Adiponectin and lipoprotein particle size

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**Objective** – Adiponectin has been postulated to affect lipid and insulin signal transduction pathways. We evaluated the relationships of plasma adiponectin with lipoprotein mean particle size and subclass concentrations, independent of obesity and insulin sensitivity.

**Research design and methods** – A cross-sectional analysis of 884 young Israeli adults who participated in the population-based Jerusalem Lipid Research Clinic (LRC) study. Lipoprotein particle size was assessed using proton NMR.

**Results** - In multivariable linear regression models that included sex, BMI, waist circumference, HOMA-IR and leptin, adiponectin was associated with mean LDL-size [standardized regression coefficient(B)=0.20, p<0.001], VLDL-size (B =-0.12, p<0.001), and HDL-size (B=0.06, p=0.013). Adiponectin was inversely related to large VLDL (p<0.001), but positively with small VLDL (p=0.02), inversely with small LDL (p<0.006), but positively with large LDL (p<0.001), and positively with large HDL (p<0.001) subclass concentrations.

**Conclusions** – Adiponectin is favorably associated with lipoprotein particle size and subclass distribution independent of adiposity and insulin sensitivity.
Adiponectin is a fat-derived adipocytokine (1, 2) that is strongly associated with insulin sensitivity and with favorable cardiovascular outcomes. As insulin resistance is associated with reduced adiponectin levels (3), we hypothesized that adiponectin may have a direct role in hepatic lipoprotein metabolism. Thus, the aim of this study was to evaluate the relationships of adiponectin with lipoprotein particle size, independent of the degree of obesity and insulin sensitivity, in a population-based sample of healthy young adults.

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RESEARCH DESIGN AND METHODS
The Jerusalem Lipid Research Clinic (LRC) prevalence study has been previously described (4). In a follow-up study of a sex-stratified random sample, we examined 570 men (73% response) and 314 women (68%) aged 28 to 32 years, as previously reported (5).

Lipoprotein particle size analysis utilized a 400 MHz proton NMR analyzer (Liposcience, Raleigh, NC, USA)(6). Adiponectin was analyzed using an in-house immunofluorometric assay (TRIFMA) as previously described (6). Plasma leptin and insulin concentrations were determined by radioimmunometric methods (Linco, St. Charles, MO, USA). Sex-specific Pearson correlations, partial correlations and multivariable linear regression modeling were employed. Regression results are expressed as standardized coefficients (B). Collinearity tolerances were acceptable.

RESULTS
The study population comprised 884 young adults (570m/314f, mean age 30.1±0.8). Men had greater body mass index (BMI) than women (25.1±3.6 vs. 23.8±3.9 kg/m², p<0.001), while women had greater adiponectin (8.9±3.4 vs. 6.4±2.6 mg/l, p<0.001) and leptin concentrations (13.2±8.3 vs. 5.1±3.5 µg/l, p<0.001).

Adiponectin was associated with lipoprotein mean particle size in both sexes. Its strongest relation in men was an inverse association with VLDL-size (r=-0.39, p<0.001) which persisted after adjustment for BMI, waist circumference and HOMA-IR (r=-0.28, p<0.001). This relation was weaker in women (r=-0.21, p<0.001) and was attenuated by adjustment (r=-0.12, p<0.05). A positive association of adiponectin with HDL- and LDL-particle size evident in both sexes (r=-0.30-0.35) remained significant after adjustment.

VLDL-, LDL- and HDL-particle sizes were introduced separately as dependent variables in backward stepwise multivariable linear regression models that included sex, BMI, waist circumference, HOMA-IR, the relevant plasma lipid or lipoprotein (triglycerides, HDL-cholesterol or LDL-cholesterol), leptin and adiponectin. Adiponectin was significantly inversely associated with VLDL size (B=-0.12, p<0.001), whereas waist circumference (B=0.12, p<0.001), HOMA-IR (B=0.09, p=0.002) and triglycerides (B=0.58, p<0.001) were positively associated (model R²=0.57).

Adiponectin was associated with larger LDL-particle size (B=0.20, p<0.001), whereas waist circumference (B = -0.31, p<0.001) and sex (male vs. female, B=-0.20, p<0.001) were associated with smaller particle size (model R²=0.32). Adiponectin (B=0.06, p=0.013) and leptin (B=-0.10, p=0.001) showed opposite associations with HDL particle size. HDL-cholesterol was strongly associated with larger HDL-particle size (B=0.58, p<0.001), whereas waist circumference (B=-0.14, p<0.001), HOMA-IR (B=-0.04, p=0.06) and male sex (B=-0.25, p<0.001) were associated with smaller HDL-particles (model R²=0.69).
We stratified the study sample into tertiles of lipoprotein subclasses and displayed the concentration of adiponectin in each tertile of a lipoprotein subclass (i.e. large, intermediate, small) while adjusting for sex, waist circumference, BMI and HOMA-IR. As shown in Figure 1A, the adiponectin concentrations differed substantially between tertiles of large VLDLs, being inversely associated with increasing concentrations of large VLDL particles (B=-0.19, p<0.001 for trend). In contrast, adiponectin was highest in the upper small VLDL tertile within each large VLDL tertile category (p=0.02 for trend). Adiponectin was highest in the upper large LDL tertile (p<0.001 for trend) (Figure 1B). In contrast, within each large LDL tertile, adiponectin decreased consistently with increasing concentrations of small LDL particles (p=0.009 for trend). Adiponectin was positively associated with concentrations of large HDLs (p<0.001 for trend, Figure 1C), but was not related to small HDL particle concentrations. Thus, adiponectin concentration showed significant contrasting associations with small and large VLDL and LDL subclasses while being associated with only large HDL and not with small HDL particles.

We modeled adiponectin and leptin separately as dependent variables in a backward stepwise procedure that included all lipoprotein subclasses, anthropometric measures (BMI and waist circumference) and HOMA-IR. In men, adiponectin was independently inversely associated with concentrations of large VLDLs and positively associated with small VLDLs and large and intermediate HDLs. Leptin was positively associated only with small VLDLs and with adiponectin on adjustment for anthropometric measures and HOMA-IR. In women, adiponectin showed an independent positive association only with large HDLs, whereas leptin was inversely associated with large VLDLs and positively with small and intermediate HDLs.

**DISCUSSION**

This analysis, in ostensibly healthy young adults, demonstrates a substantial association between plasma adiponectin and lipoprotein particle size. Adiponectin was associated with mean particle size of the three lipoprotein moieties after adjusting for measures of obesity, insulin sensitivity, leptin and the chemically-measured relevant lipoprotein (triglycerides, HDL- or LDL-cholesterol). In addition, adiponectin was significantly linked to LDL- and VLDL subclass distribution, with lower adiponectin concentrations associated with lower concentrations of large LDLs, higher concentrations of small LDLs. Such associations have been previously described in the context of insulin resistance and obesity (8); however, our findings shed further light on these relations as we show that adiponectin levels are inversely associated with the concentrations of small LDL and large VLDL particles independent of insulin sensitivity, obesity and body fat distribution.

The presence of elevated levels of small dense LDL-particles seems to be associated with increased levels of large VLDL-particles and is closely associated with insulin resistance (9). Our results show that those subjects with greater concentrations of large VLDL-particles had reduced adiponectin levels, whereas the opposite was found for small VLDL. Thus, the clustering of elevated triglycerides (due to increased large VLDL-production) and small dense LDL-particles in the context of insulin resistance and obesity may be mediated by adiponectin which is typically reduced in this clinical setting (10,11), although the cross-sectional nature of this study cannot resolve directionality. Thus, the seemingly protective role of adiponectin against cardiovascular diseases and diabetes may not necessarily be
mediated through its association with insulin sensitivity or obesity, but rather could reflect an independent effect on lipoprotein metabolism.

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Adiponectin and lipoprotein particle size

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Figure 1. Adjusted plasma adiponectin concentration as a function of lipoprotein subclass tertiles, adjusted for sex, waist circumference, BMI and HOMA-IR.
Panel A shows the cohort divided into tertiles of large VLDL concentrations. Each of these tertiles is subdivided into tertiles of small VLDL concentrations (white bars represent the lowest tertile, grey bars the middle tertile and black bars the upper tertile of small VLDL).
Panel B shows the cohort divided into tertiles of large LDL concentrations and each of these tertiles is subdivided into tertiles of small LDL concentrations (white bars represent the lowest tertile, grey bars the middle tertile and black bars the upper tertile of small LDL).
Panel C shows the cohort divided into tertiles of large HDL concentrations and each of these tertiles is subdivided into tertiles of small HDL concentrations (white bars represent the lowest tertile, grey bars the middle tertile and black bars the upper tertile of small HDL).
* p for trend <0.001 and 0.02 for large VLDL and for small VLDL median tertile values, respectively.
** p for trend <0.001 and 0.009 for large LDL and for small LDL median tertile values, respectively.
*** p for trend <0.001 for large HDL median tertile values.