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## Low HDL cholesterol is associated with increased atherogenic lipoproteins and insulin resistance in women classified with metabolic syndrome

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### Abstract

Both metabolic syndrome (MetS) and elevated LDL cholesterol (LDL-C) increase the risk for cardiovascular disease (CVD). We hypothesized that low HDL cholesterol (HDL-C) would further increase CVD risk in women having both conditions. To assess this, we recruited 89 women with MetS (25-72 y) and LDL-C  $\geq 2.6$  mmol/L. To determine whether plasma HDL-C concentrations were associated with dietary components, circulating atherogenic particles, and other risk factors for CVD, we divided the subjects into two groups: high HDL-C (H-HDL) ( $\geq 1.3$  mmol/L, n = 32) and low HDL-C (L-HDL) ( $< 1.3$  mmol/L, n = 57). Plasma lipids, insulin, adiponectin, apolipoproteins, oxidized LDL, Lipoprotein(a), and lipoprotein size and subfractions were measured, and 3-d dietary records were used to assess macronutrient intake. Women with L-HDL had higher sugar intake and glycemic load ( $P < 0.05$ ), higher plasma insulin ( $P < 0.01$ ), lower adiponectin ( $P < 0.05$ ), and higher numbers of atherogenic lipoproteins such as large VLDL ( $P < 0.01$ ) and small LDL ( $P < 0.001$ ) than the H-HDL group. Women with L-HDL also had larger VLDL and both smaller LDL and HDL particle diameters ( $P < 0.001$ ). HDL-C was positively correlated with LDL size ( $r = 0.691$ ,  $P < 0.0001$ ) and HDL size ( $r = 0.606$ ,  $P < 0.001$ ), and inversely correlated with VLDL size ( $r = -0.327$ ,  $P < 0.01$ ). We concluded that L-HDL could be used as a marker for increased numbers of circulating atherogenic lipoproteins as well as increased insulin resistance in women who are already at risk for CVD.

**Key Words:** Metabolic syndrome, heart disease risk, low HDL cholesterol, atherogenic lipoproteins, insulin resistance

### Introduction

The metabolic syndrome (MetS) is a constellation of metabolic abnormalities characterized by abdominal obesity, hyperglycemia, high blood pressure, and dyslipidemias including elevated apolipoprotein B (apo-B), high plasma triglycerides (TG), increased numbers of small, dense LDL particles, and low HDL-cholesterol (HDL-C) concentrations [1]. The cluster of these characteristics poses individuals at higher risk for both cardiovascular disease (CVD) and type 2 diabetes [2].

The inverse association between low plasma concentrations of HDL-C and atherosclerosis has been clearly established [3]. The protective effects of HDL against CVD risk are not limited to the role of this lipoprotein in reverse cholesterol transport [4]. HDL has a number of pleiotropic functions, including the transport of paraoxonase 1, an important anti-oxidant in plasma [5], as well as the promotion of cholesterol removal from macrophages [6], regulation of endothelial adhesion molecule

expression [7], anti-inflammatory effects [8], and nitric oxide promoting action [9]. What is clear from previous studies [4-9] is that elevated concentrations of HDL-C are protective against CVD and atherosclerosis through many different mechanisms. This protective role of HDL may also be extended to subjects who present other risk factors for heart disease.

Dietary carbohydrate has been shown to modulate the risk factors associated with MetS [10]. For example, carbohydrate-restricted diets decrease plasma TG [11], elevate HDL-C [12], and reduce the number of circulating small LDL [13]. Since fat accumulation in the trunk area has been shown to be associated with increased free fatty acid release, insulin resistance, and disruption of glucose metabolism [10], reductions of fat in this area are quite beneficial to reduce the risk of CVD and type 2 diabetes [2]. The primary aim of the present study was to investigate whether low concentrations of HDL-C would result in increased numbers of atherogenic lipoproteins and insulin resistance in women already at risk for CVD. Based on this,

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we evaluated several risk parameters for CVD and atherosclerosis in women having high (H-HDL) ( $>1.3$  mmol/L) versus low (L-HDL) ( $<1.3$  mmol/L) plasma HDL-C concentrations. These subjects had high LDL-C ( $>2.6$  mmol/L) and were also classified as having MetS, two conditions that increase CVD risk. A secondary objective was to determine whether dietary components were related to low concentrations of HDL-C.

## Subjects and Methods

### Study design

The subjects for this study were recruited from 3 different locations: the University of Connecticut (Storrs, CT) ( $n=29$ ), University of Florida (Jacksonville, FL) ( $n=47$ ), and University of California (Irvine, CA) ( $n=13$ ). The inclusion criteria were women with MetS, having at least 2 of the following characteristics: blood pressure  $\geq 130/85$  mm Hg or treated hypertension, waist circumference (WC)  $>88$  cm, fasting glucose  $\geq 5.6$  mmol/L, and HDL-C  $<1.3$  mmol/L, in addition to high TG ( $\geq 1.7$  mmol/L) and LDL-C  $\geq 2.6$  mmol/L.

The subjects were recruited by word of mouth, distribution e-mails, newspaper and radio advertisements, and flyers. The exclusion criteria were low LDL-C ( $<2.6$  mmol/L), TG  $<1.7$  mmol/L, age less than 25 or older than 75 y, pregnancy, lactation, thyroid problems, stroke, heart disease, or use of medication or supplements relevant to diabetes or CVD such as hypoglycemic or cholesterol lowering agents. A total of 89 women were recruited for the study. All protocols were approved by the Institutional Review Boards of the respective universities.

### Anthropometrics and blood pressure

After obtaining a consent agreement from each participant, weight and height were measured and body mass index (BMI) was calculated ( $\text{kg}/\text{m}^2$ ). WC was calculated by measuring at the superior border of the iliac crest using a flexible tape. Blood pressure (systolic and phase-V diastolic) was measured on the left arm with the subjects seated, after at least 5 minutes rest, using an automated blood pressure monitor (Omron, Healthcare Inc., Bannockburn, IL). Three separate recordings were made and the mean was used.

### Diet analysis

The subjects completed a 3-d diet record including one week-end day to assess energy, carbohydrate, fat, protein, dietary fiber, and cholesterol intake as well as glycemic load, which was automatically calculated. The dietary records were analyzed using The Nutrition Data System 9.0 (Minneapolis, MN).

### Laboratory measurements

After a 12-h overnight fast, 60 ml of fasting blood was collected from all participants. Plasma was separated from red blood cells by centrifugation at  $2000 \times g$ , aliquoted, and then frozen at  $-80^\circ\text{C}$  for further analysis. Plasma lipids, glucose, insulin, apo B, and apo A-I were measured in a certified laboratory (Northwest Lipid Research Laboratories, Seattle, WA). Plasma glucose and lipids were assayed using a Vitros 950IRC analyzer (Ortho-Clinical Diagnostics, Rochester, NY). LDL-C was determined indirectly using the Friedewald equation [14]. Apo A-I and B were analyzed by turbidimetry using an Advia 1650<sup>®</sup> (Bayer Diagnostics, Tarrytown, NY). Insulin was determined by a chemiluminescent, immunometric assay using a DPC Immulite 2000 (Diagnostics Products Corporation, Nutley, NJ). The homeostasis model assessment (HOMA) [15] was calculated as a measure of insulin resistance.

### Lipoprotein subfractions and size

H-NMR analysis was performed (LipoScience, Inc., Raleigh, NC) using a 400 MHz NMR analyzer (Bruker BioSpin Corp, Billerica, MA) as previously described [13]. NMR simultaneously quantifies  $>30$  lipoprotein subclasses that are empirically grouped into 9 smaller subclasses based on the following particle diameters: large VLDL (35-60 nm), medium VLDL (27-35 nm), small VLDL (23-27 nm), large LDL (21.2-23 nm), medium LDL (19.8-21.2 nm), small LDL (18-19.8 nm), large HDL (8.8-13 nm), medium HDL (8.2-8.8 nm), and small HDL (7.3-8.2 nm). Weighted-average lipoprotein particle sizes in diameter were calculated based on the diameter of each lipoprotein subclass multiplied by its respective relative concentration.

### Apolipoproteins A-II, C-II, C-III, and E

Apolipoproteins were measured using LINCOplex: Multiplex Biomarker Immunoassay for Luminex Instrumentation/xMAP Technology (Austin, TX). This technique uses fluorescently labeled microsphere beads with antibodies specific to each individual apolipoprotein [16]. The technique is well standardized in our laboratory [11].

### LDL oxidation and Lipoprotein(a)

LDL oxidation was measured by ELISA kits (ALPCO, Salem, NH) using the monoclonal antibody 4E6, which has been utilized in numerous clinical trials [17]. The standards and samples were read at 450 nm in a spectrophotometer (Spectramax Multimode Spectrophotometer, Sunnyvale, CA). Using a polynomial curve, concentrations of oxidized LDL were calculated and expressed as mmol/L. Plasma Lp(a) was determined in duplicate using a sandwich ELISA (Diagnostic Automation, Inc., Calabasas, CA) with a dynamic range of 0.04-5.89  $\mu\text{mol}/\text{L}$ . Absorbance was

determined using the same spectrophotometer as previously reported [18].

#### Adiponectin and intercellular adhesion molecule-1 (sICAM-1)

From fasting plasma, ICAM-1 and adiponectin were measured in duplicate in the same assay using Human CVD Panel 1 LINCOplex kits. Samples were diluted 1:100 and simultaneously quantified by using Antibody-Immobilized Beads and Luminex xMAP technology. All assays were carried out on the same day to decrease variability. The coefficient variation was 2-6%. The sensitivities for sICAM-1 and adiponectin were 9.0 pg/ml and 56.0 pg/ml, respectively, as previously reported [19].

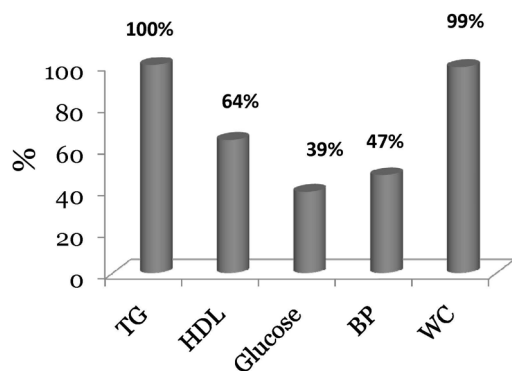
#### Statistical analysis

Data are presented as mean  $\pm$  SD for the measured parameters. Since we had different numbers of subjects in the low and high HDL-C groups, the non-parametric Mann-Whitney U test was performed to assess differences in plasma lipids, apolipoproteins, diet, inflammatory markers, and lipoprotein size and subfractions.  $P < 0.05$  was considered significant. Pearson correlations were conducted between HDL-C and the different lipoprotein sizes.

## Results

All 89 women recruited for the study were classified as having MetS. All women (100%) had high TG ( $\geq 1.7$  mmol/L), as this was one of the inclusion criteria, and 99% had WC  $> 88$  cm. Fasting blood glucose  $> 5.6$  mmol/L, high blood pressure ( $\geq 130/85$  mm Hg), and low HDL-C ( $< 1.3$  mmol/L) accounted for 39, 47, and 64% of the subjects, respectively (Fig. 1).

We divided the women into two groups according to the following concentrations of plasma HDL-C: H-HDL  $\geq 1.3$  mmol/L and L-HDL  $< 1.3$  mmol/L, to evaluate whether lower HDL-C would be associated with higher risk for atherosclerosis.



**Fig. 1.** Percent of subjects with waist circumference (WC)  $> 88$  cm, blood pressure  $> 130/85$  mm Hg; plasma glucose  $> 100$  mg/dL (5.6 mmol/L); plasma triglycerides (TG)  $> 150$  mg/dL (1.7 mmol/L) and HDL  $< 50$  mg/dL (1.3 mmol/L) in women classified with metabolic syndrome (MetS) ( $n = 89$ )

**Table 1.** Anthropometrics, blood pressure, plasma lipids, glucose, insulin and apolipoproteins (apo) of women classified with MetS having low ( $< 1.3$  mmol/L) or high ( $\geq 1.3$  mmol/L) HDL-C<sup>1)</sup>

Parameter	High HDL-C (n = 32)	Low HDL-C (n = 57)
Age (yr)	47.6 $\pm$ 9.8	46.6 $\pm$ 10.7
Weight (kg)	93.0 $\pm$ 17.0	90.3 $\pm$ 13.1
BMI (kg/m <sup>2</sup> )	34.2 $\pm$ 5.7	34.0 $\pm$ 5.0
WC (cm)	110.7 $\pm$ 11.9	107.6 $\pm$ 10.5
Systolic (mm Hg)	127.8 $\pm$ 13.3	126.8 $\pm$ 15.9
Diastolic (mm Hg)	81.0 $\pm$ 7.7	78.8 $\pm$ 8.9
TC (mmol/L)	5.96 $\pm$ 0.80	5.66 $\pm$ 0.85
LDL-C (mmol/L)	3.50 $\pm$ 0.85	3.56 $\pm$ 0.72
HDL-C (mmol/L)	1.48 $\pm$ 0.16	1.03 $\pm$ 0.16**
TG (mmol/L)	2.17 $\pm$ 0.38	2.37 $\pm$ 0.67
Glucose (mmol/L)	4.86 $\pm$ 0.70	4.97 $\pm$ 0.80
Insulin ( $\mu$ U/mL)	13.2 $\pm$ 6.2	18.8 $\pm$ 9.9*
Insulin resistance (HOMA)	3.2 $\pm$ 1.6	4.5 $\pm$ 2.8*
Adiponectin (mg/L)	16.4 $\pm$ 6.8	13.8 $\pm$ 7.4*
ICAM (mg/L)	0.094 $\pm$ 0.027	0.17 $\pm$ 0.049*
Apo B (mg/L)	1,115.3 $\pm$ 210.8	1,216.0 $\pm$ 217.4
Apo A-I (mg/L)	1711 $\pm$ 250	1415 $\pm$ 161**
Apo A-II (mg/L)	225.5 $\pm$ 83.8	168.5 $\pm$ 51.7**
Apo C-II (mg/L)	100.9 $\pm$ 34.1	87.0 $\pm$ 38.7*
Apo C-III (mg/L)	284.6 $\pm$ 99.3	252.9 $\pm$ 119.5*
Apo E (mg/L)	75.9 $\pm$ 37.2	71.5 $\pm$ 28.3

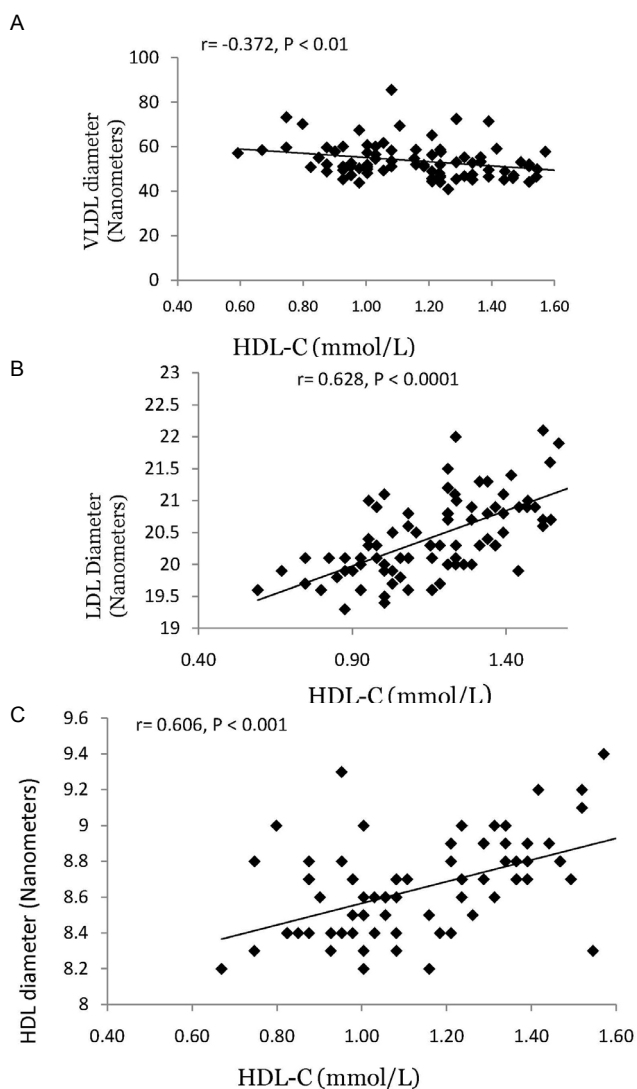
<sup>1)</sup> Values are mean  $\pm$  SD for the number of subjects indicated in parentheses. \* Significantly different at  $P < 0.05$ , \*\* Significantly different at  $P < 0.001$  as determined by Mann-Whitney U non-parametric test

**Table 2.** Total Energy, fat, carbohydrate, protein, dietary cholesterol and dietary fiber intake obtained from a 3-d dietary record of women classified with MetS having low ( $< 1.3$  mmol/L) or high ( $\geq 1.3$  mmol/L) HDL-C<sup>1)</sup>

Parameter	High HDL-C (n = 32)	Low HDL-C (n = 57)
Total energy (Kjoules/d)	8,622 $\pm$ 3,349	8,921 $\pm$ 3,374
Total fat (g/d)	101.8 $\pm$ 55.0	92.6 $\pm$ 46.9
Total fat (% energy)	38.5 $\pm$ 7.4	36.5 $\pm$ 7.7
Saturated fat (% energy)	13.5 $\pm$ 3.5	12.0 $\pm$ 3.2*
Monounsaturated fat (% energy)	14.8 $\pm$ 4.5	13.6 $\pm$ 3.1
Polyunsaturated fat (% energy)	9.5 $\pm$ 8.3	7.9 $\pm$ 3.2
Trans fatty acids (g/d)	4.4 $\pm$ 2.9	5.4 $\pm$ 3.5
Omega-3 fatty acids (g/d)	1.9 $\pm$ 1.2	1.9 $\pm$ 1.1
Total carbohydrate (g/d)	211.5 $\pm$ 101.3	234.2 $\pm$ 86.4
Carbohydrate (% energy)	42.2 $\pm$ 8.1	44.7 $\pm$ 10.0
Total sugars (g/d)	76.4 $\pm$ 48.9	103.1 $\pm$ 58.9*
Added sugars (g/d)	48.5 $\pm$ 42.0	76.4 $\pm$ 56.2*
Glycemic Load	116.4 $\pm$ 60.4	133.3 $\pm$ 53.2*
Total protein (g/d)	79.7 $\pm$ 41.7	86.1 $\pm$ 29.6
Protein (% energy)	15.9 $\pm$ 4.0	17.0 $\pm$ 4.4
Dietary cholesterol (mg/d)	321.2 $\pm$ 168.9	331.0 $\pm$ 173.
Alcohol (% energy)	3.2 $\pm$ 5.2	2.4 $\pm$ 5.8*
Total fiber (g/d)	18.3 $\pm$ 10.8	16.4 $\pm$ 8.1
Soluble fiber (g/d)	5.6 $\pm$ 3.1	5.3 $\pm$ 2.5
Insoluble fiber (g/d)	12.5 $\pm$ 8.3	10.9 $\pm$ 6.4

<sup>1)</sup> Values are mean  $\pm$  SD.

\* Indicates significantly different ( $P < 0.05$ ) as determined by Mann-Whitney non-parametric test



**Fig. 2.** Correlations between HDL-C and VLDL size (panel A) HDL size (panel B) and LDL size (panel C)

As indicated in Table 1, age, BMI, WC, weight, and systolic and diastolic blood pressure were not different between groups. Likewise, the risk factors of total cholesterol, LDL-C, apo B, apo E, and glucose were not different between the high and low HDL groups. However, HDL-C (by definition), apo A-I, apo A-II, apo C-II, and apo C-III were higher in the H-HDL group. Insulin, HOMA, adiponectin, and ICAM were lower ( $P < 0.0001$ ) in the H-HDL group (Table 1).

Regarding dietary intake, women from the H-HDL group consumed more saturated fat, as well as energy from alcohol ( $P < 0.05$ ), while they consumed less sugar and added sugar than subjects in the L-HDL group (Table 2). Other dietary components including total fat, protein, dietary cholesterol, fiber, and different types of fatty acids were not different between groups. Positive correlations were found between HDL-C and LDL size ( $r = 0.628$ ,  $P < 0.0001$ ) and HDL size ( $r = 0.606$ ,  $P < 0.0001$ ), and

**Table 3.** Number of VLDL, IDL, LDL and HDL particles according to size, apolipoproteins, VLDL, LDL and HDL diameters, LDL oxidation and Lp(a) of women classified with MetS having low ( $< 1.3$  mmol/L) or high ( $\geq 1.3$  mmol/L) HDL-C<sup>1)</sup>

Parameter	High HDL-C (n = 32)	Low HDL-C (n = 57)
VLDL diameter (nm)	51.1 ± 7.0	54.7 ± 8.1*
Total VLDL (mmol/L)	97 ± 33	104 ± 39
Large VLDL (mmol/L)	4.8 ± 3.1	8.4 ± 7.8*
Medium VLDL (mmol/L)	35.9 ± 17.5	41.6 ± 24.6
Small VLDL (mmol/L)	56.5 ± 18.6	53.6 ± 20.7
IDL (mmol/L)	65.4 ± 53.1	87.1 ± 49.7*
LDL diameter (nm)	21.0 ± 0.6	20.2 ± 0.6*
Total LDL (mmol/L)	1490 ± 366	1734 ± 345*
Large LDL (mmol/L)	513 ± 158	288 ± 169*
Small LDL (mmol/L)	932 ± 388	1,359 ± 384*
HDL diameter (nm)	8.9 ± 0.3	8.6 ± 0.2*
Total HDL (mmol/L)	39.5 ± 6.2	33.6 ± 4.9*
Large HDL (mmol/L)	8.3 ± 3.5	4.2 ± 2.4*
Medium HDL (mmol/L)	6.9 ± 5.6	6.3 ± 4.6
Small HDL (mmol/L)	24.3 ± 6.9	23.1 ± 5.3
Oxidized LDL (µg/L)	112.1 ± 94.8	118.6 ± 98.7
Lp(a) (µmol/L)	0.92 ± 0.81	0.69 ± 0.72

<sup>1)</sup> Values are mean ± SD. Values in a row with different superscripts are significantly different ( $P < 0.01$ ) as determined by Mann-Whitney non-parametric test

a negative correlation was found between HDL-C and VLDL size ( $r = -0.327$ ,  $P < 0.01$ ) (Fig. 2).

Concentrations of lipoprotein subclasses, lipoprotein size, oxidized LDL, and Lp(a), which are major biomarkers of atherosclerosis risk, were also evaluated in women with high or low concentrations of HDL-C (Table 3). The H-HDL group presented a less atherogenic lipoprotein profile with lower concentrations of large VLDL, small LDL, and IDL compared to the L-HDL group. Furthermore, lipoprotein size was modified by the concentration of HDL-C in plasma. Women with L-HDL had lower numbers of large LDL ( $P < 0.0001$ ), and therefore a smaller LDL diameter ( $P < 0.01$ ) than those from the H-HDL group. The L-HDL group also had larger VLDL ( $P < 0.05$ ) and smaller HDL ( $P < 0.05$ ) diameters than the H-HDL group (Table 3). Concentrations of oxidized LDL and Lp(a) had a wide range among participants; however, they were not different between HDL groups (Table 3).

## Discussion

In this study we evaluated the adverse effects of HDL-C in concentrations of less than 1.3 mmol/L in women with high risk for CVD, mainly a population classified with MetS and the additional risk factor of plasma LDL-C  $\geq 2.6$  mmol/L. Our data analysis suggests that HDL-C  $< 1.3$  mmol/L further increases the risk of CVD and atherosclerosis. The novel aspect of this study is that L-HDL appears to be a biomarker of elevated concentrations of circulating atherogenic lipoproteins as well as increased insulin resistance and lower concentrations of

adiponectin, all of which are key biomarkers of increased risk for type 2 diabetes and coronary heart disease. Although participants in this study had dietary habits that might increase risk for heart disease such as a high intake of *trans* fatty acids and low intakes of omega-3 fatty acids and dietary fiber, high simple sugar intake and high glycemic load were the dietary components that might have been correlated with low concentrations of HDL-C.

A main concern regarding MetS is the predisposition to glucose intolerance, insulin resistance, and diabetes [2]. The consumption of foods with a low glycemic index has been advocated for amelioration of dysfunctional glucose metabolism for more than two decades [20]. Diets based on varying degrees of carbohydrate restriction also have demonstrated efficacy in improving glucose metabolism and associated metabolic aberrations [10]. In the present study, the women with lower HDL-C (<1.3 mmol/L) consumed more sugar and had higher glycemic loads than those from the H-HDL group. Interestingly, women from the L-HDL groups also consumed less alcohol and less saturated fat. Moderate increases in alcohol have been correlated with higher HDL-C and paraoxanase-1, suggesting a protective effect against CVD [21], and all fatty acids including saturated fatty acids (with the exception of *trans* fat) have been correlated with increased HDL-C [22]. Thus, the above results are not surprising.

The women with L-HDL also had higher concentrations of plasma insulin and greater insulin resistance as determined by HOMA, along with lower concentrations of adiponectin. In agreement with our results, low adiponectin concentrations are strongly correlated with insulin resistance [23]. Data from epidemiological studies also indicate that circulating adiponectin is reduced in patients with CVD and diabetes [24]. To further support our findings that HDL-C concentrations predict insulin resistance, in a recent study, TG/HDL-C was used as a marker of insulin resistance in obese patients [25]. In the present study, plasma TG levels did not differ between groups and subjects from the H-HDL group had higher apo C-III than those from the L-HDL group. These findings suggest that the higher levels of apo C-III in the H-HDL group were related to the increased number of HDL particles available to transport this apolipoprotein. While apo C-III present in VLDL is associated with decreased lipoprotein lipase activity, apo C-III transported by HDL indicates a reservoir of this apolipoprotein [26]. Overall, subjects from the L-HDL group appear to be at greater risk for the development of diabetes as documented by more elevated insulin resistance and lower levels of adiponectin.

Subjects with L-HDL presented increased concentrations of the atherogenic lipoproteins large VLDL, small LDL, and IDL. Furthermore, these women had lower concentrations of the larger, more buoyant LDL that is considered less atherogenic [27]. In addition, impaired endothelial function can be assessed by measuring the level of molecules secreted by the endothelium, such as sICAM1 [28]. Subjects with L-HDL had increased concentrations of this adhesive molecule, which poses at higher

risk for CVD.

Abnormalities in VLDL particle size seem to be a major contributing factor to dysfunctional lipoprotein metabolism [29]. Large VLDL particles are classified as atherogenic for two main reasons: their ability to interact with macrophages in the arterial wall [30] and their easy conversion to small LDL [31]. In addition to transporting high concentrations of plasma TG, large VLDL also carry high concentrations of cholesterol (5 times more than an LDL particle) [30]. These large VLDL are taken up by macrophages through cell surface membrane-binding proteins leading to the formation of foam cells and the initiation of atherosclerosis. In addition, through the delipidation cascade, TG-rich VLDL are precursors for the formation of small, dense LDL particles and increased HDL catabolism [32]. The phenotype characterized by a predominance of small LDL particles has been termed *pattern B* and is typical of MetS and diabetes [27]. Small, dense LDL particles are considered more atherogenic due to their decreased binding to the LDL receptor, leading to increased plasma residence time and an increased susceptibility to oxidation [33]. In addition, increased levels of small LDL in plasma are associated with increased risk of coronary heart disease [33]. IDL, also known as VLDL remnants, are part of TG-rich lipoproteins and are associated with increased risk for heart disease [34]. All of these atherogenic lipoproteins were higher in women with lower concentrations of HDL-C. Another observation was that the groups of women with HDL-C > 1.3 mmol/L had lower numbers of both total HDL and large HDL particles. Since the main function of HDL is to remove cholesterol and oxysterols from extra-hepatic cells including smooth muscle cells, endothelial cells, and macrophages through ABCA1 and ABCG1 transporters [35], a higher number of particles suggest a more efficient reverse cholesterol transport and increased protection against atherosclerosis.

Both Lp(a) levels and oxidized LDL concentrations did not differ between HDL groups. Plasma Lp(a) concentrations are reported to have a strong ethnic influence and is correlated with increased risk for heart disease both through its atherogenic and prothrombotic properties [36]. Specifically, concentrations > 0.71-1.07  $\mu\text{mol/L}$  are associated with 1.5- to 3-fold increases of coronary atherosclerosis independent of plasma levels of other lipoproteins [36]. In the current study with women at high risk for CVD, levels of Lp(a) varied between 0.21 and 4.14  $\mu\text{mol/L}$ . Low HDL-C was not associated with higher concentrations of Lp(a) in this group of women. Oxidized LDL has been correlated with atherosclerosis, diabetes, and renal disease [37]. Small significant amounts of oxidized LDL have been detected in plasma by use of monoclonal antibodies specific to epitopes of oxidized apo B [17]. Among these, 4E6 recognizes MDA modified lysine epitopes and has been extensively used in human studies [17]. Similar to our findings for Lp(a), the women in the current study presented a wide range of oxidized LDL, from 1.4 to 445  $\mu\text{g/L}$ . In this group of women, concentrations of both biomarkers, Lp(a) and oxidized LDL, for increased risk of CVD,

were independent of HDL-C concentrations.

The data from this study suggest that low plasma HDL-C (< 1.3 mmol/L) is associated with increased risk of CVD and diabetes in women already at risk, and appears to be a biomarker of a greater atherogenic lipoprotein profile (decreased large VLDL and small LDL). In addition, low concentrations of HDL-C are related to a lower number of HDL particles, decreased HDL size, and decreased large HDL, conditions that suggest the existence of a less efficient reverse cholesterol transport. Finally, individuals with low concentrations of HDL-C were at increased risk for diabetes, as they presented increased insulin resistance and lower concentrations of adiponectin.

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