations decreased significantly in the mifepristone group at d 43 compared with baseline ($P = 0.03$; Table 1). This resulted in a significant decline in homeostatic model assessment of insulin resistance (HOMA-IR) (Table 1), consistent with a small increase in insulin sensitivity in the treatment group.

Lipoprotein profiles

There were no differences between the two treatment groups in fasting lipid profiles at baseline (Table 1), and no significant changes were detected in any of the lipoproteins in the placebo group on d 43 (Table 2). Oral mifepristone treatment resulted in a significant, 20% decline in total cholesterol on d 43 (Table 1) and a decline in LDL-C with the treatment ($P = 0.046$) with no change in apoB concentration (Table 2). HDL-C declined by 26% with mifepristone treatment over the 43 d ($P < 0.001$ *vs.* baseline) (Table 1). Consistent with this decline in HDL-C, serum apoA-I, the most abundant protein within HDL particles, decreased in the mifepristone-treated group (19%; $P < 0.001$ *vs.* baseline) (Table 2), as did apoA-II concentration (11%; $P = 0.033$; Table 2). Fasting triglyceride levels did not significantly change in either group (Table 1).

TABLE 2. Effects of mifepristone treatment on lipoproteins, HDL particles, and associated enzymes

	Mifepristone (n = 14)			Placebo (n = 10)		
	Day 0	Day 43	*P* (day 0 *vs.* 43)[a]	Day 0	Day 43	*P* (day 0 *vs.* 43)[a]
ApoB (mg/dl)	89 (76, 105)	89 (71, 104)	0.285	87 (80, 123)	94 (80, 114)	1.0
ApoA-I (mg/dl)	154 (146, 170)	125 (119, 135)[b]	<0.001	151 (139, 165)	160 (143, 168)	0.096
ApoA-II (mg/dl)	32.9 (30, 41)	30.0 (26, 35)[b]	0.033	30.7 (28, 35)	31.2 (28, 36)	0.527
HDL particle no. (nmol/liter)	7867 (7003, 9273)	5955 (5122, 6854)[b]	0.008	7974 (7014, 10169)	9365 (7610, 10031)	0.527
HDL_{2b} (nmol/liter)	2882 (1653, 3496)	977 (730, 1297)[b]	<0.001	2778 (1379, 3993)	3172 (1746, 4295)	0.206
HDL_{3+2a} (nmol/liter)	5330 (5046, 6224)	4990 (3889, 6123)	0.593	5984 (4431, 6426)	6057 (5553, 8072)	1.0
Pre-β-1 HDL (mg/dl)	11.4 (10.4, 16.2)	11.1 (5.4, 13.5)	0.285	12.6 (10.9, 18.6)	14.6 (10.4, 14.8)	0.527
LCAT activity (nmol/ml/h)	79 (59, 95)	61 (54, 67)	0.285	68 (45, 85)	65 (41, 91)	0.206
CETP activity (nmol/ml/h)	12.5 (0, 15)	10 (6, 20)	0.248	12.5 (3, 16)	11.5 (0, 19)	0.527

All data are presented as median (25th, 75th percentiles).

[a] $P < 0.05$ *vs.* baseline (Friedman two-way ANOVA for ranks).

[b] $P < 0.05$ for differences *vs.* placebo in change from baseline (Wilcoxon rank sum test).

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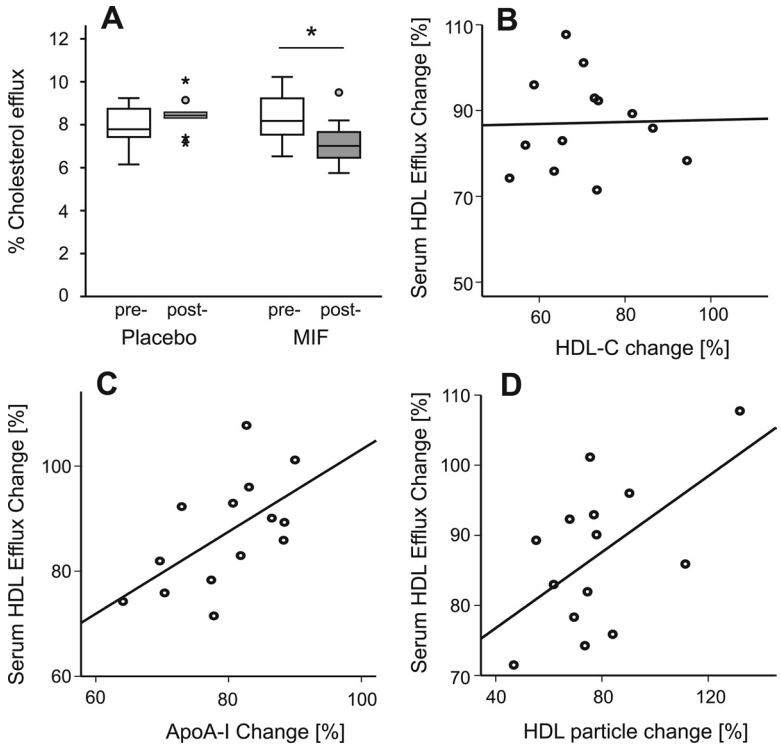

FIG. 1. Effect of mifepristone (MIF) on serum HDL sterol efflux capacity. Serum HDL sterol efflux capacity was significantly decreased by mifepristone treatment (A) (*, $P = 0.002$, treatment d 1 *vs.* d 43). Relative change of sterol efflux induced by mifepristone treatment was not correlated with relative decrease of HDL-C (r = 0.029; $P = 0.902$ (B), but significantly correlated with relative decrease of ApoA-I (r = 0.588; $P = 0.027$) (C) and HDL particles (r = 0.562; $P = 0.036$) (D). Relative changes were calculated for each subject as a change of the variable from d 1 to 43 divided by value at d 1 [(d43 − d1)/d1]. *Bars* represent median with interquartile range.

Lipoprotein subfraction analyses

To further investigate the observed decrease in HDL-C, we studied the impact of mifepristone treatment on the concentration of HDL particles measured by ion mobility (18) (Table 2). This approach directly measures lipoprotein particle concentration and is independent of apoA-I or HDL-C concentration measurements. Using this method, the total HDL particle concentration decreased by 25% (Table 2), similar to the decrease in apoA-I and HDL-C. Interestingly, mifepristone treatment exhibited potent differential effects on HDL particle subclasses. The decrease in total HDL particle number was completely accounted for by reduction in the larger HDL_{2b} particles (58%), whereas no changes were observed for the smaller HDL_{3+2a} particles (Table 2). These alterations in HDL particle concentrations occurred rapidly, within the first 2 wk of the treatment, and no further changes were observed between the d 15 and 43 (data not shown).

In parallel with the ion mobility measurements, we also evaluated the concentration of pre-β HDL particles, discoidal, and lipid-poor particles containing apoA-I. *In vitro* studies indicate that pre-β-1 HDL is an important acceptor of cellular cholesterol (11), whereas other studies found higher concentrations of pre-β-1 HDL in individuals with CVD (19, 20). There was no change in the pre-β-1 HDL in the mifepristone-treated group at d 43 compared with baseline or to the placebo group (Table 2). Similarly, the distribution of HDL particles by α-particle subtype was no different in the mifepristone group compared with placebo at either time point (data not shown).

Lastly, we investigated whether mifepristone altered LCAT and CETP activities, reasoning that these might be mechanisms whereby mifepristone lowered HDL-C and HDL particle concentration (11, 21). We found that mifepristone treatment had no impact on either LCAT or CETP enzymatic activity (Table 2).

Cholesterol efflux

Because the mifepristone treatment significantly reduced HDL-C and HDL particle concentration, we evaluated the effect of mifepristone on HDL function by measuring the ability of serum HDL to mediate cholesterol efflux from macrophages. At baseline, there were no differences in the sterol efflux capacity between the two groups, and efflux capacity correlated with serum apoA-I concentration (n = 23; r = 0.51; $P = 0.013$), total HDL particle concentration (n = 23; r = 0.53; $P = 0.009$); the efflux capacity of serum HDL also increased with increasing HDL-C (n = 23; r = 0.40; $P = 0.057$). The 6 wk of mifepristone treatment resulted in significant attenuation of HDL efflux capacity by 12% compared with no change in the placebo-treated group ($P = 0.002$) (Fig. 1A). This decrease was considerably less than the decreases in HDL-C (26%), apoA-I (19%), or total HDL particle concentration (25%). Moreover, the decrease in HDL-C with mifepristone treatment was not correlated with the decrease in the serum HDL sterol efflux capacity (Fig. 1B; r = 0.029; $P = 0.902$). In contrast, changes in both apoA-I concentration and total HDL particle concentration were significantly correlated with the change in the serum HDL sterol efflux capacity (Fig. 1, C and D; r = 0.588, $P = 0.027$; and r = 0.562, $P = 0.036$, respectively). These results indicate that HDL-C may not be the optimal surrogate measure of HDL functionality.

To further investigate the relationship between the serum HDL efflux capacity and other HDL measures, we normalized the efflux capacity to plasma HDL-C, apoA-I, and HDL particle concentration, respectively (Fig. 2). The HDL efflux capacity per unit of HDL-C, apoA-I, or HDL

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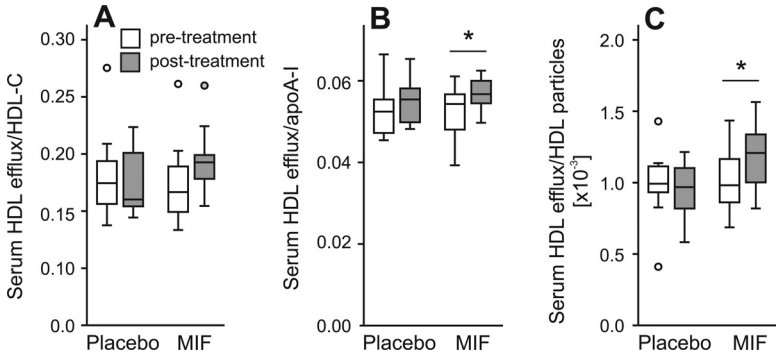

FIG. 2. Effect of mifepristone (MIF) on normalized HDL sterol efflux capacity. Mifepristone treatment improved specific serum HDL mediated efflux when normalized to HDL-C (A), apoA-I (B), or HDL particle concentrations (C). #*, $P <$ 0.01. Normalized sterol efflux was calculated by dividing % serum HDL sterol efflux by HDL-C, HDL particle, or apoA-I concentration, respectively. *Bars* represent median and interquartile range.

particle was increased by mifepristone treatment by 20, 7, and 18%, respectively (Fig. 2), suggesting that mifepristone induced changes in HDL particles that improved their ability to promote cholesterol efflux from lipid-loaded macrophages.

Relationships between changes in HDL-C, cholesterol efflux, and serum hormones

Using univariate analyses, we investigated the relationship between changes in serum hormones and changes in HDL-C and HDL particle concentration in the subjects treated with mifepristone (n = 14). There was no significant correlation between changes in total testosterone, estradiol, ACTH, cortisol, or insulin and changes in HDL-C or HDL particle concentration (data not shown). Changes in free testosterone were weakly but significantly inversely correlated with changes in total HDL particle concentration ($r = -0.54$; $P = 0.047$). We also examined whether changes in cholesterol efflux correlated with changes in any of the serum hormones. Only in the case of changes in serum cortisol did we observe a significant, negative, association with changes in sterol efflux (n = 13; $r = -0.62$; $P = 0.02$) in the mifepristone-treated group.

Discussion

Our observations indicate that administration of the glucocorticoid antagonist mifepristone significantly lowered total cholesterol, HDL-C, apoA-I, and HDL particle concentration in healthy postmenopausal women. In contrast, mifepristone treatment did not alter the concentration of pre-β-HDL particles. Although the decrease in HDL-C was accompanied by a decrease in HDL-mediated cholesterol efflux from macrophages, efflux on a per particle basis was increased by mifepristone treatment. Moreover, the change in HDL-mediated cholesterol efflux correlated with a change in HDL particle concentration but not with a change in total HDL-C.

Low levels of large HDL particles (as assessed by two-dimensional, nondenaturing electrophoresis) have been associated with CVD in a number of studies (19, 22–24) and may even be predictive of CVD risk (25). However, quantification of large and small HDL has not proven to be a more effective predictive tool for CVD risk assessment than measurement of HDL-C alone (18). On the other hand, a recent study demonstrated that serum HDL from people with similar levels of HDL-C can have dramatically different capacity to mediate cholesterol efflux from lipid-loaded macrophages (15). Furthermore, serum HDL sterol efflux capacity was strongly and negatively associated with CVD status in two different populations of subjects (14). This inverse relationship between sterol efflux and CVD persisted in multivariate models even after correction for HDL-C and apoA-I concentrations, and sterol efflux capacity was a strong independent predictor of CVD status (hazard ratio, 0.7). Moreover, recent prospective, randomized interventional studies do not support the notion that increases in HDL-C reduce CVD events (26, 27). Collectively, these studies suggest that HDL function—rather than HDL-C level—may be an important factor in determining CVD risk (14, 28, 29). Our data suggest that measurement of HDL particle concentration may reflect HDL sterol efflux capacity better than HDL-C. Furthermore, the data raise the possibility that decreases in HDL-C and HDL particle concentration induced by mifepristone treatment may be considerably mitigated by improved function of HDL. Alternatively, because the concentration of small HDL particles was not decreased by mifepristone treatment, it is possible that this population of particles promotes sterol efflux from macrophages more effectively than larger HDL particles.

How mifepristone treatment mediates changes in HDL-C and efflux capacity is unknown. Within the treatment group, increases in serum cortisol inversely correlated with cholesterol efflux. Although it has been suggested that high levels of circulating cortisol might play a role in the pathophysiology of CVD through effects on risk factors (including HDL-C) (30), interpreting the physiological relevance of increases in serum cortisol that are the consequence of a glucocorticoid antagonist (which by definition blocks cortisol action) is difficult. Antagonism of cortisol actions in peripheral tissues would be expected to reverse high triglyceride levels (4), central obesity (31, 32), and insulin resistance (33), all of which

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should raise HDL-C. Insulin resistance as measured by HOMA-IR did indeed improve with mifepristone therapy. Despite this improved insulin resistance (and unchanged triglyceride levels), however, mifepristone treatment decreased HDL-C in these healthy postmenopausal women. On the other hand, it is possible that more direct effects of glucocorticoid receptor antagonism on HDL metabolism occur in the liver and gut, such as through regulation of apoA-I gene expression (34) and secretion (35, 36).

The observed increases in serum ACTH, testosterone, and estradiol in the treatment group, resulting from blockade of both the glucocorticoid and progestin receptors by mifepristone, did not correlate with alterations in serum lipids or sterol efflux in our study, although our study may have been underpowered to observe such a relationship. On the whole, however, these hormone changes would have been expected to raise, not lower, HDL-C (37, 38). Another possibility is that cortisol or mifepristone might alter HDL protein composition (9), in turn affecting HDL function, which we did not analyze here. Finally, with regard to effects on activities of serum enzymes that affect HDL-C levels, we did not observe changes in CETP or LCAT enzymatic activity levels with treatment; however, given the observed effect size, larger studies including nearly three times the sample size enrolled here would be required for sufficient power to rule out an effect of mifepristone on these secondary endpoints.

In summary, our data indicate that whereas mifepristone treatment lowers HDL-C, HDL particle concentration, and serum HDL cholesterol efflux capacity, it fails to affect pre-β particles and small HDL (HDL_{3+2a}) particles. By decreasing specifically large HDL particles, which carry the major portion of cholesterol, mifepristone improves macrophage sterol efflux by serum HDL on a per particle basis. Because the mifepristone-induced decrease in serum HDL sterol efflux capacity strongly correlated with the decrease in HDL particles, but not with HDL-C, our data also suggest that HDL-C may not accurately reflect functional properties of HDL. Further studies will be needed to conclusively establish the relationship between the various HDL particles and the sterol efflux capacity of serum HDL, whether specific HDL particle populations associate with CVD status, and whether other factors that have been proposed to mediate the cardioprotective effects of HDL (such as its protein cargo) are altered by mifepristone treatment. The impact of mifepristone treatment on HDL in patients with Cushing's syndrome and the mechanisms underlying our observations warrant further study.

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