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Inflammatory metabolic profile of South African patients with prostate cancer

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24 **Abstract**

25 **Background:** Men with African ancestry are more likely to develop aggressive prostate cancer (PCa) and
26 to die from this disease. A distinct genomic landscape associated with specific ethnic groups may lead to
27 different metabolic adaptations and inflammatory responses that permit tumor cells to proliferate and to
28 grow. We hypothesize that a higher risk of lethal PCa in men with African ancestry may be associated with
29 high level of systemic inflammation.

30 **Methods:** In this study, plasma samples were profiled from a cohort of 41 South African men with PCa
31 using Nuclear Magnetic Resonance (NMR) spectroscopy. A total of 41 features, including metabolites,
32 lipid classes, total protein, and the inflammatory NMR markers, GlycA and GlycB were quantified from
33 each NMR spectrum. The Bruker's B.I.-LISA protocols were used to characterize 114 parameters related
34 to the lipoproteins, such as HDL, LDL and VLDL, and their relative subclasses. The unsupervised
35 KODAMA method was used to stratify the patients of our cohort based on their metabolic profile.

36 **Results:** We found that the plasma of patients with very high risk, aggressive PCa and high level of C-
37 reactive protein have a peculiar metabolic phenotype (metabotype) characterized by extremely high levels
38 of GlycA and GlycB. The inflammatory processes linked to the higher level of GlycA and GlycB are
39 characterized by a deep change of the plasma metabolome that leads the stratification of patients with PCa.
40 We also identified a not previously relationship between high values of very-high density lipoprotein and
41 low level of GlycB in a different metabotype of patients characterized by lower risk PCa.

42 **Conclusions:** Systemic inflammation plays a role in the metabolic profile of cancer. This study advances
43 our understanding of the relationship between metabolome and systemic inflammation in the context of
44 PCa and opens the door to a totally innovative approach for biomarker discovery and to the development
45 of new therapies aiming to reduce the systemic inflammation in these patients.

46 **Keywords:** Metabolomics, NMR spectroscopy, GlycA, GlycB, histidine, Prostate cancer, Africa

47

48 **Abbreviations:**

49 AA, African American; ADT, androgen-deprivation therapy; APP, acute-phase protein; BO, bilateral
50 orchidectomy; CRP, C-reactive protein; CRPC, castration-resistant prostate cancer; CSPC, castration-
51 sensitive prostate cancer EA, European American; FDR, false discovery rate; HDL, high-density
52 lipoprotein; IDL, intermediate-density lipoprotein; IL, interleukin; IFN, type I interferon; LDL, low-density
53 lipoprotein; metabotype, metabolic phenotype; mCRPC, metastatic castration-resistant prostate cancer;
54 NCCN, National Comprehensive Cancer Network; NMR, nuclear magnetic resonance; NOESY, Nuclear
55 Overhauser Effect Spectroscopy; PCa, prostate cancer; PLS, partial least-squares; PSA, prostate-specific
56 antigen; Q², predictive ability parameter; R², fit parameter; rho, correlation coefficient VLDL, very-low-
57 density lipoprotein.

58 **Background**

59 Prostate cancer (PCa) is the second most frequent cancer diagnosis made in men and the fifth leading cause
60 of death worldwide [1]. Advancing age, family history of PCa and African ancestry are among established
61 risk factors [2]. PCa in men of African descent tends to have more aggressive phenotypes compared with
62 other ethnicities [3]. Studies investigating the possible role of genetic susceptibility in affecting PCa
63 disparities are often limited to the African American (AA) population. Comparing to European American
64 (EA) men, AA men have two-thirds higher incidence and two-fold greater risk of dying of PCa[3]. The
65 hypothesis that ethnic disparity may be related to biological differences in PCa phenotype is supported by
66 recent studies conducted in Southern Africa. Men from Southern Bantu populations have a 2.1-fold and
67 4.9-fold greater risk than AAs for presenting at diagnosis a PCa with Gleason score ≥ 8 and prostate-specific
68 antigen (PSA) ≥ 20 ng/mL [4]. Recently, a pilot Whole-Genome Sequencing study conducted on six
69 African prostate tumors indicated a doubling of the mutational burden in African men compared to men of
70 European ancestry [5]. Disparities in PCa risk and aggressiveness across different ethnicities are poorly
71 understood and likely influenced by genetic factors as well as difficulties to access medical resources in
72 Africa [6].

73 The South African population is a unique blend of African and non-African ancestry [7]. Its ethnic diversity
74 represents both a challenge and an opportunity for biomedical research [8]. Currently, the major
75 ethnolinguistic groups in South Africa are Black southeastern Bantu-speakers, an admixed population
76 referred to as Coloured [9], Whites of European origin (8.9%), and an Indian population originating from
77 the Indian sub-continent (2.5%) [7].

78 Lately, more effort has been put on exploring the genetic factors contributing to PCa in men of African
79 ancestry [10-13]. Several genes have been identified as differentially methylated or expressed in PCa
80 between men of European or African ancestry [14]. The number of studies linking the association of one
81 or multiple single nucleotide polymorphisms in inflammation-related pathways to PCa risk has greatly
82 increased[15, 16]. Since inflammation may contribute to PCa development and progression to advanced

83 metastatic disease [17], polymorphisms in immune-related genes could at least partially explain the
 84 different incidence and mortality of PCa in African men [18]. AA men, compared to EA men, show an
 85 increased incidence of inflammation in biopsy specimens [19] and increased expression of immune-related
 86 genes in tumor tissues [20]. A distinct genomic landscape of PCa and immune-related genes associated
 87 with specific ethnic groups may lead to different metabolic adaptations that permit tumor cells to proliferate.
 88 While metabolic reprogramming is known to play a significant role in both PCa initiation and progression,
 89 inflammation seems to be ignored in most biomarker studies [21], including metabolomic studies.

90 Chronic inflammation has a strong impact on the human metabolome [21]. Metabolic analysis may
 91 illuminate systemic metabolic consequences of inflammation and provide novel targets for intervention.
 92 Nuclear magnetic resonance (NMR) spectroscopy is a particularly powerful technique when applied to the
 93 high-throughput analysis of biofluids such as blood [22] which can be collected with minimal impact on
 94 the participant. NMR-based metabolomics is a straightforward and useful method for the qualitative and
 95 quantitative analysis of a wide range of components in blood samples, including low-molecular-weight
 96 metabolites and lipoproteins (different for size and composition) [23]. Moreover, NMR spectroscopy
 97 allows the detection in plasma of signals arising from the glycosylation of circulating acute-phase proteins
 98 (APPs), such as fibrinogen, α 1-antichymotrypsin, haptoglobin-1, α 1-antitrypsin, complement C3 and α 1-
 99 acid glycoprotein [24].

100 The carbohydrate portions of glycoproteins containing N-acetylglucosamine and N-acetylgalactosamine
 101 (hereinafter referred to as GlycA) and N-acetylneuraminic acid (a.k.a., sialic acid; hereinafter referred to as
 102 GlycB) moieties are visible as two distinct NMR signals. GlycA and GlycB levels have been associated
 103 with common markers of inflammation such as C-reactive protein (CRP), fibrinogen, interleukin (IL)-6,
 104 tumor necrosis factor-alpha, lipoprotein-associated phospholipase A₂ and serum amyloid A [25-28]. Similar
 105 to CRP, GlycA is a marker of chronic inflammation [27]; despite the similarity, GlycA and CRP likely
 106 capture different aspects of the inflammatory response [29]. CRP is an “early” APP and the proteins that
 107 contribute the most to the GlycA and GlycB signal rise later in the acute phase response [30]. In response

108 to acute and chronic inflammatory stimuli both the concentrations of APPs and their glycan structures are
109 modified [30].

110 This study, to our knowledge, is the first to metabolic profile PCa in men from Africa. Here, we used NMR
111 spectroscopy to quantify a panel of 41 signals, including metabolites, lipid groups, proteins, and the
112 inflammatory markers GlycA and GlycB. Moreover, we used an advanced lipoprotein test based on NMR
113 spectroscopy to characterize the lipoprotein subclasses in each sample. For the first time, we reported the
114 relationship between these inflammatory biomarkers (i.e., GlycA and GlycB) and the most aggressive PCa
115 cases. We provide a clear snapshot of the metabolic alterations during the inflammatory process in PCa
116 paving the way to a better understanding of the metabolic changes occurring in PCa.

117 **Methods**

118 *Patients*

119 Participants were recruited from the Urological clinics of Groote Schuur, Eerste Rivier and New Somerset
120 Hospitals in Cape Town, South Africa. Patients scheduled to undergo transurethral resection of the prostate
121 or prostatectomy were enrolled. The diagnosis of PCa was confirmed by histopathologic examinations. The
122 protocol (*HREC454/2012*) was approved by the Human Research Ethics Committee of the Faculty of
123 Health Science, University of Cape Town, South Africa. Written consent was obtained from all the
124 participants before 5 mL of blood was collected in Vacuette® EDTA tube by medical staff. Blood plasma
125 was separated by centrifugation (1000 g for 10 min at 4°C) and stored at -80°C.

126 *NMR sample preparation and analysis*

127 Plasma samples were thawed at room temperature. An aliquot of 350 µL of a phosphate sodium buffer
128 (70 mM Na₂HPO₄; 20% (v/v) ²H₂O; 6.1 mM NaN₃; 4.6 mM sodium 3-trimethylsilyl [2,2,3,3-²H₄]-
129 propionate; pH 7.4) was added to 350 µL of each sample. The mixture was homogenized by vortexing for
130 30 s, before 600 µL of this mixture was transferred into a 5 mm NMR tube for analysis.

131 For each plasma sample, one-dimensional ¹H-NMR spectra were acquired on a Bruker 600 MHz
132 spectrometer (Bruker BioSpin) operating at 600.13 MHz proton Larmor frequency and equipped with a
133 5 mm PATXI 1H-13C-15N and 2H-decoupling probe including a z-axis gradient coil, an automatic tuning-
134 matching and an automatic and refrigerated sample changer (SampleJet). A BTO 2000 thermocouple was
135 used at the level of approximately 0.1 K on the sample to stabilize the temperature. Before starting
136 measurements, samples were kept inside the NMR probe head for at least 5 minutes to equilibrate
137 temperature at 310 K. The standard Nuclear Overhauser Effect Spectroscopy (NOESY) presat pulse
138 sequence (noesygppr1d.comp; Bruker BioSpin) was used to detect both signals of small metabolites and
139 high molecular weight macromolecules. Parameters of the experiment were: 32 scans, 98304 data points, a
140 spectral width of 18028.846 Hz, an acquisition time of 2.73 s, a relaxation delay of 4 s and a mixing time
141 of 0.01 s. Transformed spectra were automatically corrected for phase and baseline distortions using
142 Topspin 3.2 (Bruker BioSpin) and then automatically calibrated to the anomeric proton signal of α-glucose
143 at 5.24 ppm.

144 *Molecular profiling and lipoprotein quantification*

145 Lipoprotein parameters were estimated on NOESY spectra according to Bruker's B.I.-LISA protocols
146 (Bruker IVDr Lipoprotein subclass analysis) [31]. Information related to the main very-low-density
147 lipoprotein (VLDL), low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL), and high-
148 density lipoprotein (HDL) classes and to their subclasses were extrapolated. In detail, information was
149 extracted of five VLDL subclasses (from VLDL-1 to VLDL-5), six LDL sub-classes (from LDL-1 to LDL-
150 6), and four HDL-subclasses (HDL-1 to HDL-4) sorted according to increasing density and decreasing size.
151 For each class and subclass, calculated data consist of concentrations of lipids, i.e., cholesterol, free
152 cholesterol, phospholipids, and triglycerides. Instead, concentrations of apolipoproteins Apo-A1 and Apo-
153 A2 were estimated for HDL class and each relative subclass, while Apo-B concentrations are calculated for
154 VLDL, IDL classes and all LDL subclasses.

155 Identification of signals was undertaken using the SBASE database in Amix (v3.9.11; Bruker BioSpin,
156 Germany) or available assignments in the literature. The peaks of the identified metabolites were fitted by
157 a combination of a local baseline and Voigt functions based on the multiplicity of the NMR signal. GlycA
158 and GlycB signals were quantified by integrating, respectively, the areas between 2.005 and 2.054 ppm and
159 between 2.086 and 2.054 ppm above a local baseline aimed to remove the signal of the lipoproteins. Fitting
160 methods to quantify GlycA and GlycB signals were not used due to their heterogeneity and due to the
161 impossibility to completely distinguish them from the lipoprotein signal. The amide protein signals belong
162 to plasma proteins were quantified integrating the area between 6.000 and 10.000 ppm.

163 *Plasma CRP quantification*

164 The plasma concentration of CRP was determined using the Human C Reactive Protein ELISA kit (abcam,
165 ab99995). Patient plasma was diluted 1:80,000 in 1X Assay Diluent D.

166 *Statistical and data analysis*

167 Statistical analysis and graphical illustrations of the data were generated in the R (version 3.6.1) and R
168 studio (version 1.1.456) software using scripts developed in-house.

169 Wilcoxon Rank Sum test was used to compare differences in numerical covariates (e.g., age and metabolite
170 concentration). Fisher's exact test was used to assess differences between categorical variables (e.g.,
171 ethnicity). Spearman's test was used to calculate the correlation coefficient (ρ) between variables. The
172 KODAMA algorithm was used to facilitate the identification of patterns representing underlying metabolic
173 phenotypes (metabotype) on all samples in the data set. Dendrograms were performed using the KODAMA
174 output and Ward linkage. Silhouette median value being used to evaluate the optimal number of clusters
175 with the number of possible clusters varying from 2 to 10 [32]. p-values less than 0.05 were considered to
176 be significant. To account for multiple testing, a false discovery rate (FDR) of <10% was applied.

177 Discriminant analysis of metabolic profiles was performed using partial least-squares (PLS) analysis. To
178 assess the predictive ability of the PLS regression model, a 10-fold cross-validation was conducted as

179 previously described [22]. The goodness of fit parameter (R2) and the predictive ability parameter (Q2)
180 were calculated using standard definitions [33].

181

182 **Results**

183 *South African patient cohort*

184 Although a few studies have been performed to investigate the metabolic alterations in the blood of patients
185 with PCa, high-risk populations are underrepresented and limited to AAs. In this study, we recruited 41
186 South African patients with PCa in order to generate a better understanding of the metabolic changes in the
187 unique South African setting. The majority of patients were characterized by a unique mixed ancestry (61%)
188 referred to as Coloured; the rest were self-classified as Black (22%) and Whites (17%). We classified the
189 aggressiveness of cancer according to the National Comprehensive Cancer Network (NCCN) classification
190 (version 2.2020): i) very low, low, and intermediate risk; ii) high risk; and iii) very high risk. The clinical
191 and demographic features of the patients with PCa are reported in **Table 1**. Patients with regional or distant
192 metastasis were classified as a separate group. Patients that received androgen-deprivation therapy (ADT),
193 i.e., bilateral orchiectomy (BO), were considered as two distinct groups based on the evidence of
194 castration-resistant PCa (CRPC). In our cohort, we did not observe statistically significant difference among
195 ethnicity in term of NCCN classification in untreated patients, although, we reported an advanced clinical
196 stage in Black men with 71% of them classified as stage T3 or T4 compared to 19% of Coloured and 33%
197 of White men. This disparity was highlighted even by the PSA level. We reported extremely high values
198 of PSA (>100 ng/mL) in 57% of Black compared to 9% of Coloured and 33% of White. As expected, we
199 observed a higher prevalence of diabetes and hypertension in the post-BO group.

200 *GlycA and GlycB inflammatory biomarkers*

201 Growing evidence implicates chronic inflammation as a contributor to PCa development and progression
202 to advanced metastatic disease [17], and as a driver of CRPC development in ADT [34, 35]. Recently,

203 GlycA and GlycB have been identified as markers of systemic and chronic inflammation but their
204 association with PCa has not been described yet [36]. Here, we used the NMR spectroscopy to quantify the
205 signal associated with GlycA and GlycB and, for the first time, we investigated their association with the
206 aggressiveness of PCa. We noted that the values of both markers are higher in patients with very highly
207 aggressive PCa and metastatic PCa (**Fig. 1a,e**). Indeed, all patients whose GlycA and GlycB was higher
208 than the 80th percentile were diagnosed with poorly differentiated PCa (i.e., Gleason score higher than or
209 equal to 8). Although the limited number of patients who had BO did not allow for enough statistical power,
210 we observed an increased value of both GlycA and GlycB in patients with CRPC (**Fig. 1b,f**).

211 Several studies have reported the association of GlycA and GlycB with key markers of cancer stratification,
212 such as CRP [37] (**Fig. 1c,g**). Here, we report for the first time a statistically significant correlation with
213 PSA (**Fig. 1d,h**). The correlation with PSA implies that a disparity in the GlycA and GlycB related to
214 ethnicity should be expected in patients with PCa. Noteworthy, the three highest values of GlycA and GlycB
215 were found in patients that identified themselves as Black.

216 Moreover, we quantified the amides of proteins from the NMR spectra. Albumin is the most concentrated
217 protein in the plasma and consequently, the protein amides concentration is highly related to the albumin
218 level. We reported a negative correlation between the NMR inflammatory marker GlycA ($\rho=-0.41$;
219 $P=0.00747$) and GlycB ($\rho=-0.31$; $P=0.0462$) with protein amides. Albumin is known to be a negative
220 APP that decreases in concentration during inflammation. This further supports our finding that the higher
221 values of GlycA and GlycB could be associated with inflammatory process in patients with PCa.

222 *Metabolic stratification of PCa*

223 Reprogramming of metabolism is a widely accepted hallmark of cancer development [21], however the
224 metabolic changes induced by inflammation in cancer patients have not been fully characterized.
225 Metabolomics represents an essential tool for the stratification of cancer patients into groups of patients
226 with similar metabolic profiles that could share the same clinicopathologic condition (e.g., systemic

inflammation). Here, we quantified the metabolites from each plasma sample using the data collected by the NMR experiments. In order to identify potential underlying metabolic phenotypes (a.k.a. metabotype) in patients with treatment-naïve PCa, we applied the unsupervised method KODAMA method to the quantified metabolite concentrations (GlycA, GlycB, and protein amides were not considered in this analysis). We identified four different metabotypes in the KODAMA score plot (**Fig. 2a,b**) using the hierarchical clustering [38] on the KODAMA scores.

We clearly observed an association between the PCa aggressiveness (based on the NCCN classification) and the metabotypes that we rank from I to IV in order of aggressiveness. When evaluating GlycB and GlycA in the 4 metabotypes, we noted different levels of GlycB in each metabotypes with the highest levels of both GlycA and GlycB in the metabotype IV. Clinical and demographic features of the metabotypes are shown in **Table 2**.

The metabolic profile of the Metabotype IV is the most peculiar. Samples of the metabotype IV showed an unprecedentedly well-defined fingerprint that may reflect a common biologic process that drives the metabolic changes in the blood of patients with high GlycA and GlycB levels. The metabotype IV is formed almost exclusively by patients categorized as very high risk and also includes a patient with metastatic PCa (**Table 2**).

Noteworthy, a patient classified as low risk PCa based on the NCCN classification showed a metabolic profile typical of Metabotype IV but with lower level of GlycA and GlycB. This patient died only 50 days after sample collection due to pancreatic cancer. On the other hand, patients classified as very high aggressiveness but that do not belong to the Metabotype IV showed a survival time longer than 3 years and a lower Gleason score compared with patients belong to the Metabotype IV. We were unable to record the date of death for 4 out of 7 patients belong to the Metabotype IV. These patients were lost to follow-up at the hospital cancer center where they were recruited nor did they have any type of diagnostic test at a South African clinic or hospital. Considering the severe condition of these patients and the absence of registered diagnostic tests following the last visit, we assume the latter as a rough estimation of the time of survival.

252 Almost all patients of the Metabotype IV seem to have died within one year after the sample collection
253 (**Table 3**).

254 Noteworthy, we reported a few clues of possible differences of the prostate tissue inflammation among the
255 metabotype. Of 14 patients in the Metabotype III, 3 patients had mild chronic inflammation and 2 patients
256 had chronic inflammation reported on the histological exam of their prostate tissue. Of the 10 patients of
257 the Metabotype II, 2 patients had mild chronic inflammation, 3 patients had acute-on-chronic inflammation,
258 and 1 had acute prostatitis on the histological exam.

259 *Metabolic profiling of PCa metabotypes*

260 The metabolic differences discriminating between among the four metabotypes appear to be clear (**Fig. 2c**).
261 An overview of some discriminative NMR signals among the four metabotypes are showed in **Figure 3**.
262 Although we were aware of the low number of patients in this cohort, we built a supervised PLS model to
263 evaluate the accuracy of the identification of the most aggressive metabotype (i.e., Metabotype IV) using
264 the metabolic profile. Using a double cross-validation approach, we calculated an accuracy value of 91.2%,
265 with a 95% coefficient interval of 86.0%-94.1%. Next, we used the Wilcoxon rank-sum test to characterize
266 this metabotype compared to the others (**Supplementary Table 1**). As previously mentioned, the
267 Metabotype IV is characterized by higher values of the inflammatory markers GlycA ($P=7.06 \times 10^{-6}$;
268 $FDR=7.24 \times 10^{-5}$) and GlycB ($P=2.60 \times 10^{-6}$; $FDR=3.56 \times 10^{-5}$) and lower protein level ($P=9.15 \times 10^{-5}$;
269 $FDR=5.36 \times 10^{-4}$). In addition, we detected a higher level of mannose ($P=4.45 \times 10^{-3}$; $FDR=1.40 \times 10^{-2}$), an
270 important constituent of N-glycans of glycoproteins [39]. Mannose residues in N-glycans can be derived
271 from either glycogen/glucose or mannose in the blood [39]. Moreover, we detected a reduced level of amino
272 acids and their derivatives in the Metabotype IV. Among them, the reduction of histidine is the most
273 significant ($P=3.72 \times 10^{-7}$; $FDR=1.52 \times 10^{-5}$). We noted an interesting clue of the possible role of microbiota
274 in the inflammatory status of patients with PCa. We found desaminotyrosine, a metabolite produced by
275 human enteric bacteria and able to induce type I interferon (IFN) response[40], prevalent in patients with
276 very high aggressive PCa.

Similar to the previous analysis, we also investigated the rearrangement of the lipoprotein profile using the data from the application of the Bruker's B.I.-LISA protocols (**Supplementary Table 2**). The Bruker's B.I.-LISA protocols were used to characterize 114 parameters related to the lipoproteins, such as HDL, LDL and VLDL, and their relative subclasses. We observed an association between the metabotype IV and lower level of apolipoprotein Apo-A1 ($P=2.67 \times 10^{-3}$; $FDR=6.10 \times 10^{-2}$) and Apo-A2 ($P=4.04 \times 10^{-3}$; $FDR=5.32 \times 10^{-2}$), attributable to a reduced HDL particle number ($P=1.28 \times 10^{-2}$; $FDR=2.68 \times 10^{-1}$), and higher level of triglycerides in LDL of smaller size, including LDL-1 ($P=4.45 \times 10^{-3}$; $FDR=8.45 \times 10^{-2}$) and LDL-2 ($P=1.40 \times 10^{-3}$; $FDR=5.32 \times 10^{-2}$). This finding completes the snapshot of the metabotype IV as a large regulator of the blood constituents, including metabolites, proteins, and lipoproteins with several implications for the role of inflammation as a confounding factor of PCa.

The levels of inflammatory NMR markers GlycA and GlycB have been shown to be highly correlated, as previously reported in the literature [37]. In our study, we reported a Spearman's rank correlation rho of 0.59 ($P=7.16 \times 10^{-5}$). However, the biological meaning of the differences between GlycA and GlycB has not yet been fully explored. In our cohort, we observed that two distinct metatypes, Metatype II and Metatype III, had a similar level of GlycA but different levels of GlycB ($P=1.42 \times 10^{-4}$; $FDR=6.46 \times 10^{-4}$), with the latter showing the higher level (**Table S3**). Moreover, Metatype III seems to be associated with extremely reduced levels of lipids (**Fig. 3a,e**). We discovered a deep difference in the lipoprotein profile between Metatype III and Metatype II using the B.I.-LISA protocols (**Supplementary Table 4**). **Figure 4** shows a graphical representation of the lipoprotein profile changes among metatypes. Besides the evident reduction of VLDL, we also noted the lower values of Apo-A1 and Apo-A2 that could help to characterize the differences between GlycA and GlycB.

Discussion

PCa shows significant ethnic disparity among men with African ancestry representing a well-established risk factor. In a recent study, all patients who underwent a prostate biopsy from July 2008 to July 2014 in one of the hospitals included in our cohort (Groote Schuur Hospital, Cape Town) were recorded [41]. Among all patients diagnosed with PCa, 41% and 21% were classified as high and very high risk PCa (NCCN classification), respectively. Although the percentage of clinically advanced cases is already impressive if compared to American or European studies, the relative percentage of cases with very high aggressive PCa is even higher (33%) if we consider only the Black population.

In this first metabolomic study of PCa conducted on an African population, we profiled the plasma samples of men of different ancestry. We observed in men with very high aggressive PCa higher levels of the NMR inflammatory markers GlycA and GlycB, probably due to the increased concentration of positive APPs and the complexity of their glycan structures. Moreover, we noted a simultaneous reduction of the signal from protein source likely attributable to a reduction of the albumin level, a negative APP.

Interestingly, in our cohort we discovered four distinct metabolotypes associated with the aggressiveness of PCa, each one characterized by a unique metabolic fingerprint. A metabolotype identified as a subgroup of patients with very high aggressive PCa (that we named Metabolotype IV) was characterized by the highest values of GlycA and GlycB and by deep changes of the plasma metabolome. We observed a lower level of histidine that could reinforce our hypothesis of inflammatory processes underlying the Metabolotype IV. Indeed, histidine has been already associated with inflammatory processes and in particular, it has been negatively correlated with other inflammatory markers, such as IL-6 and CRP [42]. In addition, it has been reported that inflammation may alter the lipoprotein profile as well, for example modulating the HDL functions [43-45] as we observed. Recently, it has been shown that high levels of triglycerides and glucose and low levels of HDL cholesterol and Apo-A1 are related to increased PCa risk and its severity [46]. Moreover, low HDL was reported to be a risk and prognostic factor for PCa in several epidemiologic studies [47]. Elevated serum triglycerides were associated with an increased risk of PCa recurrence [48]. Lower levels of Apo-A1 and Apo-A2, and a higher level of triglycerides in LDL, reported in this study, are

325 consistent with these processes and we suggest that inflammation could be a driving factor of the lipoprotein
326 profile changes observed in Metabotype IV.

327 Although *in vitro* studies reported that aerobic glycolysis can be directly induced by an inflammatory
328 microenvironment [49, 50], we did not observe any metabolic signature of the Warburg effect in the plasma
329 of the patients with an inflammatory status associated to the PCa. Indeed, plasma metabolome do not
330 necessarily reflect the Warburg effect present in the tumor tissue. As reported in a study on metastatic
331 colorectal cancer (CRC) [22] where the Warburg effect is well-established, patients with mCRC showed
332 lower serum levels of lactate and non-statistically significant changes of glucose compared to the controls.
333 Liver gluconeogenesis [51] and insulin resistance associated with inflammation [52] may mask anaerobic
334 dissimilation of glucose and lactate production.

335 Furthermore, we highlighted two other distinct metabolotypes characterized by large differences in the
336 lipoprotein profile. We noted a higher concentration of GlycB in the Metabotype III, which is characterized
337 by a higher number of patients with very high aggressive PCa compared to the Metabotype II. No
338 differences in the concentration of GlycA and higher levels of GlycB could be due to an elevated sialylation
339 post-translational modification on glycosylated proteins. Complex biantennary glycoforms with α 2,3-sialic
340 acid have been associated with aggressive PCa [53-55]. Here, for the first time, we reported the association
341 of lower level of the inflammatory NMR biomarker GlycB with a higher concentration of VLDL. Since the
342 concentration of dietary intake can modulate the VLDL, this finding will further enrich the long-standing
343 debate over the role of dietary fat in promoting PCa [56, 57].

344 In men diagnosed with PCa, the selection of the treatment, including the type of therapy and its
345 aggressiveness, is often based on patient age and life expectancy. In an era of precision medicine, an
346 estimate of the threat of disease and the benefit and the costs of intervention within the context of the
347 patient's characteristics and desires should be taken into consideration regarding the decision of the
348 treatment. In this study, we identified a set of patients with very high aggressive PCa with extremely
349 reduced survival time. These patients, belonging to the Metabotype IV, are characterized by a similar

350 metabolic profile predictable with high accuracy. Our results postulate that this subgroup may be most
351 likely to benefit from combination therapy that associates the androgen deprivation in conjunction with
352 drugs aiming to reduce the level of systemic inflammation.

353 In this context, metabolomics could represent an invaluable tool for the stratification of patients with very
354 high aggressive PCa. The life expectancy difference highlights the need to consider an appropriate medical
355 treatment for patients within Metabotype IV. We hypothesize that these patients could largely benefit from
356 daily treatment with corticosteroids to reduce the systemic inflammation improving the overall survival,
357 along with the need for subsequent therapy. However, the only clinical settings of corticosteroids treatment
358 with proved clinical utility in PCa treatment is in combination with abiraterone. We consider the lack of
359 clinical investigation for almost all patients of the presence of distant metastasis as a limitation of this study.
360 Considering the clinical and histopathological features, we are aware that PCa could have spread to distant
361 organs in the patients with very high risk PCa belonging to the Metabotype IV. The current project is still
362 ongoing and we are recruiting more patients with PCa to further validate our results in a larger cohort.

363 **Conclusions**

364 Integrating a metabolomic analysis as a tool for patient stratification could be important to identify those
365 patients with very high risk PCa and short life expectancy, as they may benefit from therapeutic
366 interventions, targeting the lowering of systemic inflammation. Thus, further studies are necessary to better
367 characterize this group of patients and determine the costs and benefits of corticosteroid treatment in terms
368 of survival time and quality of life.

369 In conclusion, this study answers the urgent need for new precision medicine approaches aiming to profile
370 aggressive and lethal PCa in men of African ancestry. New non-invasive metabolite biomarkers are
371 necessary to improve the treatment decision, which will improve therapeutic outcomes in African patients.

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374 **Disclosure of Potential Conflicts of Interest**

375 No potential conflicts of interest were disclosed.

376

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383 **Contributions**

384 L.F.Z. conceived the project and designed experiments; S.C., M.W., and C.L. performed the experiments
385 and analysis of the data; S.C. wrote the paper with assistance and feedback of all the other co-authors. In
386 addition, all authors participated in revising the work critically for important intellectual content and
387 approved the final version.

388 **Table 1.** Clinical demographics of PCa patients

Feature	Treatment-naïve					post-BO		
	intermediate, low and very low (n=14)	high (n=7)	very high (n=12)	metastatic (n=1)	Total n=34	non-CRPC (n=4)	CRPC (n=3)	Total (n=7)
Age (year), median [95%CI]	65 [56 77]	70 [52 90]	64 [57 86]	75 [75 75]	68 [63 74]	72 [63 73]	66 [64 70]	70 [65 72]
Ancestry, n (%)								
Black	1 (7.1)	1 (14.3)	4 (33.3)	1 (100.0)	7 (20.6)	1 (25.0)	1 (33.3)	2 (28.6)
Coloured	10 (71.4)	5 (71.4)	6 (50.0)	0 (0.0)	21 (61.8)	2 (50.0)	1 (33.3)	3 (42.8)
Coloured/Black	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (14.3)
White	3 (21.4)	1 (14.3)	2 (16.7)	0 (0.0)	6 (17.6)	0 (0.0)	1 (33.3)	1 (14.3)
PSA (ng/mL), median [95%CI]	9 [3 19]	23 [17 31]	138 [29 3919]	>5000	21 [12 84]	4 [2 40]	332 [49 1128]	34 [4 188]
Diabetes, n (%)								
no	10 (71.4)	5 (71.4)	12 (100.0)	1 (100.0)	28 (82.4)	3 (75.0)	2 (66.7)	5 (71.4)
yes	4 (28.6)	2 (28.6)	0 (0.0)	0 (0.0)	6 (17.6)	1 (25.0)	1 (33.3)	2 (28.6)
Hypertension, n (%)								
no	7 (50.0)	5 (71.4)	10 (83.3)	1 (100.0)	23 (67.6)	3 (75.0)	1 (33.3)	4 (57.1)
yes	7 (50.0)	2 (28.6)	2 (16.7)	0 (0.0)	11 (32.4)	1 (25.0)	2 (66.7)	3 (42.9)
Smoker, n (%)								
no	10 (71.4)	6 (85.7)	9 (75.0)	1 (100.0)	26 (76.5)	4 (100.0)	1 (33.3)	5 (71.4)
yes	4 (28.6)	1 (14.3)	3 (25.0)	0 (0.0)	8 (23.5)	0 (0.0)	2 (66.7)	2 (28.6)

392 **Table 2.** Clinical and demographic features of the metabolotypes identified through KODAMA analysis.

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Feature	Metabotype I	Metabotype II	Metabotype III	Metabotype IV
NCCN classification, n (%)				
very low	1 (33.3)	0 (0.0)	1 (7.1)	0 (0.0)
low	0 (0.0)	1 (10.0)	1 (7.1)	1 (14.3)
intermediate	2 (66.7)	4 (40.0)	3 (21.4)	0 (0.0)
high	0 (0.0)	3 (30.0)	4 (28.5)	0 (0.0)
very high	0 (0.0)	2 (20.0)	5 (35.7)	5 (71.4)
metastatic	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)
Gleason Score, n (%)				
3+3	1 (33.3)	1 (10.0)	5 (35.7)	1 (14.3)
3+4	1 (33.3)	4 (40.0)	6 (42.9)	0 (0.0)
4+3	1 (33.3)	1 (10.0)	0 (0.0)	0 (0.0)
3+5	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)
4+5	0 (0.0)	4 (40.0)	3 (21.4)	0 (0.0)
5+4	0 (0.0)	0 (0.0)	0 (0.0)	2 (28.6)
5+5	0 (0.0)	0 (0.0)	0 (0.0)	3 (42.9)
Age, median [95%CI]	71 [64 74]	68 [56 90]	65 [51 85]	74 [59 78]
Ancestry, n(%)				
Black	1 (33.3)	1 (10.0)	2 (14.3)	3 (42.9)
Mixed ancestry	1 (33.3)	7 (70.0)	11 (78.6)	2 (28.6)
White	1 (33.3)	2 (20.0)	1 (7.1)	2 (28.6)
PSA, median [95%CI]	9 [3 9]	18 [5 233]	25 [5 126]	738 [26 5000]
Diabetes, n (%)				
no	1 (33.3)	7 (70.0)	13 (92.9)	7 (100.0)
yes	2 (66.7)	3 (30.0)	1 (7.1)	0 (0.0)
Hypertension, n (%)				
no	0 (0.0)	8 (80.0)	9 (64.3)	6 (85.7)
yes	3 (100.0)	2 (20.0)	5 (35.7)	1 (14.3)
Smoker, n (%)				
no	2 (66.7)	8 (80.0)	9 (64.3)	7 (100.0)
yes	1 (33.3)	2 (20.0)	5 (35.7)	0 (0.0)

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Table 3. Demographics and clinical features of patients belonging to the Metabotype IV and patients classified as very high aggressiveness that do not belong to the Metabotype IV.

Patient ID	Ethnicity	Collection date	Date of last visit	Date of death	Follow-up (day) ^c	Age (year)	PSA (ng/mL)	Gleason Score	DRE	NCCN classification	GlycB rank ^d	Metabotype	Note
SAPC0159	Black	2017/03/01	2017/03/23		22	75.2	>5000	5+5	T3/T4	metastatic	3	IV	
SAPC0090	Coloured	2015/05/08		2015/06/27	50	78.2	6.3	3+3	T1c	low	8	IV	a
SAPC0192	Coloured	2017/07/31		2017/12/19	141	80.9	41.68	4+5	T2a	very high	7	III	b
SAPC0080	Black	2014/10/31	2015/04/21		172	77.8	738	3+5	T4	very high	2	IV	
SAPC0180	Black	2017/06/23	2017/12/12		172	58.1	>5000	5+5	T3/T4	very high	1	IV	
SAPC0249	Coloured	2018/03/25	2019/01/07		288	63	1070	5+4	T3	very high	4	IV	
SAPC0078	White	2014/11/07		2015/10/31	358	74.3	135.79	5+5	T4	very high	5	IV	
SAPC0193	Coloured	2017/08/11	2019/04/26		623	63.5	48.85	3+4	T3	very high	9	III	
SAPC0070	Coloured	2014/01/08		2015/12/11	702	65.4	34.8	4+5	T2b	very high	13	II	
SAPC0191	White	2017/07/28		2019/08/03	736	61.8	576	5+4	T3	very high	6	IV	
SAPC0195	Black	2017/08/13	2020/01/30		900	87.2	26.53	4+5	T3	very high	11	III	
SAPC0120	Black	2016/10/03	2019/09/30		1092	82.3	289.9	4+5	T3	very high	14	II	
SAPC0108	Coloured	2016/06/27		2020/04/17	1390	56.6	96.3	3+4	T4	very high	12	III	
SAPC0076	Coloured	2014/09/26	2020/06/04		2078	63.5	140.42	3+4	T3/T4	very high	10	III	

^a The patient was diagnosed with pancreas cancer 19 day after sample collection.

^b The patient died for bone metastasis 5 months after sample collection.

^c Number of days between the date of collection and the date of last visit at hospital or the date of death (if recorded).

^d This number represent the rank of order of the GlycB values starting from the highest value.

406 **Figure 1.** GlycA concentration in (a) treatment-naïve and (b) post-treatment. (c) Correlation between
 407 GlycA and CRP. (d) Correlation between GlycA and PSA. GlycB concentration in (e) treatment-naïve and
 408 (f) post-treatment. (g) Correlation between GlycB and CRP. (h) Correlation between GlycA and PSA.

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412 **Figure 2.** KODAMA score plot of plasma PCa samples colored according to NCCN classification. The
 413 size is proportional to (a) the GlycA intensity and (b) the GlycB intensity. (c) Heatmap of the metabolic
 414 profiles.

Figure 3. (a) NMR profiles of the plasma of three different spectral regions and Box-whiskers plots of the intensity of **(b)** GlycA and **(c)** GlycB, the concentration of **(d)** histidine and the number of **(e)** VLDL particles across the four metabotypes.

429 **Table S1.** Statistical comparison between the metabolic profiles of Metabotype IV *versus* the others.

Feature	Metabotype IV, median [IQR]	Others, median [IQR]	log change	p-value	FDR
Histidine	0.647 [0.566 0.732]	1.067 [0.981 1.163]	-0.74	3.72x10 ⁻⁷	1.52x10 ⁻⁵
Lipid (beta-CH2)	0.743 [0.6 0.785]	1.12 [0.989 1.239]	-0.69	7.44x10 ⁻⁷	1.52x10 ⁻⁵
GlycB	1.706 [1.524 1.89]	0.959 [0.782 1.126]	0.89	2.60x10 ⁻⁶	3.56x10 ⁻⁵
GlycA	1.455 [1.425 1.568]	0.981 [0.892 1.075]	0.61	7.06x10 ⁻⁶	7.24x10 ⁻⁵
Valine	0.62 [0.528 0.668]	1.002 [0.915 1.244]	-0.77	6.73x10 ⁻⁵	4.60x10 ⁻⁴
Leucine	0.624 [0.487 0.764]	1.065 [0.878 1.282]	-0.81	6.73x10 ⁻⁵	4.60x10 ⁻⁴
Protein	0.9 [0.87 0.939]	1.021 [0.979 1.071]	-0.19	9.15x10 ⁻⁵	5.36x10 ⁻⁴
Alanine	0.706 [0.578 0.774]	1.075 [0.89 1.234]	-0.69	2.10x10 ⁻⁴	1.07x10 ⁻³
Isoleucine	0.616 [0.548 0.811]	1.076 [0.896 1.224]	-0.78	3.45x10 ⁻⁴	1.57x10 ⁻³
Glutamine	0.842 [0.747 0.976]	1.098 [1.007 1.203]	-0.4	1.54x10 ⁻³	6.33x10 ⁻³
Lipid (-CH3-)	0.868 [0.826 0.946]	1.062 [0.948 1.189]	-0.28	2.67x10 ⁻³	9.97x10 ⁻³
Mannose	1.368 [1.212 1.497]	1.011 [0.754 1.173]	0.46	4.45x10 ⁻³	1.40x10 ⁻²
Threonine	0.663 [0.54 0.829]	1.042 [0.783 1.49]	-0.78	4.45x10 ⁻³	1.40x10 ⁻²
2-Hydroxyvalerate	0.867 [0.853 0.938]	1.053 [0.961 1.222]	-0.3	5.22x10 ⁻³	1.53x10 ⁻²
3-Hydroxybutyrate	0.875 [0.812 0.899]	1.029 [0.965 1.196]	-0.42	6.11x10 ⁻³	1.67x10 ⁻²
Unsaturated lipid (-CH=CH-)	0.769 [0.723 0.922]	1.05 [0.904 1.332]	-0.46	8.27x10 ⁻³	1.99x10 ⁻²
Glycerol phospholipid	0.475 [0.276 0.577]	1.24 [0.552 1.922]	-1.39	8.27x10 ⁻³	1.99x10 ⁻²
Creatine	0.493 [0.362 0.765]	1.103 [0.637 1.575]	-1.08	1.10x10 ⁻²	2.51x10 ⁻²
Pyroglutamate	0.879 [0.864 0.951]	1.023 [0.96 1.239]	-0.25	1.45x10 ⁻²	2.98x10 ⁻²
Phospholipid	1.184 [1.1 1.311]	1.014 [0.926 1.118]	0.23	1.45x10 ⁻²	2.98x10 ⁻²
Desaminotyrosine	13.361 [6.036 19.946]	1 [1 1]	0.79	2.11x10 ⁻²	4.12x10 ⁻²
Isobutyrate	0.643 [0.588 0.868]	0.959 [0.848 1.202]	-0.35	3.89x10 ⁻²	7.25x10 ⁻²
Glutamate	0.702 [0.467 0.938]	1.146 [0.664 1.378]	-0.87	4.85x10 ⁻²	8.64x10 ⁻²
Pyruvate	1.781 [1.131 2.386]	1 [0.643 1.507]	0.74	8.87x10 ⁻²	1.52x10 ⁻¹
Lipid (-(-CH2-)n-)	0.825 [0.773 0.992]	1.051 [0.892 1.512]	-0.43	9.74x10 ⁻²	1.60x10 ⁻¹
Lipid (alpha-CH2)	0.518 [0.491 0.974]	0.943 [0.671 1.98]	-0.86	1.17x10 ⁻¹	1.84x10 ⁻¹
Methanol	0.919 [0.682 1.255]	1.244 [0.924 1.534]	-0.32	1.39x10 ⁻¹	2.10x10 ⁻¹
Acetoacetate	0.833 [0.643 0.94]	0.958 [0.734 1.286]	-0.52	1.77x10 ⁻¹	2.59x10 ⁻¹
Formate	1.119 [0.951 1.352]	1.023 [0.767 1.158]	0.24	2.57x10 ⁻¹	3.63x10 ⁻¹
2-Hydroxybutyrate	0.989 [0.854 1.332]	0.933 [0.759 1.196]	0.21	2.75x10 ⁻¹	3.76x10 ⁻¹
3-Hydroxyisovalerate	0.484 [0.392 2.729]	0.663 [0.489 3.622]	-0.03	3.35x10 ⁻¹	4.29x10 ⁻¹
Lipid (=CH-CH2-CH=)	0.95 [0.819 1.004]	1.05 [0.86 1.102]	-0.1	3.35x10 ⁻¹	4.29x10 ⁻¹
Glycine	1.003 [0.886 1.145]	1.109 [0.979 1.193]	-0.14	4.27x10 ⁻¹	5.30x10 ⁻¹
Cholesterol backbone	1.047 [0.912 1.146]	1.131 [0.93 1.324]	-0.11	5.04x10 ⁻¹	6.08x10 ⁻¹
Glucose	1.015 [0.865 1.093]	1 [0.898 1.247]	-0.13	5.59x10 ⁻¹	6.51x10 ⁻¹
Lactate	1.388 [0.738 1.634]	0.869 [0.698 1.334]	0.27	5.88x10 ⁻¹	6.51x10 ⁻¹
Citrate	0.969 [0.8 1.195]	1.042 [0.805 1.285]	-0.1	5.88x10 ⁻¹	6.51x10 ⁻¹
Creatinine	0.974 [0.873 1.149]	1.004 [0.807 1.14]	-0.03	7.39x10 ⁻¹	7.97x10 ⁻¹
Phenylalanine	0.958 [0.821 1.113]	0.95 [0.799 1.111]	0.03	8.03x10 ⁻¹	8.44x10 ⁻¹
Acetate	1.053 [0.873 1.247]	1.004 [0.902 1.255]	-0.05	9.01x10 ⁻¹	9.23x10 ⁻¹
Tyrosine	1.077 [0.87 1.186]	1.043 [0.865 1.148]	0.01	1.00	1.00

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Table S2. Statistical comparison between the lipoprotein profiles of Metabotype IV *versus* the others.

Feature	Metabotype IV, median [IQR]	Others, median [IQR]	log change	p-value	FDR
Main_Parameters_Apo.A2	25.01 [22.865 26.685]	30.93 [27.97 33.845]	-0.32	1.04x10 ⁻³	5.32x10 ⁻²
HDL_Subfractions_Apo.A2_HDL.4	12.15 [11.055 13.345]	18.24 [14.905 21.8]	-0.6	1.04x10 ⁻³	5.32x10 ⁻²
LDL_Subfractions_Triglycerides_LDL.2	3.59 [3.33 3.905]	2.08 [1.825 2.755]	0.63	1.40x10 ⁻³	5.32x10 ⁻²
MainFractions_Apo.A2_HDL	26.37 [24.41 28.245]	31.88 [29.105 34.71]	-0.31	1.87x10 ⁻³	5.34x10 ⁻²
HDL_Subfractions_Apo.A1_HDL.4	54.24 [48.105 57.045]	64.89 [60.085 76.18]	-0.35	2.67x10 ⁻³	6.10x10 ⁻²
LDL_Subfractions_Triglycerides_LDL.1	8.15 [7.56 9.31]	5.15 [3.7 7.2]	0.57	4.45x10 ⁻³	8.45x10 ⁻²
HDL_Subfractions_Phospholipid_HDL.4	18.97 [18.37 19.995]	22.74 [20.58 27.65]	-0.33	5.22x10 ⁻³	8.50x10 ⁻²
MainFractions_Triglycerides_LDL	25.6 [23.305 28.935]	20.07 [16.775 23.18]	0.38	8.27x10 ⁻³	1.18x10 ⁻¹
MainFractions_Phospholipids_IDL	2.88 [2.1 5.535]	7.1 [4.77 11.555]	-1.13	9.37x10 ⁻³	1.19x10 ⁻¹
HDL_Subfractions_Cholesterol_HDL.4	13.87 [11.825 14.56]	18.07 [14.09 22.105]	-0.44	1.06x10 ⁻²	1.21x10 ⁻¹
VLDL_Subfractions_Cholesterol_VLDL.1	2.71 [1.69 4.625]	7.76 [4.5 17.16]	-1.52	1.43x10 ⁻²	1.48x10 ⁻¹
Main_Parameters_Cholesterol	151.07 [139.25 184.675]	209.08 [183.615 223.545]	-0.32	1.89x10 ⁻²	1.78x10 ⁻¹
VLDL_Subfractions_FreeCholesterol_VLDL.1	0.5 [0.355 1.135]	2.84 [1.345 6.68]	-2.05	2.02x10 ⁻²	1.78x10 ⁻¹
LDL.5_Particle_Number	122.22 [44.215 173.705]	235.58 [150.9 303.1]	-0.82	2.75x10 ⁻²	2.08x10 ⁻¹
LDL_Subfractions_Apo.B_LDL.5	6.72 [2.43 9.555]	12.96 [8.3 16.67]	-0.82	2.75x10 ⁻²	2.08x10 ⁻¹
VLDL_Subfractions_Phospholipids_VLDL.1	2.89 [2.05 3.805]	7.51 [3.32 14.185]	-1.4	3.09x10 ⁻²	2.08x10 ⁻¹
VLDL_Subfractions_Cholesterol_VLDL.2	2.38 [1.82 2.735]	3.91 [2.935 5.69]	-0.73	3.32x10 ⁻²	2.08x10 ⁻¹
LDL_Subfractions_Cholesterol_LDL.5	7.27 [1.465 11.7]	18.74 [10.945 22.83]	-1.01	3.32x10 ⁻²	2.08x10 ⁻¹
LDL_Subfractions_Phospholipids_LDL.5	4.84 [1.515 6.935]	9.85 [6.405 11.875]	-0.87	3.47x10 ⁻²	2.08x10 ⁻¹
VLDL_Subfractions_Triglycerides_VLDL.1	11.87 [9.42 26.39]	39.89 [19.25 92.63]	-1.53	4.35x10 ⁻²	2.36x10 ⁻¹
HDL_Subfractions_Triglycerides_HDL.1	4.34 [3.785 5.4]	3.33 [2.37 3.97]	0.4	4.35x10 ⁻²	2.36x10 ⁻¹
MainFractions_Apo.A1_HDL	126.51 [118.6 131.56]	138.97 [133.715 147.975]	-0.14	4.85x10 ⁻²	2.51x10 ⁻¹
Main_Parameters_Apo.A1	128.49 [120.11 132.985]	137.99 [131.55 147.33]	-0.13	5.98x10 ⁻²	2.97x10 ⁻¹
LDL_Subfractions_FreeCholesterol_LDL.5	2.63 [1.05 3.635]	4.45 [2.98 5.795]	-0.66	7.36x10 ⁻²	3.50x10 ⁻¹
HDL_Subfractions_Triglycerides_HDL.2	2.14 [1.96 2.345]	1.7 [1.365 2.22]	0.32	8.07x10 ⁻²	3.68x10 ⁻¹
LDL_Subfractions_Triglycerides_LDL.3	2.9 [2.51 3.37]	2.4 [1.705 2.765]	0.36	8.45x10 ⁻²	3.68x10 ⁻¹
LDL_Subfractions_Cholesterol_LDL.4	1.51 [0 4.95]	8.94 [2.735 13.525]	-1.03	8.71x10 ⁻²	3.68x10 ⁻¹
MainFractions_Triglycerides_IDL	4.43 [2.895 7.345]	9.89 [5.215 25.96]	-1.39	9.25x10 ⁻²	3.70x10 ⁻¹
Main_Parameters_LDL.Chol	70.08 [51.52 95.205]	104.2 [82.98 116.18]	-0.38	9.74x10 ⁻²	3.70x10 ⁻¹
MainFractions_Cholesterol_LDL	70.08 [51.52 95.205]	104.2 [82.98 116.18]	-0.38	9.74x10 ⁻²	3.70x10 ⁻¹
LDL_Subfractions_Triglycerides_LDL.4	2.58 [2.16 2.75]	1.55 [1.11 2.14]	0.56	1.06x10 ⁻¹	3.88x10 ⁻¹
MainFractions_Phospholipids_VLDL	14.07 [11.885 23.41]	23.72 [16.435 39.41]	-0.56	1.17x10 ⁻¹	3.95x10 ⁻¹
LDL.4_Particle_Number	34.66 [0 72.185]	119.2 [41.125 153.81]	-0.85	1.24x10 ⁻¹	3.95x10 ⁻¹
LDL_Subfractions_Phospholipids_LDL.4	2.14 [0 3.865]	5.41 [1.77 7.75]	-0.82	1.24x10 ⁻¹	3.95x10 ⁻¹
LDL_Subfractions_Apo.B_LDL.4	1.91 [0 3.97]	6.56 [2.265 8.46]	-0.85	1.24x10 ⁻¹	3.95x10 ⁻¹
MainFractions_FreeCholesterol_VLDL	6.27 [5.155 10.92]	11.05 [7.74 17.9]	-0.58	1.25x10 ⁻¹	3.95x10 ⁻¹
LDL.Chol_HDL.Chol	1.48 [0.985 1.8]	1.82 [1.61 2.12]	-0.23	1.47x10 ⁻¹	3.95x10 ⁻¹
MainFractions_Cholesterol_VLDL	13.65 [11.82 24.19]	26.46 [17.545 40.495]	-0.57	1.51x10 ⁻¹	3.95x10 ⁻¹
LDL_Subfractions_Phospholipids_LDL.6	12.38 [9.875 17.385]	16.01 [13.97 19.735]	-0.31	1.51x10 ⁻¹	3.95x10 ⁻¹
HDL_Subfractions_Phospholipid_HDL.1	25.88 [23.355 28.415]	20.69 [14.64 27.49]	0.29	1.51x10 ⁻¹	3.95x10 ⁻¹
MainFractions_FreeCholesterol_LDL	22.25 [19.255 27.525]	28.41 [24.245 32.88]	-0.21	1.60x10 ⁻¹	3.95x10 ⁻¹
Main_Parameters_Apo.B100	69.62 [62.005 86.355]	91.46 [76.055 99.625]	-0.21	1.63x10 ⁻¹	3.95x10 ⁻¹
Total_Particle_Number	1265.89 [1127.435 1570.115]	1663.05 [1382.915 1811.48]	-0.21	1.63x10 ⁻¹	3.95x10 ⁻¹
MainFractions_Triglycerides_VLDL	60.34 [40.14 72.935]	74.42 [49.29 161.715]	-0.77	1.63x10 ⁻¹	3.95x10 ⁻¹
LDL_Subfractions_Cholesterol_LDL.6	21.31 [16.14 31.8]	29.34 [24.955 37.035]	-0.38	1.63x10 ⁻¹	3.95x10 ⁻¹
LDL_Subfractions_Apo.B_LDL.1	13.34 [12.155 13.715]	11.37 [8.335 13.33]	0.18	1.73x10 ⁻¹	3.95x10 ⁻¹
LDL_Particle_Number	1006.8 [780.95 1357.535]	1329.88 [1132.015 1424.785]	-0.23	1.77x10 ⁻¹	3.95x10 ⁻¹
LDL.1_Particle_Number	242.55 [220.995 249.37]	206.72 [151.57 242.355]	0.18	1.77x10 ⁻¹	3.95x10 ⁻¹
MainFractions_Phospholipids_LDL	46.19 [37.155 58.085]	59.18 [47.095 66.04]	-0.2	1.77x10 ⁻¹	3.95x10 ⁻¹
MainFractions_Apo.B_LDL	55.37 [42.95 74.66]	73.14 [62.255 78.36]	-0.23	1.77x10 ⁻¹	3.95x10 ⁻¹
HDL_Subfractions_Cholesterol_HDL.1	22.1 [19.685 24.495]	18.78 [13.855 23.415]	0.24	1.77x10 ⁻¹	3.95x10 ⁻¹
VLDL_Subfractions_FreeCholesterol_VLDL.2	1.17 [1.04 1.43]	1.91 [1.16 2.945]	-0.58	1.87x10 ⁻¹	4.09x10 ⁻¹
MainFractions_FreeCholesterol_IDL	2.34 [1.975 4.385]	4.03 [2.78 5.695]	-0.46	2.01x10 ⁻¹	4.28x10 ⁻¹
LDL.6_Particle_Number	325.77 [240.52 484.46]	450.55 [346.56 564.44]	-0.39	2.06x10 ⁻¹	4.28x10 ⁻¹
LDL_Subfractions_Apo.B_LDL.6	17.92 [13.23 26.645]	24.78 [19.06 31.045]	-0.39	2.06x10 ⁻¹	4.28x10 ⁻¹
VLDL_Subfractions_Phospholipids_VLDL.2	2.64 [2.47 3.195]	3.8 [2.465 5.65]	-0.47	2.17x10 ⁻¹	4.41x10 ⁻¹
Main_Parameters_Triglycerides	96.35 [80.635 122.505]	114.96 [91.02 219.825]	-0.57	2.39x10 ⁻¹	4.78x10 ⁻¹
MainFractions_Triglycerides_HDL	10.91 [9.755 11.665]	8.54 [7.13 12.12]	0.14	2.57x10 ⁻¹	4.92x10 ⁻¹
LDL_Subfractions_FreeCholesterol_LDL.4	1.95 [0.545 2.795]	2.95 [1.57 4.345]	-0.5	2.59x10 ⁻¹	4.92x10 ⁻¹
HDL_Subfractions_Apo.A2_HDL.3	5.51 [5.165 5.945]	6.01 [5.535 6.885]	-0.19	2.59x10 ⁻¹	4.92x10 ⁻¹
MainFractions_Cholesterol_IDL	9.21 [7.735 15.675]	14.04 [9.71 18.84]	-0.35	2.75x10 ⁻¹	5.14x10 ⁻¹
LDL_Subfractions_FreeCholesterol_LDL.1	6.71 [5.86 7.34]	5.47 [4.495 7.21]	0.11	2.87x10 ⁻¹	5.28x10 ⁻¹
VLDL_Subfractions_FreeCholesterol_VLDL.5	0.39 [0.35 1.16]	0.93 [0.575 1.565]	-0.59	2.94x10 ⁻¹	5.32x10 ⁻¹
LDL_Subfractions_Phospholipids_LDL.1	13.28 [11.57 13.34]	11.24 [8.925 13.96]	0.1	3.07x10 ⁻¹	5.46x10 ⁻¹
Main_Parameters_HDL.Chol	52.7 [48.33 54.175]	55.73 [51.2 62.19]	-0.1	3.35x10 ⁻¹	5.79x10 ⁻¹

MainFractions_Cholesterol_HDL	52.7 [48.33 54.175]	55.73 [51.2 62.19]	-0.1	3.35x10 ⁻¹	5.79x10 ⁻¹
LDL_Subfractions_FreeCholesterol_LDL.6	5.21 [3.14 7.33]	6.48 [5.045 7.83]	-0.27	3.49x10 ⁻¹	5.90x10 ⁻¹
LDL_Subfractions_FreeCholesterol_LDL.2	5.75 [5.19 6.645]	5.35 [4.185 6.4]	0.18	3.57x10 ⁻¹	5.90x10 ⁻¹
HDL_Subfractions_Triglycerides_HDL.3	2.21 [2.09 2.45]	1.71 [1.585 2.645]	0.17	3.57x10 ⁻¹	5.90x10 ⁻¹
HDL_Subfractions_FreeCholesterol_HDL.3	1.4 [1.325 1.605]	1.67 [1.375 1.95]	-0.1	3.71x10 ⁻¹	6.04x10 ⁻¹
VLDL_Subfractions_Triglycerides_VLDL.2	9.48 [9.145 12.295]	12.3 [7.77 20.28]	-0.35	4.03x10 ⁻¹	6.26x10 ⁻¹
VLDL_Subfractions_Triglycerides_VLDL.4	11.24 [8.685 16.995]	10.15 [7.525 13.39]	0.26	4.03x10 ⁻¹	6.26x10 ⁻¹
HDL_Subfractions_Triglycerides_HDL.4	2.8 [2.31 3.11]	2.86 [2.515 3.98]	-0.23	4.03x10 ⁻¹	6.26x10 ⁻¹
VLDL_Subfractions_FreeCholesterol_VLDL.3	1.11 [1.075 2.275]	2.12 [1.16 3.395]	-0.42	4.06x10 ⁻¹	6.26x10 ⁻¹
Apo.B100_Apo.A1	0.57 [0.475 0.68]	0.66 [0.56 0.745]	-0.06	4.18x10 ⁻¹	6.35x10 ⁻¹
VLDL_Subfractions_Cholesterol_VLDL.3	2.67 [2.43 5.15]	4.67 [2.66 6.615]	-0.32	4.56x10 ⁻¹	6.84x10 ⁻¹
VLDL_Subfractions_Triglycerides_VLDL.5	3.31 [2.555 3.66]	3.01 [2.105 3.655]	0.19	4.69x10 ⁻¹	6.94x10 ⁻¹
HDL_Subfractions_FreeCholesterol_HDL.2	1.75 [1.495 1.82]	1.57 [1.03 1.81]	0.1	5.23x10 ⁻¹	7.55x10 ⁻¹
HDL_Subfractions_Apo.A2_HDL.1	2.93 [2.72 3.645]	2.86 [2.115 3.64]	0.12	5.23x10 ⁻¹	7.55x10 ⁻¹
HDL_Subfractions_Cholesterol_HDL.3	8.81 [8.215 9.26]	9 [8.4 10.165]	-0.05	5.31x10 ⁻¹	7.55x10 ⁻¹
LDL_Subfractions_Triglycerides_LDL.6	5.11 [4.85 7.015]	5.12 [4.355 6.655]	0.11	5.37x10 ⁻¹	7.55x10 ⁻¹
HDL_Subfractions_FreeCholesterol_HDL.4	2.4 [2.05 2.655]	2.42 [1.69 3.47]	-0.22	5.59x10 ⁻¹	7.76x10 ⁻¹
VLDL_Subfractions_Phospholipids_VLDL.3	3.31 [2.965 5.41]	4.97 [3.11 6.755]	-0.22	5.65x10 ⁻¹	7.76x10 ⁻¹
LDL.2_Particle_Number	166.03 [151.33 208.055]	164.37 [139.665 205.405]	0.11	5.88x10 ⁻¹	7.84x10 ⁻¹
LDL_Subfractions_Apo.B_LDL.2	9.13 [8.32 11.44]	9.04 [7.68 11.295]	0.11	5.88x10 ⁻¹	7.84x10 ⁻¹
VLDL_Subfractions_FreeCholesterol_VLDL.4	1.52 [1.075 3.665]	2.14 [1.405 3.315]	-0.14	6.09x10 ⁻¹	7.84x10 ⁻¹
LDL_Subfractions_Triglycerides_LDL.5	2.56 [2.1 3.05]	2.94 [2.205 3.695]	-0.07	6.09x10 ⁻¹	7.84x10 ⁻¹
HDL_Subfractions_Apo.A1_HDL.1	27.97 [26.28 30.92]	26.72 [18.345 36.18]	0.15	6.17x10 ⁻¹	7.84x10 ⁻¹
VLDL_Subfractions_Cholesterol_VLDL.4	4.74 [3.605 8.725]	5.18 [4.165 7.74]	0	6.39x10 ⁻¹	7.84x10 ⁻¹
VLDL_Subfractions_Cholesterol_VLDL.5	1.21 [0.59 1.64]	1.51 [0.84 1.88]	-0.06	6.39x10 ⁻¹	7.84x10 ⁻¹
VLDL_Particle_Number	146.61 [124.635 216.05]	174.76 [125.635 260.425]	-0.19	6.47x10 ⁻¹	7.84x10 ⁻¹
IDL_Particle_Number	85.1 [82.795 107.41]	93.81 [66.715 125.515]	-0.12	6.47x10 ⁻¹	7.84x10 ⁻¹
MainFractions_Apo.B_VLDL	8.06 [6.855 11.885]	9.61 [6.91 14.32]	-0.19	6.47x10 ⁻¹	7.84x10 ⁻¹
MainFractions_Apo.B_IDL	4.68 [4.555 5.905]	5.16 [3.67 6.9]	-0.12	6.47x10 ⁻¹	7.84x10 ⁻¹
LDL_Subfractions_Cholesterol_LDL.3	9.6 [6.69 12.495]	13.12 [7.465 15.125]	-0.13	6.70x10 ⁻¹	8.04x10 ⁻¹
MainFractions_FreeCholesterol_HDL	10.57 [10.04 12.075]	9.66 [9.24 12.95]	0.06	7.01x10 ⁻¹	8.24x10 ⁻¹
HDL_Subfractions_FreeCholesterol_HDL.1	3.98 [3.115 4.53]	3.75 [2.475 4.825]	0.02	7.01x10 ⁻¹	8.24x10 ⁻¹
LDL_Subfractions_Phospholipids_LDL.2	8.86 [8.125 10.94]	8.8 [7.225 11.315]	0.09	7.39x10 ⁻¹	8.60x10 ⁻¹
LDL_Subfractions_Apo.B_LDL.3	6.96 [5.93 8.795]	7.25 [5.035 9.525]	0.11	7.66x10 ⁻¹	8.79x10 ⁻¹
LDL.3_Particle_Number	126.58 [107.8 159.865]	131.79 [91.545 173.235]	0.11	7.71x10 ⁻¹	8.79x10 ⁻¹
LDL_Subfractions_FreeCholesterol_LDL.3	3.79 [3.595 4.735]	4.21 [2.53 5.035]	0.15	7.98x10 ⁻¹	8.97x10 ⁻¹
LDL_Subfractions_Cholesterol_LDL.2	14.59 [13.305 19.375]	16.89 [13.345 20.83]	-0.01	8.03x10 ⁻¹	8.97x10 ⁻¹
HDL_Subfractions_Apo.A1_HDL.3	24.55 [22.595 25.55]	23.88 [22.23 27.09]	-0.05	8.15x10 ⁻¹	9.02x10 ⁻¹
HDL_Subfractions_Phospholipid_HDL.2	13.27 [12.895 14.535]	14.26 [10.64 15.715]	0.07	8.35x10 ⁻¹	9.15x10 ⁻¹
VLDL_Subfractions_Phospholipids_VLDL.5	1.36 [1 2.415]	1.97 [1.055 2.29]	-0.06	8.48x10 ⁻¹	9.21x10 ⁻¹
HDL_Subfractions_Apo.A1_HDL.2	19.84 [15.235 20.365]	17.65 [15.25 20.59]	0.02	8.98x10 ⁻¹	9.66x10 ⁻¹
VLDL_Subfractions_Phospholipids_VLDL.4	4.86 [3.69 7.7]	4.87 [3.93 6.625]	0.1	9.15x10 ⁻¹	9.75x10 ⁻¹
LDL_Subfractions_Phospholipids_LDL.3	6.36 [5.4 7.79]	7.63 [4.78 8.565]	0.05	9.32x10 ⁻¹	9.76x10 ⁻¹
HDL_Subfractions_Cholesterol_HDL.2	8.36 [7.58 8.71]	8.56 [6.67 10.025]	-0.01	9.34x10 ⁻¹	9.76x10 ⁻¹
VLDL_Subfractions_Triglycerides_VLDL.3	9.86 [9.56 16.12]	11.94 [9.045 19.305]	-0.03	9.66x10 ⁻¹	9.84x10 ⁻¹
MainFractions_Phospholipids_HDL	73.02 [67.19 75.64]	72.67 [65.82 80.31]	-0.01	9.67x10 ⁻¹	9.84x10 ⁻¹
LDL_Subfractions_Cholesterol_LDL.1	20.18 [17.485 21.32]	18.55 [14.815 24.82]	-0.07	9.67x10 ⁻¹	9.84x10 ⁻¹
HDL_Subfractions_Phospholipid_HDL.3	14.39 [13.985 15.93]	14.33 [13.63 16.59]	0	9.83x10 ⁻¹	9.92x10 ⁻¹
HDL_Subfractions_Apo.A2_HDL.2	3.83 [3.415 4.015]	3.61 [3.125 4.435]	-0.06	1.00	1.00

439 **Table S3.** Statistical comparison between the metabolic profiles of Metabotype II *versus* Metabotype III.

Feature	Metabotype II, median [IQR]	Metabotype III, median [IQR]	log change	p-value	FDR
Pyroglutamate	1.28 [1.226 1.343]	0.974 [0.943 1.011]	0.45	1.02x10 ⁻⁶	1.05x10 ⁻⁵
2-Hydroxyvalerate	1.263 [1.21 1.325]	0.961 [0.93 0.997]	0.45	1.02x10 ⁻⁶	1.05x10 ⁻⁵
Unsaturated lipid (-CH=CH-)	1.388 [1.331 1.469]	0.917 [0.838 0.998]	0.64	1.02x10 ⁻⁶	1.05x10 ⁻⁵
Lipid (-(-CH2-)n-)	1.569 [1.51 1.742]	0.892 [0.776 1.003]	0.91	1.02x10 ⁻⁶	1.05x10 ⁻⁵
Lipid (-CH3-)	1.226 [1.183 1.272]	0.948 [0.938 1.034]	0.33	2.04x10 ⁻⁶	1.67x10 ⁻⁵
Lipid (alpha-CH2)	2.144 [1.957 2.631]	0.671 [0.494 0.863]	1.76	7.14x10 ⁻⁶	4.18x10 ⁻⁵
Glycerol phospholipid	2.084 [1.919 2.643]	0.552 [0.401 0.809]	1.73	7.14x10 ⁻⁶	4.18x10 ⁻⁵
Threonine	1.596 [1.341 1.786]	0.783 [0.741 0.96]	0.96	4.59x10 ⁻⁵	2.35x10 ⁻⁴
GlycB	0.72 [0.661 0.783]	0.978 [0.903 1.167]	-0.5	1.42x10 ⁻⁴	6.46x10 ⁻⁴
Lipid (beta-CH2)	1.288 [1.193 1.36]	1.032 [0.9 1.121]	0.36	1.98x10 ⁻⁴	8.11x10 ⁻⁴
3-Hydroxybutyrate	1.28 [1.183 1.351]	0.979 [0.949 1.017]	0.4	3.73x10 ⁻⁴	1.39x10 ⁻³
Lipid (=CH-CH2-CH=)	1.111 [1.08 1.156]	0.957 [0.818 1.046]	0.28	1.49x10 ⁻³	5.10x10 ⁻³
Cholesterol backbone	1.324 [1.228 1.43]	1.021 [0.851 1.149]	0.37	1.91x10 ⁻³	6.03x10 ⁻³
Methanol	0.978 [0.826 1.148]	1.41 [1.091 1.572]	-0.46	2.20x10 ⁻²	6.45x10 ⁻²
Pyruvate	1.362 [0.789 1.844]	0.918 [0.581 1.09]	0.75	4.17x10 ⁻²	1.14x10 ⁻¹
Phenylalanine	1.003 [0.942 1.072]	0.834 [0.669 1.01]	0.28	4.84x10 ⁻²	1.24x10 ⁻¹
Isoleucine	1.146 [1.062 1.254]	0.911 [0.777 1.182]	0.28	8.41x10 ⁻²	2.03x10 ⁻¹
GlycA	1.019 [0.96 1.073]	0.913 [0.827 0.979]	0.13	9.56x10 ⁻²	2.18x10 ⁻¹
Creatine	1.401 [0.958 1.613]	0.931 [0.45 1.289]	0.33	1.08x10 ⁻¹	2.34x10 ⁻¹
Mannose	1.11 [0.949 1.27]	0.977 [0.714 1.076]	0.25	1.22x10 ⁻¹	2.51x10 ⁻¹
Valine	1.01 [0.936 1.284]	0.956 [0.806 1.069]	0.19	1.38x10 ⁻¹	2.68x10 ⁻¹
3-Hydroxyisovalerate	0.568 [0.479 0.635]	1.799 [0.498 4.786]	-1.53	1.72x10 ⁻¹	3.21x10 ⁻¹
Glucose	1.011 [0.925 1.471]	0.911 [0.889 1.124]	0.28	1.92x10 ⁻¹	3.41x10 ⁻¹
Glutamine	1.157 [1.108 1.204]	1.07 [0.999 1.213]	0.07	2.35x10 ⁻¹	4.01x10 ⁻¹
Acetate	0.956 [0.895 1.125]	1.149 [0.959 1.291]	-0.15	3.41x10 ⁻¹	5.59x10 ⁻¹
Formate	0.979 [0.758 1.077]	1.051 [0.716 1.253]	-0.18	3.71x10 ⁻¹	5.85x10 ⁻¹
Glutamate	0.802 [0.46 1.302]	1.175 [0.814 1.223]	-0.23	4.03x10 ⁻¹	6.12x10 ⁻¹
Glycine	1.083 [0.933 1.158]	1.151 [0.99 1.195]	-0.1	4.37x10 ⁻¹	6.39x10 ⁻¹
Histidine	1.101 [1.029 1.16]	1.053 [0.967 1.151]	0.04	4.72x10 ⁻¹	6.67x10 ⁻¹
Creatinine	1.023 [0.906 1.071]	0.922 [0.754 1.126]	0.09	5.08x10 ⁻¹	6.72x10 ⁻¹
Leucine	1.069 [0.941 1.199]	0.94 [0.842 1.145]	0.1	5.08x10 ⁻¹	6.72x10 ⁻¹
Tyrosine	1.044 [0.928 1.109]	0.918 [0.784 1.1]	0.11	5.46x10 ⁻¹	6.99x10 ⁻¹
Alanine	1.019 [0.856 1.274]	0.95 [0.879 1.126]	0.16	5.85x10 ⁻¹	7.27x10 ⁻¹
2-Hydroxybutyrate	1.024 [0.746 1.18]	0.865 [0.806 1.063]	0.04	7.96x10 ⁻¹	9.31x10 ⁻¹
Protein	1.013 [0.962 1.072]	1.028 [0.993 1.064]	0	7.96x10 ⁻¹	9.31x10 ⁻¹
Desaminotyrosine	1 [1 1]	1 [1 1]	-1.32	8.37x10 ⁻¹	9.31x10 ⁻¹
Citrate.left	1.003 [0.778 1.279]	1.035 [0.819 1.249]	-0.01	8.41x10 ⁻¹	9.31x10 ⁻¹
Lactate	0.79 [0.689 1.483]	0.82 [0.709 1.035]	0.21	8.86x10 ⁻¹	9.31x10 ⁻¹
Acetoacetate	0.943 [0.724 1.231]	0.904 [0.659 1.244]	-0.18	8.86x10 ⁻¹	9.31x10 ⁻¹
Phospholipid	1.006 [0.925 1.143]	1.035 [0.934 1.099]	-0.02	9.31x10 ⁻¹	9.55x10 ⁻¹
Isobutyrate	0.972 [0.895 1.035]	0.918 [0.817 1.3]	-0.15	9.77x10 ⁻¹	9.77x10 ⁻¹

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Table S4. Statistical comparison between the lipoprotein profiles of Metabotype II *versus* Metabotype III.

Feature	Metabotype II, median [IQR]	Metabotype III, median [IQR]	log change	p-value	FDR
Main_Parameters_Triglycerides	240.36 [214.002 299.433]	91.02 [71.323 111.78]	1.52	2.04x10 ⁻⁶	8.14x10 ⁻⁵
MainFractions_Triglycerides_VLDL	170.69 [159.183 210.132]	49.29 [38.688 69.142]	1.79	4.08x10 ⁻⁶	8.14x10 ⁻⁵
MainFractions_Triglycerides_IDL	26.57 [25.95 36.155]	5.215 [2.48 8.28]	2.47	4.08x10 ⁻⁶	8.14x10 ⁻⁵
VLDL_Subfractions_Triglycerides_VLDL.1	107.94 [80.3 134.335]	19.25 [11.785 27.652]	2.45	4.08x10 ⁻⁶	8.14x10 ⁻⁵
VLDL_Subfractions_Phospholipids_VLDL.1	15.77 [13.717 19.802]	3.32 [2.45 4.475]	-2.2	4.08x10 ⁻⁶	8.14x10 ⁻⁵
VLDL_Particle_Number	278.955 [243.442 330.25]	125.635 [95.94 150.235]	1.26	7.14x10 ⁻⁶	8.14x10 ⁻⁵
MainFractions_Cholesterol_VLDL	43.075 [37.285 54.905]	17.545 [11.707 21.458]	1.42	7.14x10 ⁻⁶	8.14x10 ⁻⁵
MainFractions_Cholesterol_IDL	18.84 [15.553 28.68]	9.94 [6.973 12.57]	1.22	7.14x10 ⁻⁶	8.14x10 ⁻⁵
MainFractions_Apo.B_VLDL	15.34 [13.39 18.163]	6.91 [5.275 8.262]	1.26	7.14x10 ⁻⁶	8.14x10 ⁻⁵
VLDL_Subfractions_Cholesterol_VLDL.1	20.4 [16.017 25.052]	4.5 [2.473 6.945]	2.2	7.14x10 ⁻⁶	8.14x10 ⁻⁵
Main_Parameters_Apo.B100	105.6 [96.73 118.345]	80.055 [67.373 86.227]	0.45	3.06x10 ⁻⁵	2.49x10 ⁻⁴
Total_Particle_Number	1920.06 [1758.775 2151.845]	1455.6 [1224.988 1567.833]	0.45	3.06x10 ⁻⁵	2.49x10 ⁻⁴
MainFractions_Phospholipids_VLDL	43.66 [36.663 48.425]	16.435 [12.775 21.26]	1.35	3.06x10 ⁻⁵	2.49x10 ⁻⁴
VLDL_Subfractions_Triglycerides_VLDL.2	23.955 [18.545 28.067]	7.77 [6.612 12.015]	1.31	3.06x10 ⁻⁵	2.49x10 ⁻⁴
VLDL_Subfractions_FreeCholesterol_VLDL.3	3.745 [3.165 4.645]	1.16 [0.662 1.658]	1.65	4.59x10 ⁻⁵	3.27x10 ⁻⁴
VLDL_Subfractions_Phospholipids_VLDL.2	6.555 [5.123 7.707]	2.465 [1.86 3.462]	1.22	4.59x10 ⁻⁵	3.27x10 ⁻⁴
MainFractions_Phospholipids_IDL	13.195 [11.277 13.99]	4.77 [3.405 5.865]	1.44	6.03x10 ⁻⁵	4.04x10 ⁻⁴
VLDL_Subfractions_FreeCholesterol_VLDL.1	6.905 [6.43 8.595]	1.345 [0.48 1.855]	2.52	7.68x10 ⁻⁵	4.87x10 ⁻⁴
MainFractions_FreeCholesterol_VLDL	19.25 [17.398 23.538]	7.74 [5.652 9.453]	1.43	9.83x10 ⁻⁵	5.90x10 ⁻⁴
MainFractions_FreeCholesterol_IDL	5.695 [4.505 8.465]	2.78 [1.777 3.518]	1.32	1.40x10 ⁻⁴	6.73x10 ⁻⁴
VLDL_Subfractions_FreeCholesterol_VLDL.2	3.625 [2.663 3.978]	1.16 [0.845 1.642]	1.55	1.41x10 ⁻⁴	6.73x10 ⁻⁴
Main_Parameters_Cholesterol	242.43 [216.257 251.153]	186.395 [170.765 211.16]	0.33	1.42x10 ⁻⁴	6.73x10 ⁻⁴
VLDL_Subfractions_Cholesterol_VLDL.3	7.59 [6.305 9.242]	2.66 [1.96 4.388]	1.34	1.42x10 ⁻⁴	6.73x10 ⁻⁴
VLDL_Subfractions_FreeCholesterol_VLDL.4	3.19 [2.522 5.088]	1.405 [1.087 1.878]	1.38	1.42x10 ⁻⁴	6.73x10 ⁻⁴
VLDL_Subfractions_Triglycerides_VLDL.4	14.33 [12.16 18.005]	7.765 [5.54 9.773]	0.96	1.98x10 ⁻⁴	9.02x10 ⁻⁴
VLDL_Subfractions_Phospholipids_VLDL.4	6.795 [5.648 8.938]	3.99 [2.865 4.717]	0.88	2.74x10 ⁻⁴	1.20x10 ⁻³
IDL_Particle_Number	123.81 [97.71 174.377]	72.05 [59.055 92.15]	0.93	3.73x10 ⁻⁴	1.32x10 ⁻³
LDL.6_Particle_Number	627.52 [479.947 741.995]	346.56 [249.407 458.607]	0.82	3.73x10 ⁻⁴	1.32x10 ⁻³
MainFractions_Apo.B_IDL	6.805 [5.375 9.592]	3.965 [3.25 5.067]	0.93	3.73x10 ⁻⁴	1.32x10 ⁻³
LDL_Subfractions_Phospholipids_LDL.6	20.9 [18.147 23.673]	14.04 [11.262 17.135]	0.59	3.73x10 ⁻⁴	1.32x10 ⁻³
LDL_Subfractions_Apo.B_LDL.6	34.515 [26.395 40.81]	19.06 [13.715 25.225]	0.82	3.73x10 ⁻⁴	1.32x10 ⁻³
HDL_Subfractions_Triglycerides_HDL.4	4.755 [3.945 4.992]	2.58 [2.433 2.852]	0.77	3.73x10 ⁻⁴	1.32x10 ⁻³
VLDL_Subfractions_Cholesterol_VLDL.2	7.385 [5.69 7.815]	2.935 [2.002 3.798]	1.24	3.95x10 ⁻⁴	1.32x10 ⁻³
VLDL_Subfractions_Phospholipids_VLDL.3	7.77 [6.45 8.297]	3.11 [2.045 3.857]	1.21	3.95x10 ⁻⁴	1.32x10 ⁻³
VLDL_Subfractions_Triglycerides_VLDL.3	21.9 [18.928 23.95]	9.045 [5.5 11.13]	1.15	4.41x10 ⁻⁴	1.44x10 ⁻³
LDL_Subfractions_Cholesterol_LDL.6	39.615 [33.542 44.188]	24.98 [18.985 31.68]	0.68	5.04x10 ⁻⁴	1.60x10 ⁻³
VLDL_Subfractions_Phospholipids_VLDL.5	2.29 [2.138 2.743]	1.16 [0.915 1.9]	0.87	6.12x10 ⁻⁴	1.89x10 ⁻³
VLDL_Subfractions_FreeCholesterol_VLDL.5	1.66 [1.202 2.345]	0.575 [0.302 0.908]	1.49	6.70x10 ⁻⁴	2.01x10 ⁻³
VLDL_Subfractions_Cholesterol_VLDL.4	7.425 [5.768 11.15]	4.54 [3.668 5.143]	0.9	8.84x10 ⁻⁴	2.58x10 ⁻³
VLDL_Subfractions_Triglycerides_VLDL.5	3.69 [3.298 3.907]	2.235 [1.852 2.918]	0.6	1.15x10 ⁻³	3.28x10 ⁻³
HDL_Subfractions_Triglycerides_HDL.3	2.645 [2.518 2.85]	1.61 [1.395 1.738]	0.71	3.84x10 ⁻³	1.07x10 ⁻²
Apo.B100_Apo.A1	0.755 [0.705 0.815]	0.59 [0.442 0.62]	0.4	4.46x10 ⁻³	1.21x10 ⁻²
MainFractions_Triglycerides_HDL	12.12 [10.488 13.635]	7.795 [6.595 9.035]	0.63	5.91x10 ⁻³	1.57x10 ⁻²
HDL_Subfractions_Apo.A2_HDL.3	6.88 [6.22 7.827]	5.75 [5.462 6.303]	0.3	9.97x10 ⁻³	2.58x10 ⁻²
LDL_Subfractions_Triglycerides_LDL.6	6 [5.397 7.277]	4.94 [4.215 5.12]	0.34	1.08x10 ⁻²	2.74x10 ⁻²
LDL_Subfractions_FreeCholesterol_LDL.6	8.035 [6.635 8.848]	5.59 [4.115 7.095]	0.54	1.18x10 ⁻²	2.92x10 ⁻²
VLDL_Subfractions_Cholesterol_VLDL.5	1.88 [1.268 1.94]	0.935 [0.682 1.532]	0.73	1.28x10 ⁻²	3.10x10 ⁻²
MainFractions_Apo.A2_HDL	34.53 [34.012 39.838]	31.145 [28.988 33.998]	0.21	1.51x10 ⁻²	3.58x10 ⁻²
HDL_Subfractions_FreeCholesterol_HDL.2	1.365 [1.005 1.562]	1.705 [1.578 2.295]	-0.48	1.77x10 ⁻²	4.12x10 ⁻²
LDL.Chol_HDL.Chol	2.05 [1.872 2.608]	1.66 [1.365 1.948]	0.35	2.20x10 ⁻²	4.92x10 ⁻²
HDL_Subfractions_Cholesterol_HDL.2	7.945 [6.66 8.887]	10.025 [8.425 11.535]	-0.32	2.20x10 ⁻²	4.92x10 ⁻²
Main_Parameters_Apo.A2	33.51 [31.752 39.38]	30.085 [28.275 32.907]	0.19	3.06x10 ⁻²	6.46x10 ⁻²
LDL_Subfractions_Triglycerides_LDL.4	1.215 [1.105 1.64]	2.055 [1.572 2.675]	-0.75	3.06x10 ⁻²	6.46x10 ⁻²
LDL_Subfractions_FreeCholesterol_LDL.3	3.41 [1.525 3.945]	4.775 [4.383 5.195]	-0.61	3.06x10 ⁻²	6.46x10 ⁻²
Main_Parameters_HDL.Chol	52.805 [51.48 56.24]	60.32 [55.983 66.567]	-0.19	3.58x10 ⁻²	7.16x10 ⁻²
MainFractions_Cholesterol_HDL	52.805 [51.48 56.24]	60.32 [55.983 66.567]	-0.19	3.58x10 ⁻²	7.16x10 ⁻²
HDL_Subfractions_Apo.A1_HDL.3	25.605 [24.717 30.588]	23 [22.125 27.023]	0.17	3.58x10 ⁻²	7.16x10 ⁻²
LDL.5_Particle_Number	300.27 [247.382 321.38]	166.685 [140.99 271.045]	0.53	4.17x10 ⁻²	7.93x10 ⁻²
LDL_Subfractions_Apo.B_LDL.5	16.515 [13.608 17.677]	9.165 [7.755 14.91]	0.53	4.17x10 ⁻²	7.93x10 ⁻²
HDL_Subfractions_Apo.A2_HDL.4	21.8 [19.265 23.615]	16.905 [14.2 20.055]	0.27	4.17x10 ⁻²	7.93x10 ⁻²
MainFractions_FreeCholesterol_HDL	9.35 [9.21 10.613]	11.74 [9.867 13.873]	-0.26	4.33x10 ⁻²	8.10x10 ⁻²
LDL_Particle_Number	1413.735 [1368.12 1703.253]	1290.365 [1040.328 1393.905]	0.27	4.84x10 ⁻²	8.76x10 ⁻²
MainFractions_Apo.B_LDL	77.755 [75.245 93.675]	70.965 [57.218 76.665]	0.27	4.84x10 ⁻²	8.76x10 ⁻²
LDL_Subfractions_Cholesterol_LDL.5	22.305 [19.075 26.078]	11.495 [8.82 21.198]	0.57	6.43x10 ⁻²	1.11x10 ⁻¹
LDL_Subfractions_FreeCholesterol_LDL.2	4.665 [3.745 5.432]	6.255 [4.715 6.837]	-0.34	6.43x10 ⁻²	1.11x10 ⁻¹
HDL_Subfractions_FreeCholesterol_HDL.1	3.275 [2.427 4.258]	4.425 [3.698 5.373]	-0.43	6.43x10 ⁻²	1.11x10 ⁻¹

LDL_Subfractions_Triglycerides_LDL.1	6.215 [4.98 7.638]	4.66 [3.415 6.215]	0.41	8.41x10 ⁻²	1.39x10 ⁻¹
LDL_Subfractions_Phospholipids_LDL.2	8.03 [5.698 9.823]	10.935 [8.47 11.943]	-0.36	8.41x10 ⁻²	1.39x10 ⁻¹
HDL_Subfractions_Apo.A1_HDL.1	21.315 [17.885 26.622]	33.085 [21.492 38.812]	-0.39	8.41x10 ⁻²	1.39x10 ⁻¹
HDL_Subfractions_Apo.A2_HDL.2	4.045 [3.553 4.988]	3.53 [2.595 4.11]	0.37	9.50x10 ⁻²	1.54x10 ⁻¹
LDL_Subfractions_Phospholipids_LDL.5	11.475 [10.037 13.707]	6.905 [5.468 11.435]	0.45	9.56x10 ⁻²	1.54x10 ⁻¹
MainFractions_Phospholipids_HDL	71.915 [67.455 74.508]	78.99 [71.608 86.375]	-0.14	1.22x10 ⁻¹	1.94x10 ⁻¹
MainFractions_Triglycerides_LDL	21.375 [19.322 24.075]	19.655 [15.92 20.8]	0.16	1.38x10 ⁻¹	2.15x10 ⁻¹
LDL_Subfractions_Triglycerides_LDL.2	1.91 [1.802 2.067]	2.6 [1.895 3.11]	-0.41	1.43x10 ⁻¹	2.18x10 ⁻¹
HDL_Subfractions_Triglycerides_HDL.2	2.015 [1.69 2.253]	1.62 [1.118 1.89]	0.37	1.43x10 ⁻¹	2.18x10 ⁻¹
LDL_Subfractions_Cholesterol_LDL.2	15.645 [10.517 18.652]	20.02 [14.985 21.427]	-0.28	1.54x10 ⁻¹	2.25x10 ⁻¹
HDL_Subfractions_Phospholipid_HDL.1	18.775 [15.248 20.535]	24.395 [19.26 30.763]	-0.3	1.54x10 ⁻¹	2.25x10 ⁻¹
HDL_Subfractions_Phospholipid_HDL.2	13.235 [10.63 14.83]	15.465 [12.505 17.177]	-0.17	1.54x10 ⁻¹	2.25x10 ⁻¹
LDL_Subfractions_Triglycerides_LDL.5	3.56 [2.708 3.827]	2.725 [2.22 3.428]	0.25	1.69x10 ⁻¹	2.43x10 ⁻¹
LDL_Subfractions_FreeCholesterol_LDL.5	5.4 [4.493 6.308]	3.31 [2.975 5.9]	0.28	1.88x10 ⁻¹	2.66x10 ⁻¹
LDL_Subfractions_Phospholipids_LDL.3	6.72 [1.825 8.13]	7.88 [5.605 8.828]	-0.45	1.92x10 ⁻¹	2.66x10 ⁻¹
HDL_Subfractions_Cholesterol_HDL.1	16.89 [13.783 19.642]	20.415 [15.768 26.862]	-0.28	1.92x10 ⁻¹	2.66x10 ⁻¹
HDL_Subfractions_Apo.A1_HDL.4	73.3 [63.96 83.575]	66.545 [59.858 75.243]	0.13	2.35x10 ⁻¹	3.23x10 ⁻¹
Main_Parameters_LDL.Chol	109.14 [99.135 134.655]	103.665 [83.118 113.803]	0.19	2.59x10 ⁻¹	3.39x10 ⁻¹
LDL.2_Particle_Number	152.755 [114.168 179.565]	187.735 [143.328 209.08]	-0.25	2.59x10 ⁻¹	3.39x10 ⁻¹
MainFractions_Cholesterol_LDL	109.14 [99.135 134.655]	103.665 [83.118 113.803]	0.19	2.59x10 ⁻¹	3.39x10 ⁻¹
LDL_Subfractions_Apo.B_LDL.2	8.4 [6.28 9.872]	10.325 [7.88 11.495]	-0.25	2.59x10 ⁻¹	3.39x10 ⁻¹
Main_Parameters_Apo.A1	143.235 [136.41 150.05]	138.3 [133.245 148.077]	0.07	3.12x10 ⁻¹	4.04x10 ⁻¹
HDL_Subfractions_Triglycerides_HDL.1	3.54 [2.73 3.958]	2.96 [1.878 3.848]	0.49	3.41x10 ⁻¹	4.36x10 ⁻¹
HDL_Subfractions_FreeCholesterol_HDL.3	1.68 [1.388 1.87]	1.76 [1.452 2.138]	-0.16	3.96x10 ⁻¹	5.00x10 ⁻¹
LDL.3_Particle_Number	131.705 [37.047 171.588]	146.74 [108.203 174.567]	-0.36	4.03x10 ⁻¹	5.00x10 ⁻¹
LDL_Subfractions_Apo.B_LDL.3	7.245 [2.04 9.44]	8.07 [5.95 9.598]	-0.36	4.03x10 ⁻¹	5.00x10 ⁻¹
LDL_Subfractions_Cholesterol_LDL.3	11.685 [2.105 15.377]	13.41 [8.697 15.675]	-0.35	4.29x10 ⁻¹	5.26x10 ⁻¹
HDL_Subfractions_Cholesterol_HDL.3	8.86 [8.398 10.175]	9.345 [8.625 10.555]	-0.05	4.72x10 ⁻¹	5.72x10 ⁻¹
LDL_Subfractions_Triglycerides_LDL.3	2.395 [1.852 2.637]	2.475 [1.735 2.86]	-0.11	5.78x10 ⁻¹	6.94x10 ⁻¹
MainFractions_Phospholipids_LDL	59.635 [54.36 74.132]	59.185 [49.73 65.748]	0.09	5.85x10 ⁻¹	6.95x10 ⁻¹
HDL_Subfractions_Phospholipid_HDL.3	15.095 [14.135 16.82]	14.365 [13.732 17.263]	0.01	6.25x10 ⁻¹	7.35x10 ⁻¹
LDL_Subfractions_FreeCholesterol_LDL.4	3.065 [1.095 4.562]	3.54 [2.478 4.383]	-0.14	6.82x10 ⁻¹	7.93x10 ⁻¹
LDL_Subfractions_Cholesterol_LDL.1	18.885 [15.72 25.035]	20.14 [13.995 26.125]	-0.03	7.52x10 ⁻¹	8.66x10 ⁻¹
HDL_Subfractions_Apo.A1_HDL.2	17.805 [15.957 21.42]	18.075 [15.985 20.025]	0.06	8.38x10 ⁻¹	9.43x10 ⁻¹
HDL_Subfractions_Phospholipid_HDL.4	26.26 [20.55 29.66]	23.26 [21.895 27.84]	0.06	8.41x10 ⁻¹	9.43x10 ⁻¹
LDL.4_Particle_Number	132.31 [49.392 180.19]	126.205 [59.178 156.785]	0.11	8.61x10 ⁻¹	9.43x10 ⁻¹
LDL_Subfractions_Phospholipids_LDL.4	5.705 [1.485 8.697]	6.23 [3.59 7.855]	-0.08	8.61x10 ⁻¹	9.43x10 ⁻¹
LDL_Subfractions_Apo.B_LDL.4	7.275 [2.72 9.91]	6.945 [3.255 8.625]	0.11	8.61x10 ⁻¹	9.43x10 ⁻¹
LDL.1_Particle_Number	202.365 [170.42 230.515]	209.91 [145.275 253.295]	-0.05	8.86x10 ⁻¹	9.53x10 ⁻¹
LDL_Subfractions_Apo.B_LDL.1	11.13 [9.37 12.68]	11.545 [7.992 13.93]	-0.05	8.86x10 ⁻¹	9.53x10 ⁻¹
MainFractions_FreeCholesterol_LDL	28.15 [25.31 36.675]	29.46 [25.735 33.145]	0	9.07x10 ⁻¹	9.57x10 ⁻¹
HDL_Subfractions_Apo.A2_HDL.1	2.71 [2.225 3.487]	2.98 [2.49 3.685]	0.13	9.07x10 ⁻¹	9.57x10 ⁻¹
MainFractions_Apo.A1_HDL	140.92 [136.89 147.097]	142.275 [135.855 148.532]	0.01	9.31x10 ⁻¹	9.65x10 ⁻¹
HDL_Subfractions_FreeCholesterol_HDL.4	3.055 [1.69 3.705]	2.53 [2.2 3.5]	-0.01	9.31x10 ⁻¹	9.65x10 ⁻¹
LDL_Subfractions_Cholesterol_LDL.4	10.105 [2.053 16.44]	10.1 [5.402 13.578]	0.04	9.53x10 ⁻¹	9.70x10 ⁻¹
LDL_Subfractions_Phospholipids_LDL.1	10.87 [9.21 13.52]	11.94 [8.565 14.76]	-0.09	9.53x10 ⁻¹	9.70x10 ⁻¹
LDL_Subfractions_FreeCholesterol_LDL.1	5.19 [4.58 7.225]	6.675 [4.457 7.775]	-0.09	9.77x10 ⁻¹	9.77x10 ⁻¹
HDL_Subfractions_Cholesterol_HDL.4	19.02 [14.853 22.042]	18.695 [16.23 22.68]	0.01	9.77x10 ⁻¹	9.77x10 ⁻¹

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578

Figures

Figure 1

GlycA concentration in (a) treatment-naïve and (b) post-treatment. (c) Correlation between GlycA and CRP. (d) Correlation between GlycA and PSA. GlycB concentration in (e) treatment-naïve and (f) post-treatment. (g) Correlation between GlycB and CRP. (h) Correlation between GlycA and PSA.

Figure 2

KODAMA score plot of plasma PCa samples colored according to NCCN classification. The size is proportional to (a) the GlycA intensity and (b) the GlycB intensity. (c) Heatmap of the metabolic profiles.

Figure 3

(a) NMR profiles of the plasma of three different spectral regions and Box-whiskers plots of the intensity of (b) GlycA and (c) GlycB, the concentration of (d) histidine and the number of (e) VLDL particles across the four metabotypes.

	HDL-1	HDL-2	HDL-3	HDL-4	LDL-1	LDL-2	LDL-3	LDL-4	LDL-5	LDL-6	VLDL-1	VLDL-2	VLDL-3	VLDL-4	VLDL-5	
Metabotype IV versus others				↓												Apolipoproteins
																Phospholipids
																Free Cholesterol
																Cholesterol
					↑	↑										Triglycerides

Metabotype II versus Metabotype III			↑	↑					↓	↑↑	↑↑↑					Apolipoproteins
										↑↑	↑↑↑	↑↑↑	↑↑	↑↑	↑↑	Phospholipids
		↓					↓			↑	↑↑↑	↑↑↑	↑↑↑	↑↑	↑↑	Free Cholesterol
										↑↑	↑↑↑	↑↑↑	↑↑	↑↑	↑	Cholesterol
			↑	↑↑			↓		↑		↑↑↑	↑↑↑	↑↑	↑↑	↑	Triglycerides

Lower: ↓ 0.001 < p-value < 0.01

Higher: ↑ 0.001 < p-value ≤ 0.01

↑↑ 0.0001 < p-value ≤ 0.001

↑↑↑ 0.00001 < p-value ≤ 0.0001

Figure 4

Graphics illustration of the changes of the lipoprotein profile.