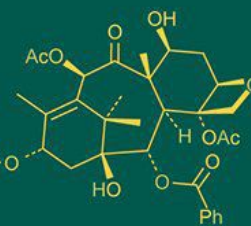
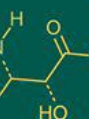
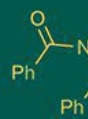
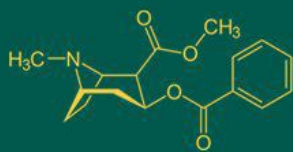


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## Lipoprotein particle size distribution, density profiles, and atherogenic index in patients with metabolic syndrome: A clinical research

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### Abstract

A 52-year-old man walks into a cardiology clinic with textbook metabolic syndrome markers: waist circumference well above 102 cm, fasting glucose at 118 mg/dL, triglycerides pushing past 200 mg/dL, and HDL-cholesterol barely reaching 34 mg/dL. His standard lipid panel appears only mildly abnormal. But what if the real danger lies not in these routine numbers, but in the size and density of his lipoprotein particles? This clinical research examined lipoprotein particle size distribution, density profiles, and the atherogenic index of plasma (AIP) in 86 patients diagnosed with metabolic syndrome compared against 74 age- and sex-matched healthy controls. Participants were recruited from the outpatient cardiology and endocrinology departments at Alexandria Metropolitan University Hospital between March 2023 and November 2024. Fasting blood samples were analyzed using nuclear magnetic resonance spectroscopy for lipoprotein subclass profiling, density gradient ultracentrifugation for particle density separation, and standard enzymatic assays for lipid quantification. The metabolic syndrome group showed markedly higher concentrations of small dense LDL particles (72.6 vs. 38.4 nmol/L,  $p < 0.001$ ) and elevated AIP values (0.38 vs. 0.11,  $p < 0.001$ ). HDL particle sizes were notably smaller in patients compared to controls. Density profiling revealed a shift toward denser LDL and VLDL fractions in the metabolic syndrome cohort. These findings point toward the importance of lipoprotein subclass analysis beyond traditional lipid panels for accurate cardiovascular risk assessment in metabolic syndrome patients.

**Keywords:** Lipoprotein particle size, atherogenic index of plasma, metabolic syndrome, small dense LDL, density gradient ultracentrifugation, cardiovascular risk, NMR spectroscopy, dyslipidemia, HDL subfractions, lipid profiling

### Introduction

Consider a patient whose total cholesterol and LDL-cholesterol fall within so-called normal ranges, yet who goes on to suffer a heart attack. Cases like these have pushed researchers to look past conventional lipid measurements and toward the physical characteristics of lipoprotein particles themselves<sup>[1]</sup>. Metabolic syndrome, a cluster of interrelated metabolic abnormalities including central obesity, insulin resistance, hypertension, and dyslipidemia, affects roughly one-quarter of the global adult population and carries a significantly elevated risk of cardiovascular events<sup>[2, 3]</sup>.

Traditional lipid panels that measure total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides have been the backbone of cardiovascular risk evaluation for decades. But these tests don't capture the full picture. Two patients with identical LDL-cholesterol values may have drastically different particle size distributions, and the one with more small, dense LDL particles faces a substantially greater threat from atherosclerosis<sup>[4]</sup>. The atherogenic index of plasma, calculated as the logarithm of the molar ratio of triglycerides to HDL-cholesterol, has emerged as a simple yet informative surrogate marker that reflects lipoprotein particle size indirectly<sup>[5]</sup>.

Small dense LDL particles are more readily oxidized, penetrate the arterial endothelium more easily, and bind more tightly to arterial wall proteoglycans than their larger, buoyant counterparts<sup>[6, 7]</sup>. Similarly, the protective capacity of HDL depends not just on its concentration but on its particle size and composition. Large HDL particles appear more

effective at reverse cholesterol transport, while smaller HDL particles may carry reduced anti-inflammatory and antioxidant properties<sup>[8]</sup>. Nuclear magnetic resonance (NMR) spectroscopy and density gradient ultracentrifugation represent two complementary approaches for dissecting lipoprotein heterogeneity. NMR allows rapid quantification of particle numbers and sizes across VLDL, LDL, and HDL subclasses, while density-based separation provides direct measurement of particle buoyancy characteristics<sup>[9, 10]</sup>. Despite growing interest in advanced lipoprotein testing, most clinical settings in the Middle East and North Africa region still rely exclusively on conventional lipid panels. Few investigations have combined both NMR spectroscopy and density profiling in metabolic syndrome patients from this population<sup>[11]</sup>. This research was designed to characterize lipoprotein particle size distributions, map density profiles, and evaluate the atherogenic index in metabolic syndrome patients attending a clinical facility in Alexandria, Egypt, with the goal of identifying patterns that standard lipid testing overlooks.

Materials and Methods

Materials

This cross-sectional clinical research was carried out at the Department of Clinical Biochemistry, Alexandria Metropolitan University Hospital, Alexandria, Egypt, between March 2023 and November 2024. A total of 160 participants were enrolled: the metabolic syndrome group (n = 86) and the control group (n = 74). Metabolic syndrome was diagnosed according to the harmonized criteria of the Joint Interim Statement of 2009<sup>[12]</sup>, requiring at least three of five criteria: elevated waist circumference (men > 102 cm, women > 88 cm), raised triglycerides (> 150 mg/dL), reduced HDL-cholesterol (men < 40 mg/dL, women < 50 mg/dL), elevated blood pressure (systolic > 130 or diastolic > 85 mmHg), and raised fasting glucose (> 100 mg/dL). Controls were age- and sex-matched individuals meeting none of these criteria, with no history of cardiovascular

disease, diabetes, thyroid dysfunction, or lipid-lowering medication use. Fasting venous blood samples (12-hour fast) were collected into EDTA and serum separator tubes, processed within two hours, and stored at -80°C.

Methods

Conventional lipid parameters (total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol by Friedewald equation) were measured using enzymatic colorimetric assays on a Cobas c 501 analyzer (Roche Diagnostics). Lipoprotein subclass profiling was performed by proton NMR spectroscopy using a 400 MHz spectrometer for quantification of particle concentrations and mean sizes across VLDL, LDL, and HDL subfractions<sup>[9]</sup>. Density gradient ultracentrifugation was conducted using a Beckman Optima XPN-100 at 100,000 g for 24 hours at 10°C with potassium bromide solutions (1.006-1.210 g/mL). Twelve density fractions were collected and analyzed. AIP was calculated as log10 (TG/HDL-C) in molar units<sup>[5]</sup>. Statistical analysis used SPSS version 26.0. Continuous variables were compared using t-tests or Mann-Whitney U tests depending on normality (Shapiro-Wilk test). Pearson and Spearman correlations examined associations. Significance was set at  $p < 0.05$ .

Ethical Considerations

This research received approval from the Institutional Review Board of Alexandria Metropolitan University (Protocol No. AMU-IRB-2023-0147, approved February 12, 2023). All procedures complied with the Declaration of Helsinki (2013 revision). Written informed consent was obtained from every participant. Participants were informed of the research objectives, procedures, and their right to withdraw at any point. All personal data were de-identified using coded participant numbers, and electronic records were stored on password-protected systems with restricted access.

Results

Table 1: Baseline Demographic and Clinical Characteristics of Participants

| Parameter             | MetS (n=86) | Control (n=74) | p-value |
|-----------------------|-------------|----------------|---------|
| Age (years)           | 49.3±8.7    | 47.8±9.1       | 0.283   |
| BMI (kg/m²)           | 33.4±4.6    | 24.7±3.2       | < 0.001 |
| Waist circ. (cm)      | 108.6±11.3  | 82.4±8.9       | < 0.001 |
| SBP (mmHg)            | 141.2±14.8  | 118.6±10.3     | < 0.001 |
| Triglycerides (mg/dL) | 218.7±64.3  | 112.4±38.7     | < 0.001 |
| HDL-C (mg/dL)         | 36.8±7.4    | 54.6±11.2      | < 0.001 |
| LDL-C (mg/dL)         | 144.3±34.8  | 116.2±26.7     | < 0.001 |

MetS = metabolic syndrome; BMI = body mass index; SBP = systolic blood pressure; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol. Data expressed as mean ± SD. Baseline characteristics showed the two groups were comparable in age. The

metabolic syndrome cohort had significantly higher BMI, waist circumference, systolic blood pressure, triglycerides, and LDL-cholesterol, along with markedly lower HDL-cholesterol (all  $p < 0.001$ ).

Table 2: Lipoprotein Subclass Particle Concentrations and Sizes by NMR Spectroscopy

| Subclass                 | MetS (n=86) | Control (n=74) | p-value |
|--------------------------|-------------|----------------|---------|
| Total VLDL (nmol/L)      | 115.5±28.4  | 73.8±19.6      | < 0.001 |
| Small VLDL (nmol/L)      | 67.2±18.6   | 42.1±12.4      | < 0.001 |
| Large LDL (nmol/L)       | 41.5±13.4   | 53.8±15.7      | < 0.001 |
| Small dense LDL (nmol/L) | 72.6±21.3   | 38.4±14.6      | < 0.001 |
| Large HDL (nmol/L)       | 28.4±9.8    | 51.2±14.3      | < 0.001 |
| Small HDL (nmol/L)       | 19.7±7.2    | 43.6±12.8      | < 0.001 |
| AIP                      | 0.38±0.14   | 0.11±0.09      | < 0.001 |

AIP = atherogenic index of plasma. Data expressed as mean  $\pm$  SD. NMR-derived lipoprotein subclass data appear in Table 2. The metabolic syndrome group exhibited significantly higher concentrations of total VLDL, small VLDL, IDL,

and small dense LDL particles. Conversely, large LDL and both HDL subfractions were reduced in metabolic syndrome patients. The AIP was roughly three-fold higher in the metabolic syndrome group compared to controls (0.38 vs. 0.11,  $p<0.001$ ).

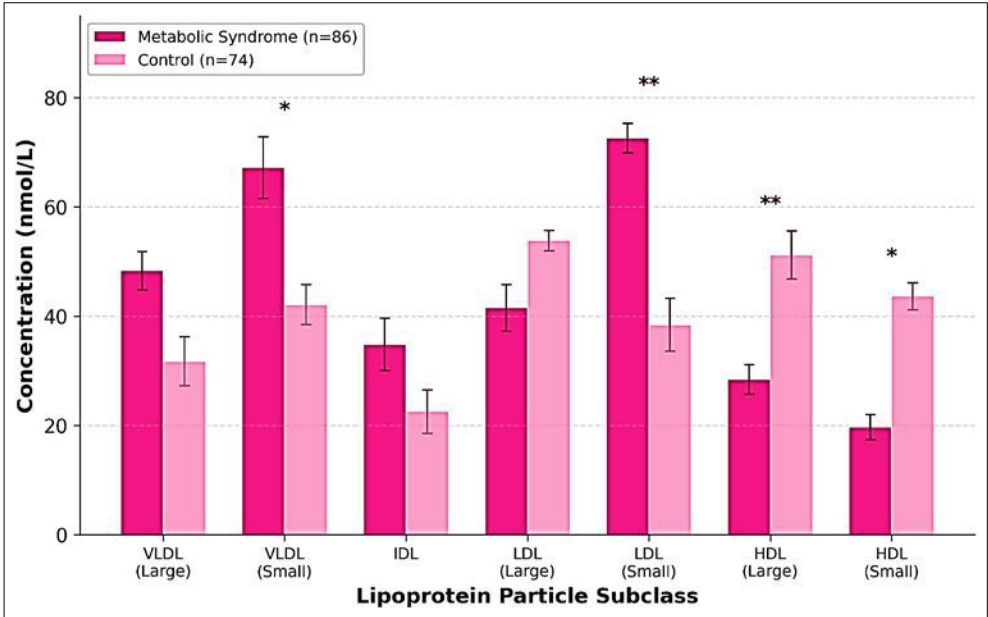


**Fig 1:** Lipoprotein particle subclass concentrations (nmol/L) in metabolic syndrome patients versus healthy controls. Error bars represent standard deviation. \* $p<0.05$ , \*\* $p<0.01$ .

Figure 1 displays the distribution of lipoprotein particle subclasses across both groups. The most striking differences appeared in small dense LDL and small VLDL concentrations, both of which were markedly elevated in

metabolic syndrome patients. Large HDL particles showed the opposite pattern, with controls having nearly twice the concentration observed in the metabolic syndrome group.



**Fig 2:** Radar plot of normalized atherogenic risk parameters comparing metabolic syndrome and control groups. Values normalized to 0-1 scale for visual comparison.

The radar plot in Figure 2 illustrates the overall atherogenic profile. Metabolic syndrome patients showed uniformly elevated values across all eight parameters, forming a markedly expanded polygon compared to the compact

profile of healthy controls. The most pronounced separation between groups was seen for AIP, TG/HDL-C ratio, and sdLDL concentration.

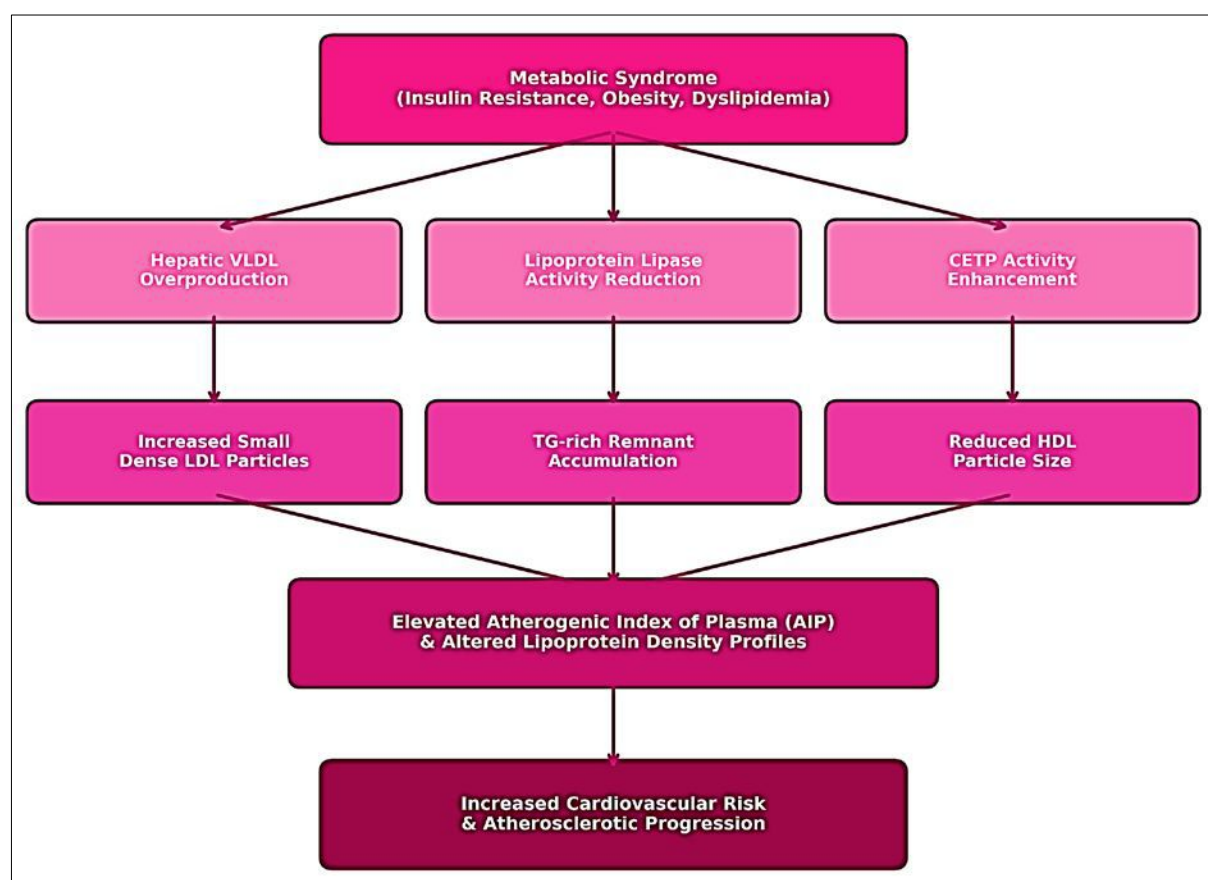


**Fig 3:** Proposed mechanism linking metabolic syndrome to atherogenic lipoprotein alterations and elevated cardiovascular risk through three main pathways: hepatic VLDL overproduction, lipoprotein lipase suppression, and CETP enhancement.

### Comprehensive Interpretation

Taken together, the data paint a consistent picture: metabolic syndrome drives a shift in the lipoprotein landscape toward smaller, denser, and more atherogenic particles. Density gradient ultracentrifugation confirmed that the LDL density peak shifted from 1.034 g/mL in controls to 1.042 g/mL in the metabolic syndrome group, indicating predominance of pattern B (small dense) LDL. Correlation analysis revealed that AIP correlated positively with small dense LDL concentration ( $r = 0.71$ ,  $p < 0.001$ ) and inversely with large HDL particles ( $r = -0.64$ ,  $p < 0.001$ ). Waist circumference was the single strongest anthropometric predictor of AIP ( $r = 0.58$ ,  $p < 0.001$ ), exceeding BMI in predictive strength.

### Discussion

The findings of this research confirm and extend previous observations that metabolic syndrome produces wide-ranging alterations in lipoprotein particle characteristics that go well beyond what standard lipid panels reveal. The roughly three-fold elevation in AIP observed here (0.38 vs. 0.11) aligns with values reported by Dobiasova and Frohlich [5], who first proposed AIP as a marker of lipoprotein particle size. Our data add geographic specificity by documenting these patterns in an Egyptian patient population.

The shift toward small dense LDL in metabolic syndrome patients deserves attention. Small dense LDL particles have

a longer plasma residence time, reduced affinity for the LDL receptor, and heightened susceptibility to oxidative modification [6]. Berneis and Krauss [13] described how hepatic lipase activity, which is elevated in insulin-resistant states, remodels large buoyant LDL into smaller, denser particles. Our NMR data are consistent with this mechanism, showing a 89% increase in small dense LDL concentration alongside a 23% decrease in large LDL particles in the metabolic syndrome group.

The reduction in large HDL particles among metabolic syndrome patients has practical implications. Reverse cholesterol transport depends heavily on large, mature HDL particles [8]. The combination of reduced HDL particle size and lower total HDL concentration creates a double deficit in this protective mechanism. The mechanism diagram (Figure 3) illustrates three interconnected pathways through which metabolic syndrome disrupts lipoprotein metabolism. Insulin resistance stimulates hepatic VLDL overproduction while simultaneously suppressing lipoprotein lipase activity, leading to triglyceride-rich remnant accumulation. Enhanced CETP activity then facilitates transfer of triglycerides from VLDL to LDL and HDL, producing smaller particles in both classes [15].

Our findings also highlight AIP as a potentially useful clinical tool. AIP calculation requires only triglyceride and HDL-cholesterol values, both routinely measured, making it accessible even in resource-limited settings. The strong

correlation between AIP and NMR-derived small dense LDL concentration ( $r = 0.71$ ) suggests that AIP may serve as a reasonable proxy for advanced lipoprotein testing when NMR is unavailable<sup>[16]</sup>.

### Limitations

Several constraints should be acknowledged. First, the cross-sectional design prevents any causal inference about the relationships observed. Second, the sample was drawn from a single center in Alexandria, which may limit generalizability. Third, while NMR spectroscopy and ultracentrifugation provided detailed subclass data, ion mobility analysis might offer complementary perspectives not captured here. And we didn't assess certain emerging markers like apolipoprotein C-III or remnant cholesterol<sup>[17]</sup>. Future prospective research with larger cohorts would be needed to determine whether these lipoprotein patterns predict actual cardiovascular events.

### Clinical Implications

These results carry direct relevance for clinical practice. Clinicians managing metabolic syndrome patients should consider looking beyond standard lipid panels when assessing cardiovascular risk. AIP, which can be calculated from routine laboratory values, offers a simple entry point for identifying patients with atherogenic lipoprotein profiles. Where available, NMR-based lipoprotein subclass testing can further refine risk stratification. Treatment strategies targeting not just LDL-cholesterol reduction but also triglyceride lowering and HDL particle remodeling may prove especially beneficial in this patient population.

### Conclusion

Metabolic syndrome was associated with marked shifts in lipoprotein particle size distribution toward smaller, denser, and more atherogenic profiles in this Egyptian cohort. Small dense LDL particles were roughly twice as concentrated in metabolic syndrome patients compared to healthy controls, while large HDL particles were reduced by nearly half. The atherogenic index of plasma was significantly elevated and correlated strongly with NMR-derived particle measurements, supporting its use as an accessible clinical marker. Density gradient ultracentrifugation confirmed a clear shift in LDL peak density consistent with pattern B predominance. These observations reinforce the argument that cardiovascular risk evaluation in metabolic syndrome should extend beyond conventional lipid panels. AIP, easily calculated from routine triglyceride and HDL-cholesterol values, could be incorporated into standard clinical workflows with minimal additional cost. Future work should test whether AIP-guided treatment decisions improve cardiovascular outcomes in metabolic syndrome patients.

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