**Effect of metformin on metabolites and relation with myocardial infarct size and left ventricular ejection fraction after myocardial infarction**

Metabolic biomarkers of LVEF after STEMI

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Subject terms:

- Biomarkers
- Myocardial infarction
- Heart failure
- Lipids and cholesterol
- Secondary prevention

**Background:** Left ventricular ejection fraction (LVEF) and infarct size (ISZ) are key predictors of long-term survival after myocardial infarction (MI). However, little is known about the biochemical pathways driving left ventricular dysfunction after MI. To identify novel biomarkers predicting post-MI LVEF and ISZ, we performed metabolic profiling in the GIPS-III randomized clinical trial. We also investigated the metabolic footprint of metformin, a drug associated with improved post-MI left ventricular function in experimental studies.

**Methods and Results:** Participants were ST-elevated MI (STEMI) patients who were randomly assigned to receive metformin or placebo for 4 months. Blood samples were obtained on admission, 24 h and 4 months post-MI. 233 metabolite measures were quantified using nuclear magnetic resonance (NMR) spectrometry. LVEF and ISZ were assessed 4 months post-MI. 24 h post-MI measurements of HDL triglycerides (HDL-TG) predicted LVEF (β=1.90 [95% CI: 0.82, 2.98]; $p=6.4x10^{-4}$) and ISZ (β=-0.41; 95% CI: -0.60, -0.21]; $p=3.2x10^{-5}$). Additionally, 24 h post-MI measurements of medium HDL-TG (β=-0.40 [95% CI: -0.60, -0.20]; $p=6.4x2x10^{-5}$), small HDL-TG (β=-0.34 [95% CI: -0.53, -0.14]; $p=7.3x10^{-4}$) and the triglyceride content of very large HDL (β=-0.38 [95% CI: -0.58, -0.18]; $p=2.7x10^{-4}$) were associated with ISZ. After the 4-month treatment, the phospholipid content of very large HDL was lower in metformin vs. placebo treated patients (28.89% vs. 38.79%; $p=7.5x10^{-5}$); alanine levels were higher in the metformin group (0.46 mmol/L vs. 0.44 mmol/L; $p=2.4x10^{-4}$).

**Conclusions:** HDL triglyceride concentrations predict post-MI LVEF and ISZ. Metformin increases alanine levels and reduces the phospholipid content in very large HDL particles.

**Clinical Trial Registration:** NCT01217307 (https://clinicaltrials.gov/show/NCT01217307)

**Key words:** metabolomics, metformin, left ventricular function, infarct size, prognosis

## Introduction

Myocardial Infarction (MI) is one of the leading causes of global morbidity and mortality. While the survival after MI has improved due to ameliorated treatment strategies, including primary percutaneous interventions, the long-term outcome of MI in general remains poor with a 1-year risk for recurrent cardiovascular (CV) disease of over 10%.[1] Left ventricular ejection fraction (LVEF) and infarct size (ISZ) are key predictors of long-term prognosis after MI.[2,3]. However, treatment options for left ventricular dysfunction are limited and the biochemical mechanisms driving functional decline of the myocardium after MI are largely unknown.

Metformin, which is commonly used in the treatment of diabetes and more recently in insulin resistant conditions, has been found to preserve LVEF and to reduce ISZ in non-diabetic animal models of MI.[4] The GIPS-III clinical trial was designed to study the effects of metformin therapy on LVEF in non-diabetic ST segment Elevation MI (STEMI) patients undergoing PCI. However, in contrast to preclinical findings, metformin did not improve LVEF compared with placebo 4 months post-MI.[5]

This result may be explained by interindividual differences in metformin response, raising the possibility that metformin is effective in a subgroup of CV patients. Metabolic profiling has emerged as a powerful tool to explore drug effects and factors influencing drug response.[6-8] Metabolomics is a relatively novel field in 'omics' sciences, which uses high-throughput technologies, such as nuclear magnetic resonance (NMR) spectroscopy, to concurrently quantify a large number of small molecules in different tissues. While recent studies reported changes in lipid and amino acid concentrations after metformin treatment[9-11], no study has yet used large-scale metabolic platforms to investigate the effects of metformin on a wide range of metabolite measures at a time. Furthermore, metabolic profiling has been performed to improve diagnosis and prediction of CV events.[12,13] A recent study identified

metabolic profiles which discriminate heart failure patients from healthy controls.[14] Metabolic profiling may thus help identify novel biomarkers of left ventricular function and ISZ to improve risk stratification in MI patients.

Metabolite concentrations can vary greatly over time and are highly sensitive to environmental influences. Lipid profiles have been shown to change shortly after MI and only gradually return to baseline after several weeks.[15] The predictive value of a biomarker may thus vary over time. We therefore studied metabolic markers of LVEF, ISZ and metformin response in the GIPS-III cohort at three different time points: baseline (on admission), 24 h post-MI and 4 months post-MI.

The objective of this ancillary study of the GIPS-III trial was to evaluate the effect of metformin on metabolic profiles in non-diabetic STEMI patients and to identify prognostic markers, which predict LVEF and ISZ 4 months post-MI. Furthermore we tested whether metformin improved LVEF and ISZ in subgroups of patients, as identified by metabolic profiling.

## Methods

*Study population*

The GIPS-III study is a randomized trial that included 380 non-diabetic patients undergoing primary PCI for ST segment elevation myocardial infarction. Participants received a 4-month regimen with either metformin 500mg 2dd1 or matching placebo 2dd1. The design of the study has been previously described in more detail.[4,5] All patients provided written informed consent. The study complied with the Declaration of Helsinki and was approved by the ethics committee of the University Medical Center Groningen (the Netherlands) and national authorities (NCT01217307). The primary outcome measure was LVEF, the secondary

outcome measure was ISZ. Both measures were assessed 4 months post-MI by MRI as described below.

*Laboratory Measurements*

Non-fasting blood samples were obtained on admission (N=339), 24 h post-MI (N=329) and 4 months post-MI (N=316). Serum and EDTA-anticoagulated plasma samples were stored at − 80 °C until analyzed. Metabolic profiling was performed using a high-throughput $^{1}$H NMR metabolomics platform.[16] We obtained a total of 233 serum metabolite concentrations and ratios, including 168 lipoprotein subclass measures, 45 lipid related measures, 5 glycolysis related metabolites, 9 amino acids, 3 ketone bodies, 2 fluid balance related metabolites and 1 inflammatory marker. An overview of all metabolite measures is given in Supplemental Table 1.

*Cardiac Magnetic Resonance imaging (CMR)*

LVEF and ISZ were measured by cardiac magnetic resonance imaging (MRI) 4 months after MI as previously described in detail.[4,5] Independent cardiologists analyzed all MRI data and assessed LVEF and ISZ, blinded for treatment assignment.

*Statistical analysis*

Missing metabolite measures were imputed using random forest imputation as implemented in the R package missForest.[17] Since most metabolite measures showed skewed distributions, they were normalized using rank-based inverse normal transformation within each time point separately. Spearman's correlation coefficients were calculated from the metabolite concentrations for each time point (baseline, 24 h post-MI and 4 months post-MI) and plotted

using the corrplot function of the corrplot package of R. The correlation plots are presented in Supplemental Figure 1.

Since many metabolites were highly correlated, principal component analysis (PCA) was applied to estimate the number of independent tests for multiple testing correction, using the prcomp-function in R. To additionally account for multiple testing at different time points, principal components (PCs) were calculated across all three time points. The first 68 PCs explained over 95% of the variation in the metabolite data, yielding an adjusted significance level of $p<0.05/68 = 0.00074$.

Unpaired t-tests were performed to assess the effect of metformin treatment on metabolite measures. To identify biomarkers predictive of LVEF and ISZ, we analyzed all metabolite measures at each time point separately, using linear regression adjusted for known predictors of ventricular function and medication use: age, sex, baseline N-terminal prohormone of brain natriuretic peptide (NTproBNP) levels, baseline creatine kinase (CK)-MB levels, myocardial blush grade, metformin treatment and statin treatment (4 months post-MI). To meet the assumption of normality of residuals, we tested different transformations. Since square-root transformation provided the best results, ISZ was square-root transformed. In addition we performed stratified analyses for LVEF and ISZ. According to current guidelines[18], LVEF 52%-72% was categorized as normal ventricular function; LVEF 41-51% was defined as mildly abnormal and LVEF<41% as abnormal for men. Categories were LVEF 54%-74% for normal ventricular function, LVEF 41-53% as mildly abnormal and LVEF<41% for abnormal for women. ISZ was stratified by tertiles to obtain the same number of strata as with LVEF. Associations of metabolite measures with LVEF categories and ISZ tertiles were assessed using multinomial logistic regression, which provides pairwise comparisons between each level of the outcome variable and a reference level. Finally we added the interaction term of metformin treatment and metabolite measure to the linear

regression models to identify subgroups of patients in whom metformin was effective. R (version 3.02 or higher, http://www.r-project.org/) was used for all statistical analyses.

## Results

*Patient characteristics and metabolite measures*

A total of 380 patients received either metformin placebo treatment. Of these, 109 did not undergo MRI 4 months post-MI or did not provide utilizable scans due to insufficient quality. Details on metformin/placebo treatment, clinical parameters and conventional lipid and (apo)lipoprotein measures have been published elsewhere.[5,11] Briefly, metformin treatment resulted in a modest decrease in low-density lipoprotein cholesterol (LDL-C) without significant effects on total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, apolipoprotein B (apoB) and apolipoprotein A-I (apoA1) when the values after 4 months and after 24 h were compared (data not shown).[14] Metabolic profiles were quantified in a total of 376 patients. Baseline, 24 h post-MI and 4-month post-MI measurements were available from 339, 326 and 316 patients, respectively. Premature dropout was neither related to metformin treatment, nor to mortality as none of the participants died before MRI.[5] A summary of all metabolite measures can be found in Supplemental Table 1. The correlation matrices revealed substantial correlation within lipoprotein subclasses, between amino acids and between fatty acids (Supplemental Figure 1).

*Association of metabolites with LVEF and ISZ*

Results for all metabolite measures tested are shown in Supplemental Tables 2-7. None of the metabolite measures was significantly associated with LVEF 4 months post-MI. No baseline

metabolite measure predicted LVEF. Patients with higher HDL-TG levels 24 h post-MI showed significantly better LVEF (β=1.90 [95% CI: 0.82, 2.97]; $p=6.4x10^{-4}$) after adjustment for metformin treatment, age, sex, baseline NTproBNP levels, baseline CK-MB levels, myocardial blush grade and statin use (Table 1A). When LVEF was entered as categorical variable (normal, mildly abnormal, abnormal ventricular function), 24 h post-MI measurements of HDL-TG (OR=0.36 [95% CI: 0.21, 0.61]; $p=1.8x10^{-4}$), medium (M-) HDL-TG (OR=0.37 [95% CI: 0.22, 0.63]; $p=2.3x10^{-4}$) and small (S-) HDL-TG (OR=0.35 [95% CI: 0.20, 0.61]; $p=2.1x10^{-4}$) significantly predicted normal vs. abnormal LVEF 4 months post-MI (Figure 1, Table 2). Notably, all HDL-TG related measures showed a positive association with LVEF, suggesting a beneficial effect of increased triglyceride content in HDL. We found no association of 24-h post-MI measurements with mildly abnormal LVEF relative to normal LVEF. In addition, 24 h post-MI measurements of triglycerides (OR=0.39 [95% CI: 0.23, 0.66]; $p=5.2x10^{-4}$) and the cholesterol (OR=2.52 [95% CI: 1.48, 4.30]; $p=6.6x10^{-4}$) in very small (XS-) very low-density lipoprotein (VLDL) particles was associated with abnormal LVEF compared to normal left ventricular function. Finally, baseline measurements of the TG to total lipids ratio in large (L) LDL predicted abnormal LVEF (OR=0.37 [95% CI: 0.21, 0.65]; $p=6.2x10^{-4}$). Addition of a treatment x metabolite interaction term did not reveal any patient subgroup in whom metformin improved LVEF (Table 1B).

We did not find any association between metabolite measures and ISZ at baseline and 4 months post-MI. In the adjusted model, HDL-TG (β=-0.41 [95% CI: -0.60, -0.22]; p=3.2x10-5), M-HDL-TG (β=-0.40 [95% CI: -0.60, -0.21]; $p=6.4x2x10^{-5}$), XL-HDL-TG% (β=-0.38 [95% CI: -0.58, -0.18]; $p=2.7x10^{-4}$) and S-HDL-TG (β=-0.34 [95% CI: -0.54, -0.15]; $p=7.3x10^{-4}$) were significantly associated with ISZ 24 h post-MI (Table 1A). In addition, phenylalanine (β=0.38 [95% CI: 0.18, 0.58]; $p=1.9x10^{-4}$) and albumin (β=-0.33 [95% CI: -0.52, -0.15]; $p=5.2x10^{-4}$) reached significance in the unadjusted model, but not in the adjusted

model. Similarly, 24 h post-MI measurements of HDL-TG (OR=0.48 [95% CI: 0.33, 0.69]; $p=9.2x10^{-5}$), M-HDL-TG (OR=0.46 [95% CI: 0.31, 0.67]; $p=6.2x10^{-5}$), S-HDL-TG (OR=0.51 [95% CI: 0.35, 0.74]; $p=3.9x10^{-4}$) and XL-HDL-TG% (OR=0.49 [95% CI: 0.33, 0.72]; $p=3.2x10^{-4}$) predicted ISZ, when the first tertile was compared to the third tertile (Figure 2, Table 2). Again, our findings suggest a beneficial effect of higher HDL-TG levels. We found no significant treatment x metabolite interactions (Table 1B).

As shown in Supplemental Table 8, HDL-TG, M-HDL-TG, S-HDL-TG and XL-HDL-TG% increased between baseline and 24 h post-MI and remained relatively stable between 24 h and 4 months post-MI, except for XL-HDL-TG% which showed a moderate gain. Similar to HDL-TG, serum triglyceride levels increased between baseline and 24 h post-MI, but were decreased 4 months after MI.

*Effects of metformin of metabolic profiles*

Results for all metabolite measures are shown in Supplemental Table 9. To assess baseline differences in metabolic profiles, we compared metabolite measures between the treatment group and controls at baseline. We did not find any difference between the two groups at baseline. Table 3 summarizes metabolic measurements for 24 h post-MI and 4 months post-MI. 24 h post-MI, after the first doses of the treatment had been administered, both alanine (median: 0.49 mmol/L vs. 0.46 mmol/L; $p=9.0x10^{-4}$) and pyruvate (median: 0.16 mmol/L vs. 0.14 mmol/L; p=0.001) displayed trends towards increased concentrations in the metformin group. After the 4-month treatment period, alanine levels were significantly elevated in metformin-treated patients (median: 0.46 mmol/L vs. 0.44 mmol/L; $p=2.4x10^{-4}$) as shown in Table 3. In addition, the phospholipids to total lipids ratio in very large high density lipoprotein (XL-HDL) particles (XL-HDL-PL%) was significantly reduced in the metformin group (median: 28.89% vs. 38.79%; $p=7.5x10^{-5}$).

## Discussion

We used $^{1}$H NMR spectrometry-based metabolite measures to evaluate the effects of metformin on metabolic profiles of non-diabetic MI patients and to study prognostic metabolites predicting LVEF and ISZ 4 months post-MI. Moreover we investigated whether metabolic profiling could be used to identify subgroups of patients in whom metformin was effective. After the 4-month treatment period, we found higher alanine levels and lower XL-HDL-PL% in metformin-treated patients as compared to controls. Remarkably, higher triglyceride levels in HDL and several HDL subfractions measured 24 h post-MI were associated with favorable outcome as inferred from higher LVEF and smaller ISZ 4 months post-MI. Moreover, categorical analysis of LVEF revealed that besides HDL-TG, the composition of XS-VLDL (24 h post-MI) and L-LDL (baseline) was associated with abnormal left ventricular function 4 months post-MI. We could not identify metabolic profiles associated with treatment benefits from metformin.

Similar to our results, the CAMERA study, a clinical trial investigating the effects of metformin on different amino acids, found substantially increased alanine levels 18 months after treatment onset.[13] Alanine plays a crucial role in the alanine-glucose cycle, in which alanine released by muscle tissue is transported to the liver before it is converted into pyruvate for gluconeogenesis. Findings from animal studies suggest that metformin reduces gluconeogenesis by inhibiting hepatic alanine uptake[19] and by hampering fat-induced changes in the glycolysis metabolic pathway[20]. As a result of reduced uptake into the liver, blood alanine levels may rise in metformin-treated patients. Interestingly, we observed a trend towards increased alanine levels in the metformin group 24 h post-MI,,suggesting rapid effects of metformin on gluconeogenesis.

Numerous randomized controlled trials have studied the effects of metformin treatment on lipid levels in patients with type 2 diabetes. A recent study in diabetic patients found that metformin lowered total cholesterol and LDL-C.[21] Another study in patients at risk for diabetes reported changes in lipoprotein subclasses after one year of metformin treatment, with reduced particle concentrations of small LDL and elevated large LDL, small HDL and large HDL.[9] In our recent report, we observed modest decreases in LDL cholesterol, no change in apolipoprotein B, and as a result a small decrease in LDL particle size.[11] In the present study which used a different NMR-based method, only the phospholipid content of large HDL particles was decreased in response to metformin.

We also tested whether lipoprotein characteristics and metabolite measures at baseline, 24 h post-MI and 4 months post-MI were associated with 4 months post-MI LVEF and ISZ. We found that increased HDL-TG levels measured 24 h post-MI were associated with a greater LVEF. In addition, decreased HDL-TG, M-HDL-TG, XL-HDL-TG% and S-HDL-TG measured 24 h post-MI predicted higher ISZ. Categorical analysis of LVEF and ISZ provided similar results with more favorable outcomes for patients with higher HDL-TG levels. No metabolite measure showed a significant interaction with metformin treatment, suggesting that there was no metabolic subgroup of patients in whom metformin was effective.

Our findings suggest beneficial effects of higher triglyceride levels in HDL and in HDL subfractions measured 24 h post-MI on ISZ and LVEF. Clinical studies identified low admission triglyceride levels as a risk factor for recurrent CV events and mortality in STEMI patients.[22,23] Likewise, low triglyceride levels are associated with a poor prognosis in stroke patients.[24] This contrasts with findings from large-scale case-control and prospective cohort studies indicating that hypertriglycemia is a strong predictor of CV events, even independent of cholesterol levels.[25,26] These epidemiological findings, however, apply to individuals who were not studied during the course of an acute coronary event. Similarly paradoxical findings

have been obtained for plasma cholesterol levels. While hypercholesterolemia is an established CV risk factor in the general population, admission LDL-C levels < 70 mg/dl are associated with higher mortality and incidence of heart failure in statin-naïve STEMI patients.[27] The pathogenic mechanisms underlying recurrent CV events shortly after an acute event are still poorly understood. It is possible that in the acute setting HDL-TG plays a distinct role on CV outcome .

VLDL is the most important triglyceride carrier in plasma. The triglyceride content of VLDL showed substantial correlation with HDL-TG 24 h post-MI (Supplemental Figure 1 B). However, only the triglyceride content of very small VLDL particles was associated with LVEF categories. In addition, the TG content of large LDL particles at baseline predicted abnormal LVEF 4 months post-MI. Inhibition of fatty acid uptake by relocation of FAT/CD36 may reduce intracellular fatty acid concentrations[28], resulting in increased extracellular fatty acid levels and diminished lipolysis of lipid-bound triglycerides. This may initially lead to triglyceride enrichment of VLDL and LDL particles, which subsequently transfer excess triglycerides to HDL particles in exchange for cholesteryl esters by the action of cholesteryl ester transfer protein (CETP), thereby increasing the triglyceride content in HDL.[29] In line with this, blood samples of MI patients collected immediately after diagnosis show strong triglyceride enrichment of $HDL_2$ particles.[30] Higher plasma HDL-TG levels could thus be consequent to inhibition of fatty acid uptake, and coincide with diminished fatty acid oxidation and prevention of further myocardial damage.[31] Larger triglyceride-rich particles are converted to small VLDL subfractions as a result of lipase-mediated delipidation[32], suggesting that triglyceride enrichment may be secondary to initial triglyceride uptake of large VLDL. Larger VLDL particles may be delipidated rapidly, which may explain why the association of triglycerides with LVEF was limited to very small VLDL 24 h post-MI. Similarly, a major proportion of LDL-TG is derived from large VLDL[32], which

may partly result from CETP-mediated delipidation of large VLDL. Taken together, early metabolic changes after MI could reflect adaptive mechanisms that promote functional recovery.

While we observed associations of LVEF categories with 24 h post-MI measurements of XS-VLDL-TG% and XS-VLDL-C%, these metabolite measures did not significantly predict LVEF when LVEF was analyzed as a continuous variable. However, the regression model with continuous outcome assumes linearity between metabolite measures and LVEF, whereas categorical analysis of LVEF in combination with multinomial logistic regression renders the model sensitive to non-linear associations. As shown in Figure 2 A, HDL-TG, M-HDL-TG and S-HDL-TG follow a linear trend across the three LVEF categories, whereas XS-VLDL-TG% and XS-VLDL-C% display non-linear trends.

*Limitations*

The GIPS-III trial was originally designed to assess differences in LVEF between metformin treated patients controls. We conducted 68 independent tests, raising the possibility that our study was not powered to detect smaller changes. However, we were able to detect a significant effect for alanine levels, which were only slightly increased in the metformin group (median difference: 0.03 mmol/L), demonstrating sufficient power to perform a metabolic profiling analysis. In addition, all patients received intravenous heparin before PCI when baseline blood samples were drawn. Heparin stimulates lipolysis and hence acutely reduces plasma triglyceride levels[33], which is in line with the marked increase in triglyceride levels between baseline and the other time points (Supplemental Table 8). STEMI patients routinely receive heparin before PCI, rendering the results for baseline measurements relevant to clinical settings. These findings measurements should nevertheless be interpreted with caution. Moreover, we performed metabolic profiling in non-fasting blood samples,

warranting further research to substantiate our findings under fasting conditions. However, the NMR platform used in our study mainly quantifies lipid measures, which change only slightly after food consumption and show similar associations with cardiovascular risk in fasting and non-fasting individuals.[34]

*Conclusions*

In summary, our study suggests that metformin treatment started directly after presentation with STEMI produces changes in alanine and XL-HDL-PL% as assessed after 4 months. Higher triglyceride levels in HDL and in HDL subfractions measured 24 h post-MI were predictive of better LVEF and smaller ISZ 4 months post-MI. HDL-TG may thus serve as an early biomarker of left ventricular dysfunction in STEMI patients. However, further studies are required to substantiate the clinical significance of HDL-TG in CV risk prediction and to investigate the biological mechanism underlying associations of metabolic biomarkers with recurrent CV events. Our findings emphasize the utility of high-throughput metabolic profiling as a tool to study drug effects and to identify prognostic biomarkers of LVEF and ISZ.

**Sources of Funding**

The GIPS-III trial was supported by grant number 95103007, ZonMw, the Netherlands Organization for Health Research and Development, The Hague, the Netherlands. Metabolic profiling was supported by the Biobanking and Biomolecular Resources Research Infrastructure, the Netherlands (BBMRI-NL). Daniel Kofink is supported by the FP EU project CVgenes@target (HEALTH-F2-2013-601456). Folkert W. Asselbergs is supported

by a Dekker scholarship-Junior Staff Member 2014T001 – Netherlands Heart Foundation and

UCL Hospitals NIHR Biomedical Research Centre.

**Disclosure**

None

**References**

1. Sabate M, Cequier A, Iñiguez A, Serra A, Hernandez-Antolin R, Mainar V, et al. Everolimus-eluting stent versus bare-metal stent in ST-segment elevation myocardial infarction (EXAMINATION): 1 year results of a randomised controlled trial. *Lancet*. 2012;380:1482-1490.
2. El Aidi H, Adams A, Moons KG, Den Ruijter HM, Mali WP, Doevendans PA, et al. Cardiac magnetic resonance imaging findings and the risk of CV events in patients with recent myocardial infarction or suspected or known coronary artery disease: a systematic review of prognostic studies. *J Am Coll Cardiol*. 2014;63:1031-1045.
3. Wu E, Ortiz JT, Tejedor P, Lee DC, Bucciarelli-Ducci C, Kansal P, et al. Infarct size by contrast enhanced cardiac magnetic resonance is a stronger predictor of outcomes than left ventricular ejection fraction or end-systolic volume index: prospective cohort study. *Heart*. 2008;94:730-736.
4. Lexis CPH, van der Horst ICC, Lipsic E, van der Harst P, van der Horst-Schrivers ANA, Wolffenbuttel BHR, et al. Metformin in non-diabetic patients presenting with ST elevation myocardial infarction: rationale and design of the glycometabolic intervention as adjunct to primary percutaneous intervention in ST elevation myocardial infarction (GIPS)-III trial. *Cardiovasc Drugs Ther Spons Int Soc Cardiovasc Pharmacother.* 2012;26: 417–426.

5. Lexis CP, van der Horst IC, Lipsic E, Wieringa WG, de Boer RA, van den Heuvel AF, et al. Effect of metformin on left ventricular function after acute myocardial infarction in patients without diabetes: the GIPS-III randomized clinical trial. *JAMA*. 2014;311:1526-1535.
6. Krauss RM, Zhu H, Kaddurah-Daouk R. Pharmacometabolomics of statin response. *Clin Pharmacol Ther*. 2013;94:562-565.
7. Maiso P, Huynh D, Moschetta M, Sacco A, Aljawai Y, Mishima Y, et al. Metabolic signature identifies novel targets for drug resistance in multiple myeloma. *Cancer Res*. 2015;75:2071-2082.
8. Winnike J, Li Z, Wright F, Macdonald J, O'Connell T, Watkins P. Use of pharmaco-metabonomics for early prediction of acetaminophen-induced hepatotoxicity in humans. *Clin Pharmacol Ther*. 2010;88:45-51.
9. Goldberg R, Temprosa M, Otvos J, Brunzell J, Marcovina S, Mather K, et al. Lifestyle and metformin treatment favorably influence lipoprotein subfraction distribution in the Diabetes Prevention Program. *J Clin Endocrinol Metab*. 2013;98:3989-3998.
10. Preiss D, Rankin N, Welsh P, Holman R, Kangas A, Soininen P, et al. Effect of metformin therapy on circulating amino acids in a randomized trial: the CAMERA study. *Diabetic Med*. 2016;33:1569-1574.
11. Eppinga RN, Hartman MH, van Veldhuisen DJ, Lexis CP, Connelly MA, Lipsic E, et al. Effect of Metformin Treatment on Lipoprotein Subfractions in Non-Diabetic Patients with Acute Myocardial Infarction: A Glycometabolic Intervention as Adjunct to Primary Coronary Intervention in ST Elevation Myocardial Infarction (GIPS-III) Trial. *PLOS ONE*. 2016;11:e0145719.
12. Vaarhorst AA, Verhoeven A, Weller CM, Böhringer S, Göraler S, Meissner A, et al. A metabolomic profile is associated with the risk of incident coronary heart disease. *Am Heart J*. 2014;168:45-52.
13. Würtz P, Raiko JR, Magnussen CG, Soininen P, Kangas AJ, Tynkkynen T, et al. High-throughput quantification of circulating metabolites improves prediction of subclinical atherosclerosis. *Eur Heart J*. 2012;33:2307-2316.
14. Wang J, Li Z, Chen J, Zhao H, Luo L, Chen C, et al. Metabolomic identification of diagnostic plasma biomarkers in humans with chronic heart failure. *Mol Biosyst.* 2013;9:2618-2626.
15. Rosenson RS. Myocardial injury: the acute phase response and lipoprotein metabolism. *J. Am Coll Cardiol.* 1993;22:933-940.
16. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet.* 2015;8:192–206.
17. Stekhoven DJ, Bühlmann P. MissForest – non-parametric missing value imputation for mixed-type data. *Bioinformatics*. 2012;28:112-118.
18. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of CV Imaging. *J Am Soc Echocardiogr.* 2015;28:1-39. e14.
19. Komori T, Hotta N, Kobayashi M, Sakakibara F, Koh N, Sakamoto N. Biguanides may produce hypoglycemic action in isolated rat hepatocytes through their effects on L-alanine transport. *Diabetes Re. Clin Pr*. 1993;22:11-17.
20. Song S, Andrikopoulos S, Filippis C, Thorburn AW, Khan D, Proietto J. Mechanism of fat-induced hepatic gluconeogenesis: effect of metformin. *Am J Physiol Endocrinol Metab.* 2001;281:E275-E282.
21. Xu T, Brandmaier S, Messias AC, Herder C, Draisma HH, Demirkan A, et al. Effects of

metformin on metabolite profiles and LDL cholesterol in patients with type 2 diabetes. *Diabetes Care*. 2015;38:1858-1867.
22. Cheng Y-T, Liu T-J, Lai H-C, Lee W-L, Ho H-Y, Su C-S, et al. Lower serum triglyceride level is a risk factor for in-hospital and late major adverse events in patients with ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention- a cohort study. *BMC Cardiovasc. Disord*. 2014;14:143.
23. Khawaja OA, Hatahet H, Cavalcante J, Khanal S, Al-Mallah MH. Low admission triglyceride and mortality in acute coronary syndrome patients. *Cardiol J*. 2011;18:297-303.
24. Weir CJ, Sattar N, Walters MR, Lees KR. Low triglyceride, not low cholesterol concentration, independently predicts poor outcome following acute stroke. *Cerebrovasc Dis*. 2003;16:76-82.
25. Nordestgaard BG, Benn M, Schnohr P, Tybjærg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA*. 2007;298:299-308.
26. Sarwar N, Danesh J, Eiriksdottir G, Sigurdsson G, Wareham N, Bingham S, et al. Triglycerides and the risk of coronary heart disease 10 158 incident cases among 262 525 participants in 29 western prospective studies. *Circulation*. 2007;115:450-458.
27. Oduncu V, Erkol A, Kurt M, Tanboğa İH, Karabay CY, Şengül C, et al. The prognostic value of very low admission LDL-cholesterol levels in ST-segment elevation myocardial infarction compared in statin-pretreated and statin-naive patients undergoing primary percutaneous coronary intervention. *Int J Cardiol*. 2013;167:458-463.
28. Heather LC, Pates KM, Atherton HJ, Cole MA, Ball DR, Evans RD, et al. Differential translocation of the fatty acid transporter, FAT/CD36, and the glucose transporter, GLUT4, coordinates changes in cardiac substrate metabolism during ischemia and reperfusion. *Circ Heart Fail*. 2013;6:1058-1066.
29. Kappelle PJ, van Tol A, Wolffenbuttel BH, Dullaart RP. Cholesteryl ester transfer protein inhibition in CV risk management: ongoing trials will end the confusion. *Cardiovasc Ther.* 2011;29:e89-99.
30. Cho K-H, Shin D-G, Baek S-H, Kim J-R. Myocardial infarction patients show altered lipoprotein properties and functions when compared with stable angina pectoris patients. *Exp Mol Med*. 2009;41:67-76.
31. Liu Q, Docherty JC, Rendell JC, Clanachan AS, Lopaschuk GD. High levels of fatty acids delay the recovery of intracellular pH and cardiac efficiency in post-ischemic hearts by inhibiting glucose oxidation. *J Am Coll Cardiol*. 2002;39:718-725.
32. Packard CJ and Shepherd J. Lipoprotein heterogeneity and apolipoprotein B metabolism. *Arterioscler Thromb Vasc Biol.* 1997;17:3542-3556.
33. Brunner MP, Shah SH, Craig DM, Stevens RD, Muehlbauer MJ, Bain JR, et al. Effect of heparin administration on metabolomic profiles in samples obtained during cardiac catheterization. *Circ Cardiovasc Genet*. 2011;4:695-700.
34. Nordestgaard, BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points – a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. *Eur Heart J.* 2016;37:1944-1958.

**Tables and Figures**

**A**

| Metabolite | Unadjusted model β [95% CI] | p | Adjusted model β [95% CI] | p |
|---|---|---|---|---|
| **LVEF** (N=245) | | | | |
| HDL-TG | 1.84 [0.78, 2.89] | $7.4x10^{-4}$ | 1.90 [0.82, 2.97] | **$6.4x10^{-4}$** |
| M-HDL-TG | 1.70 [0.65, 2.75] | 0.002 | 1.65 [0.55, 2.74] | 0.003 |
| XL-HDL-TG% | 1.67 [0.56, 2.77] | 0.003 | 1.82 [0.68, 2.96] | 0.002 |
| S-HDL-TG | 1.51 [0.45, 2.57] | 0.006 | 1.68 [0.58, 2.78] | 0.003 |
| Albumin | 1.10 [0.04, 2.16] | 0.044 | 1.25 [0.10, 2.40] | 0.034 |
| Phenylalanine | -0.90 [-2.01, 0.21] | 0.113 | -0.55 [-1.68, 0.58] | 0.344 |
| **ISZ** (N=231) | | | | |
| HDL-TG | -0.42 [-0.60, -0.24] | **$1.2x10^{-5}$** | -0.41 [-0.60, -0.22] | **$3.2x10^{-5}$** |
| M-HDL-TG | -0.42 [-0.60, -0.23] | **$1.4x10^{-5}$** | -0.40 [-0.60, -0.21] | **$6.4x10^{-5}$** |
| XL-HDL-TG% | -0.37 [-0.56, -0.18] | **$1.9x10^{-4}$** | -0.38 [-0.58, -0.18] | **$2.7x10^{-4}$** |
| S-HDL-TG | -0.33 [-0.52, -0.14] | **$6.8x10^{-4}$** | -0.34 [-0.54, -0.15] | **$7.3x10^{-4}$** |
| Albumin | -0.33 [-0.52, -0.15] | **$5.2x10^{-4}$** | -0.33 [-0.54, -0.13] | 0.002 |
| Phenylalanine | 0.38 [0.18, 0.58] | **$1.9x10^{-4}$** | 0.34 [0.14, 0.55] | 0.001 |

**B**

| Metabolite | Unadjusted model β [95% CI] | p | Adjusted model β [95% CI] | p |
|---|---|---|---|---|
| **LVEF** (N=245) | | | | |
| Treatment X | | | | |
| HDL-TG | 1.15 [-0.96, 3.26] | 0.285 | 0.94 [-1.18, 3.06] | 0.387 |
| M-HDL-TG | 1.09 [-1.01, 3.19] | 0.311 | 0.82 [-1.30, 2.94] | 0.449 |
| XL-HDL-TG% | 0.71 [-1.50, 2.92] | 0.529 | 0.60 [-1.61, 2.82] | 0.593 |
| S-HDL-TG | 0.73 [-1.42, 2.87] | 0.506 | 0.68 [-1.46, 2.82] | 0.534 |
| Albumin | 0.36 [-1.77, 2.49] | 0.740 | 0.38 [-1.74, 2.51] | 0.725 |
| Phenylalanine | -1.02 [-3.25, 1.22] | 0.373 | -1.24 [-3.51, 1.03] | 0.286 |
| **ISZ** (N=231) | | | | |
| Treatment X | | | | |
| HDL-TG | -0.07 [-0.44, -0.30] | 0.701 | -0.04 [-0.41, 0.34] | 0.856 |
| M-HDL-TG | -0.19 [-0.56, 0.18] | 0.316 | -0.14 [-0.52, 0.24] | 0.465 |
| XL-HDL-TG% | 0.04 [-0.35, 0.42] | 0.849 | 0.04 [-0.35, 0.44] | 0.827 |
| S-HDL-TG | -0.04 [-0.42, 0.35] | 0.857 | -0.03 [-0.41, 0.36] | 0.891 |
| Albumin | -0.06 [-0.43, 0.32] | 0.762 | -0.05 [-0.43, 0.33] | 0.804 |
| Phenylalanine | 0.01 [-0.39, 0.40] | 0.977 | 0.04 [-0.36, 0.45] | 0.834 |

**Table 1A:** Association of selected metabolite measures with LVEF and ISZ 24 h post-MI. Results are shown for the unadjusted model and the adjusted model including age, sex, treatment, statin use, CKMB, NTproBNP and MBG as covariates. **Table 1B:** Association of treatment x metabolite interaction for selected metabolites. Results are shown for the unadjusted model (main effects and interaction term) and adjusted model including age, sex, statin use, CKMB, NTproBNP and MBG as covariates. Effects significant after correction for multiple testing ($p<7.4x10^{-4}$) are highlighted in bold. CI: confidence interval; LVEF: left ventricular ejection fraction; ISZ: infarct size; HDL-TG: triglycerides in HDL particles; M-HDL-TG: triglycerides in medium HDL particles; XL-HDL-TG%: triglycerides to total lipids ratio in very large HDL particles; S-HDL-TG: triglycerides in small HDL particles.

| **Metabolite** | OR [95% CI] | p | OR [95% CI] | p |
|---|---|---|---|---|
| **LVEF** | **normal vs. mildly abnormal** | | **normal vs. abnormal** | |
| Baseline | | | | |
| L-LDL-TG% | 1.05 [0.76, 1.45] | 0.774 | 0.37 [0.21, 0.65] | **$6.2x10^{-4}$** |
| 24 h post-MI | | | | |
| HDL-TG | 0.70 [0.51, 0.96] | 0.027 | 0.36 [0.21, 0.61] | **$1.8x10^{-4}$** |
| M-HDL-TG | 0.72 [0.52, 1.00] | 0.050 | 0.37 [0.22, 0.63] | **$2.3x10^{-4}$** |
| S-HDL-TG | 0.74 [0.53, 1.02] | 0.062 | 0.35 [0.20, 0.61] | **$2.1x10^{-4}$** |
| XS-VLDL-TG% | 0.83 [0.61, 1.14] | 0.247 | 0.39 [0.23, 0.66] | **$5.2x10^{-4}$** |
| XS-VLDL-C% | 1.10 [0.82, 1.49] | 0.523 | 2.52 [1.48, 4.30] | **$6.6x10^{-4}$** |
| **ISZ** | **1st tertile vs. 2nd tertile** | | **1st tertile vs. 3rd tertile** | |
| 24 h post-MI | | | | |
| HDL-TG | 0.78 [0.55, 1.11] | 0.169 | 0.48 [0.33, 0.69] | **$9.2x10^{-5}$** |
| M-HDL-TG | 0.88 [0.62, 1.25] | 0.472 | 0.46 [0.31, 0.67] | **$6.2x10^{-5}$** |
| S-HDL-TG | 0.92 [0.64, 1.30] | 0.621 | 0.51 [0.35, 0.74] | **$3.9x10^{-4}$** |
| XL-HDL-TG% | 0.75 [0.52, 1.07] | 0.116 | 0.49 [0.33, 0.72] | **$3.2x10^{-4}$** |

**Table 2:** Associations of metabolite measures 24 h post-MI with LVEF categories (normal, mildly abnormal, abnormal) and ISZ (tertiles) categories, adjusted for age, sex, treatment, statin use, CKMB, NTproBNP and MBG. Results for pairwise comparisons are given. Metabolites with at least one significant pairwise between-group comparison are shown. Effects significant after correction for multiple testing ($p<7.4x10^{-4}$) are highlighted in bold. CI: confidence interval; LVEF: left ventricular ejection fraction; ISZ: infarct size; L-LDL-TG%: triglyceride to total lipids ratio in large LDL particles; HDL-TG: triglycerides in HDL particles; M-HDL-TG: triglycerides in medium HDL particles; S-HDL-TG: triglycerides in small HDL particles; XL-HDL-TG%: triglycerides to total lipids ratio in very large HDL particles; XS-VLDL-TG%: triglycerides to total lipids ratio in very small VLDL particles; XS-VLDL-C%: cholesterol to total lipids ratio in very small VLDL particles.

| | Placebo | Metformin | |
|---|---|---|---|
| **Metabolite** | Median (IQR) | Median (IQR) | p |
| **24 h** | N=170 | N=159 | |
| Alanine in mmol/l | 0.46 (0.09) | 0.49 (0.09) | $9.0 \times 10^{-4}$ |
| Pyruvate in mmol/l | 0.14 (0.05) | 0.16 (0.07) | 0.001 |
| XL-HDL-PL% | 36.11 (17.52) | 33.98 (14.65) | 0.908 |
| **4 months** | N=159 | N=157 | |
| Alanine in mmol/l | 0.44 (0.08) | 0.46 (0.09) | $\mathbf{2.4 \times 10^{-4}}$ |
| Pyruvate in mmol/l | 0.10 (0.04) | 0.11 (0.04) | 0.006 |
| XL-HDL-PL% | 38.79 (19.50) | 28.89 (23.90) | $\mathbf{7.5 \times 10^{-5}}$ |

**Table 3:** Effects of treatment on selected metabolite measures 24 h post-MI and 4 months post-MI. Significant effects ($p<7.4 \times 10^{-4}$) are highlighted in bold. IQR: inter-quartile range; XL-HDL-PL%: phospholipids to total lipids ratio in very large HDL particles.

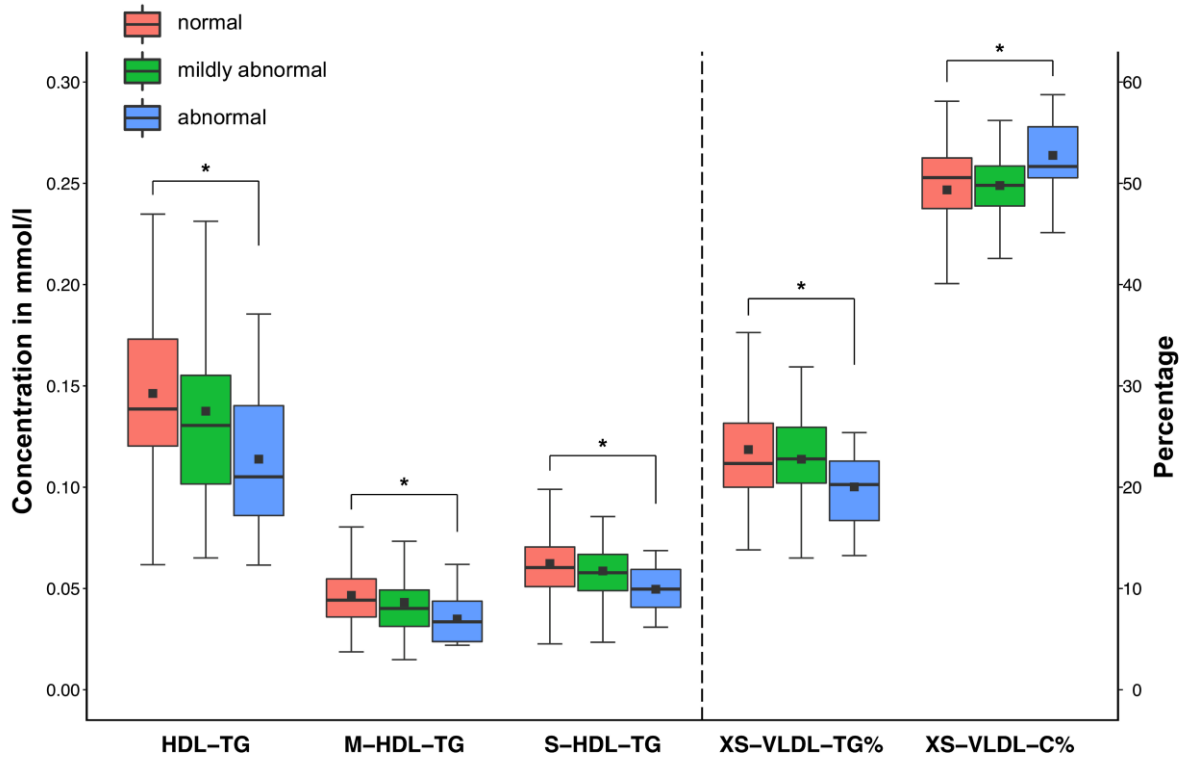


**Figure 1:** Box plots comparing selected metabolite measures (24 h post-MI) between distinct LVEF categories. For all plots, the lower and the upper margins represent the first and third quartile, respectively. Vertical lines indicate median values; squares indicate mean values. The whiskers represent the lowest and the highest value within 1.5 IQR. Differences between categories were assessed using multinomial logistic regression adjusted for treatment, age, sex, NTproBNP levels, CK-MB levels, myocardial blush grade, statin use. Asterisks indicate effects significant after correction for multiple testing ($p<7.4x10^{-4}$). IQR: inter-quartile range; LVEF: left ventricular ejection fraction; ISZ: infarct size; HDL-TG: triglycerides in HDL particles; M-HDL-TG: triglycerides in medium HDL particles; XL- S-HDL-TG: triglycerides in small HDL particles; XS-VLDL-TG%: triglycerides to total lipids ratio in very small VLDL particles; XS-VLDL-C%: cholesterol to total lipids ratio in very small VLDL particles.

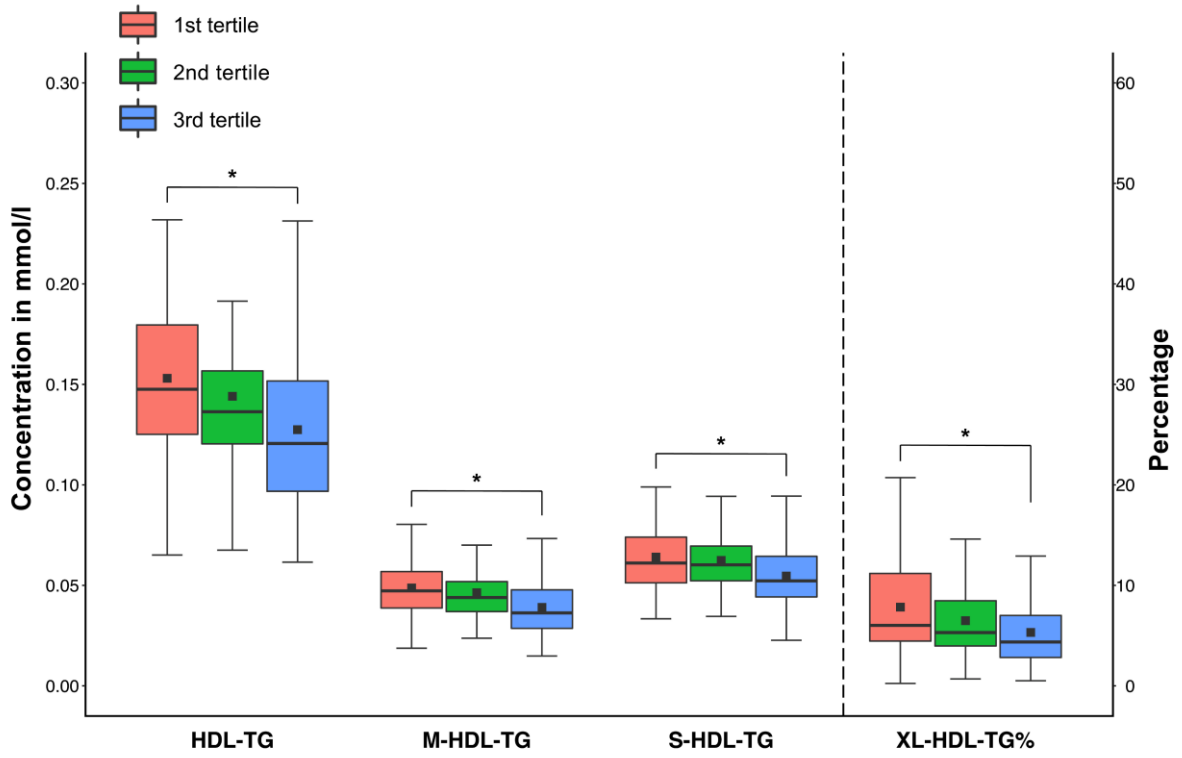


**Figure 2:** Box plots comparing selected metabolite measures (24 h post-MI) between ISZ tertiles. For all plots plots, the lower and the upper margins represent the first and third quartile, respectively. Vertical lines indicate median values; squares indicate mean values. The whiskers represent the lowest and the highest value within 1.5 IQR. Differences between categories were assessed using multinomial logistic regression adjusted for treatment, age, sex, NTproBNP levels, CK-MB levels, myocardial blush grade, statin use. Asterisks indicate effects significant after correction for multiple testing ($p<7.4x10^{-4}$). IQR: inter-quartile range; LVEF: left ventricular ejection fraction; ISZ: infarct size; HDL-TG: triglycerides in HDL particles; M-HDL-TG: triglycerides in medium HDL particles; XL- S-HDL-TG: triglycerides in small HDL particles; XL-HDL-TG: triglyceride to total lipids ratio in very large HDL particles.