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Computational modeling of lipoprotein metabolism

Schalkwijk, D.B. van

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Author: Schalkwijk, Daniël Bernardus van

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General discussion

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In this PhD thesis the development, validation and application of the Particle Profiler computational model is described. This model was developed with the aim of analyzing measured detailed lipoprotein profiles, to obtain information about human lipoprotein metabolism that can be used for diagnostic purposes. This general discussion includes the main findings, methodological considerations related to Particle Profiler, a comparison of Particle Profiler with other modeling studies, future research recommendations and a general conclusion.

Main findings

This section includes the main findings presented in this PhD thesis with regard to Particle Profiler. The first section summarizes the knowledge gained during model development and validation, while the second section summarizes the use Particle Profiler had in various applications.

Model Development and Validation

The second chapter of this PhD thesis presents a first version of the Particle Profiler model. Particle Profiler models how the rate of lipoprotein metabolic processes depends on the size of the lipoprotein particle. The model was applied to existing preanalyzed stable isotope tracer data presented by Packard et al. (78). This gave four main findings (125). The model implementation was able to reproduce the original metabolic fluxes calculated by Packard et al., requiring only six parameters to describe all modeled lipolysis and uptake processes. The Particle Profiler results in chapter [2] were able to predict the LDL size shift that was measured in the original study, only using reported flux data measured in four density classes by Packard et al. Therefore, the model predicts a change that is actually measured, which gives confidence in the model's realism. Furthermore, Particle Profiler was able to indicate relevant differences in physiology between the groups, such as a difference in higher hepatic lipase activity that is known to cause smaller LDL particles. This finding indicates a possible mechanism for the shift in LDL peak size. Finally, the potential for modeling the effects of genetic variants was demonstrated by simulating reductions in ApoB-related uptake affinity and lipolysis affinity. These simulations gave results that correspond to the observed lipoprotein profiles in subjects with such deficiencies. These four findings encouraged further investigation.

In the third chapter, we further developed and calibrated Particle Profiler model parameters using literature data on metabolic fluxes and lipoprotein profiles from subjects with specific genetic conditions. The development step consisted in replacing the function for particle attachment for the liver with a new function that better matched the observed hepatic lipoprotein uptake in lipoprotein flux studies. In this stage, we also further

reduced the number of parameters to five. The calibration step consisted in estimating the model ‘constants’ (those parameters that remain the same for all subjects) using subjects with different genetic backgrounds: LPL deficiency, the apoE 3/3 genotype, and the apoE 2/2 genotype. In these subjects specific processes stand out clearly, which makes it possible to estimate the model constants. We subsequently performed technical model validation using data on lipoprotein concentrations and metabolic fluxes: the model could fit a range of normolipidemic and dyslipidemic subjects from fifteen out of sixteen studies equally well, with an average $9\pm5\%$ fit error; only one study showed a larger fit error. This result constitutes a corroboration of the model, which is the best possible technical model validation available.

Model Application

The first model application was an initial clinical validation also in chapter three. There, we showed that a new diagnostic marker based on VLDL metabolic ratios calculated by the Particle Profiler model, better distinguished dyslipidemic from normolipidemic subjects than established markers like triglycerides, HDL cholesterol, and LDL cholesterol. This diagnostic marker is called VLDL performance, and is the average of two metabolic ratios: lipolysis/production in VLDL and uptake/production in VLDL. Two additional model-based markers clearly distinguished subjects with genetic variants apoE 2/2, LPL -/-, homozygous familial hypercholesterolemia, and familial defective apoB from other normolipidemic and dyslipidemic subjects. (149) This indicates that Particle Profiler-derived diagnostics reflect specific traits of dyslipidemia and genetic deficiencies.

The second application in chapter 4 concerned a nutritional study, which investigated the metabolic effects of dietary medium chain fatty acids (MCFA) versus long chain fatty acids, especially linoleic acid. This application showed that combining Particle Profiler-derived flux ratios with stable isotope-determined VLDL apoB production rates can be used to determine absolute VLDL production, lipolysis and uptake rates. The study showed no significant difference in VLDL production between treatments. However, it did show a difference in lipolysis and uptake, which was attributed to PPAR activation by linoleic acid.

The third application in chapter 5 was to the GOLDN study, in which we identified three subgroups based on clustering of the lipoprotein profiles. Analysis of the cluster centroid lipoprotein profiles using Particle Profile showed differences in the two metabolic ratios that constitute VLDL performance. The ‘VLDL performance’ marker was high in the first ‘healthy’ subgroup, lower in the second ‘intermediate’ subgroup, and lowest in the third ‘unhealthy’ subgroup. The difference between the second and third versus the first and second subgroups showed a larger influence of extrahepatic lipolysis

dysfunction versus liver uptake dysfunction. The reported dyslipidemia, therefore, seems to involve two stadia in which extrahepatic lipolysis dysfunction becomes progressively more important compared to liver uptake dysfunction. (139) The subgroups also show difference in lipid response to fibrate therapy, where both the ‘intermediate’ and ‘unhealthy’ subgroup show improved lipid values.

The final application in chapter 6 was to lipoprotein profiles measured using NMR in the Framingham Heart study. It is possible to construct many different Particle Profiler-based diagnostics by taking ratios of all processes modeled in Particle Profiler, and evaluating these ratios for various particle size ranges. In this study, we used the following three size ranges: VLDL to LDL, VLDL only, IDL and LDL. From all possible Particle Profiler-based diagnostics thus derived, we selected those that consistently contributed to cardiovascular disease risk prediction, using a nonlinear SVM-type statistical approach. We found that two lipoprotein metabolic ratios significantly improved the area under the curve of the ROC curve for general cardiovascular disease, and therefore significantly improve the risk prediction for cardiovascular disease. The first ratio we found is the ‘extrahepatic lipolysis ratio’. This ratio is especially important for predicting risk in people that take blood pressure medication: if people take this medication and extrahepatic lipolysis does not function well, the risk of cardiovascular disease dramatically increases. The second ratio is the ‘hepatic turnover ratio’. This ratio is especially important when a person has a LDL particle number that indicates the borderline between low and medium risk categories. If the liver works well in such a person, risk is low; if the liver does not work well, the person belongs to the medium risk category, which often entails therapeutic intervention. Therefore, we have found two lipoprotein metabolic ratios that significantly improve the existing diagnostic power for general cardiovascular disease, a finding that can really make a difference for people’s lives. For clinical application, further validation is required.

Methodological Considerations Related to Particle Profiler

This section treats two issues that came up during the project and that still require further thought and investigation. The first issue is about how to quantify single particle lipolysis. This issue was dealt with briefly in chapter [2], but a lengthier discussion is useful for examining in detail what can be done about it. The second issue concerns the precise size-dependency of lipoprotein metabolic process rates. This issue comes up as a consequence of the work done in this PhD thesis, and discussing it is useful to trace a path for future investigation.

Quantifying Single Particle Lipolysis

The first methodological issue is that of quantifying single particle lipolysis. In the process of designing a model for interpreting detailed lipoprotein profile measurements, several assumptions need to be made to base the mathematical equations on. Making these assumption explicit is one of the key parts of the learning processes involved in the mathematical modeling of biology. The assumptions make clear what the current state of our knowledge about the system is, and often point to new scientific questions. The Particle Profiler model is based on thinking in terms of processes affecting single lipoprotein particles, and explicitly takes into account the size of the particles. One of the questions that appeared was how the particle lipolysis process, during which the particle loses fat to other tissues, relates to the size of the lipoprotein particle. In this process, there are two steps, as described in chapter [2]. The first step describes how fast the lipolysis process occurs for a particle of a given size. The second step describes how the size of the particle changes after lipolysis. I would like to discuss the assumptions we made for the second step.

In an earlier single particle-based model by Adiels (20), the particle size decrease by lipolysis has been treated as a stochastic variable. For our model, stochasticity was not an option, because of the computational overhead it entails. Therefore, we sought for experimental data to underpin the size change through lipolysis, but we found none. Since the LPL protein lipolyzes the triglycerides in the core of the lipoprotein particle (107), we related the size change due to lipolysis to the triglyceride content of the particle. This approach poses two problems, the first being the relation between particle size and triglyceride content, and the second being the manner in which triglycerides are taken out. Because of the scarcity of the data, we had to solve the first problem by assuming that a particle of a given size always has the same triglyceride content, where the relation between triglyceride content and particle size is based on an earlier published model that is extensively linked to experimental data (101). For the second problem, we considered two manners in which triglycerides might be taken out, as a constant number of triglycerides per lipolysis step or as a constant fraction of triglycerides per lipolysis step. In both these cases, the model would be parameterizable with a single parameter, which is the maximal number we could hope to estimate with the available data. We were able to see that taking out a constant number of triglycerides per lipolysis step led to inconsistencies with the experimental data, whereas taking out a fixed fraction of triglycerides was compatible with the experimental data. Therefore, for this problem we decided to use a fixed fraction loss of triglycerides per lipolysis step.

As the reader will note, these last arguments are largely inspired by practical considerations and the solution is limited by scarcity of experimental data. Therefore, in this area the model leads to an experimental challenge:

quantifying how the size and composition of a single lipoprotein particle changes during a lipolysis event. The most feasible approach to this problem would be a combination of experiments and modeling. The experiments would have to be stable isotope labeling studies, in which apoB is labeled, for instance through a labeled leucine infusion. Then at several timepoints after the infusion, plasma samples should be taken and lipoprotein profiles run on these samples. The different lipoprotein subfractions (how many subfractions there are depends on the measurement method used) should be analyzed for their biochemical constituents: apoB, free cholesterol, cholesterol ester, phospholipids, triglycerides and total protein. This analysis will allow Particle Profiler to optimally take into account the lipoprotein composition. In addition, the apoB stable isotope enrichment should be measured for determining the particle production, lipolysis and uptake fluxes. The methodology for such a study is comparable to that of earlier stable isotope studies (50). These data form the ideal dataset for applying a version of Particle Profiler to that can take into account lipoprotein tracer data. This version of the model is yet to be developed. This methodology will lead to improved quantitative understanding of lipoprotein lipolysis.

Precise Size-Dependency of Lipoprotein Metabolic Process Rates

A second issue relates to the new concept that the Particle Profiler model introduces: the relation between the rate of lipoprotein metabolic processes and particle size. It is possible to further refine our understanding of how lipoprotein metabolic process rates (production, lipolysis, and uptake) depend on the size of the lipoprotein particle. In this PhD thesis we have been able to work with apoB kinetics data from many dyslipidemic subjects, mostly published by Packard *et al.* The only drawback these data have from the perspective of the Particle Profiler model, is the fact that they do not include more than four lipoprotein subfractions. The studies described above, in which apoB kinetics are measured in multiple subfractions, are also necessary to refine our understanding of how lipoprotein metabolism rates depend on particle size. Such studies will need to be carried out in subjects with different genetic backgrounds, similar to the study presented in chapter [3], where we used subjects with LPL deficiency, apoE 3/3 and apoE 2/2 backgrounds. Such studies will allow us to refine our understanding of how lipoprotein metabolic process rates depend on particle size.

One example where refinement is possible, is in the process of LDL uptake through the LDL receptor. It is known that apoB changes its configuration when the lipoprotein particle changes its size, and that this configuration change affects its interaction with the LDL receptor.(114) Put differently, the affinity of the LDL receptor for LDL particles changes with the size of the particle. However, the currently available data do not contain

enough information to include this effect in the model, which is why it is currently not included. Future studies of the type described above would help to refine our understanding of how the apoB-dependent LDL uptake varies with the size of the particle. Next to furthering our understanding, this knowledge would also help to further improve the model-based diagnostics.

Model Application

Particle Profiler can in principle be applied to lipoprotein profiles including VLDL through LDL lipoproteins measured by any method. Several such methods are available, as discussed in the Introduction chapter of this thesis under: “What methods are available for measuring lipoproteins and to what data types do these methods lead?” As Figure 1.2 illustrates, these methods are hard to compare directly, because they subdivide the lipoprotein size spectrum into different subclasses. However, Particle Profiler can analyze all these data types and always give the same output parameters. Therefore, it is possible to compare the Particle Profiler output based on different measurement methods.

Such a comparison is interesting, but not trivial, since things can go wrong at several levels. First of all, the measurement methods can give mutually inconsistent results. But secondly, the measurement methods can measure the same particle and report different sizes. This method-dependent bias in size measure needs to be corrected for. The bias can be detected by comparing the lipoprotein compositions in each size fraction. Therefore, this size bias-related error can be minimized by measuring the lipoprotein composition in each subfraction (phospholipids, free cholesterol, cholesterol ester, triglycerides and total protein). Clearly, the different lipoprotein profiling measures would have to be carried out on the same samples. It would be very interesting to perform this study from a technical point of view.

Further applications of Particle Profiler are only limited by the domains in which lipoproteins perform an important role. Chapter [6] of this thesis demonstrates that Particle Profiler has produced new diagnostic markers that improve cardiovascular disease prediction. This area could be carried further by looking at specific cardiovascular endpoints, or by further validating the model in other cohort studies. It would also be interesting to relate Particle Profiler parameters to genetic variants, especially those parameters that are important as diagnostic markers. Other possible domains of application include diabetes type II prediction and obesity research.

Particle Profiler Compared to Other Modeling Studies

Modeling can be used to investigate lipoprotein metabolism in various ways. Here, we will discuss several modeling methodologies and their relation with Particle Profiler.

Modeling in the determination of subclass kinetics

Since lipoprotein subclasses can be physically separated e.g. by ultracentrifugation or FPLC, they are amenable for kinetic studies employing tracers. Isotopically labeled precursors of lipoprotein constituents (fatty acids, cholesterol, lipids, or amino acids) can be supplied by means of intravenous infusion, and the kinetics of accumulation of the isotope in different lipoprotein fractions in humans can be followed in samples taken at different time points (46,91). The kinetic constants that characterize the different processes in which lipoproteins take part, then have to be derived by mathematical modeling of these processes and non-linear least squares fitting of predicted vs. measured isotopic enrichments. Typically, the models used are multicompartment models that include pools of precursors in plasma and various body compartments, pools of assembled (nascent) lipoproteins e.g. in the liver, and pools of subclasses of VLDL, IDL, and LDL in plasma (50,51). The kinetic processes typically include synthesis of pool constituents, exchange reactions between the various pools, and irreversible loss of pool constituents. In most cases, first-order kinetics of reactions is assumed. Estimated parameters include pool sizes and first-order rate constants that can be combined to calculate fractional synthetic rates, fractional catabolic rates and/or fractional transfer rates. These parameters can then be related to environmental factors (e.g. interventions) and disease, providing possible mechanistic clues to observed phenotypic responses. The use and applications of these models particularly in studies on the nutrition/metabolic syndrome has recently been reviewed in (228). Tracer studies combined with kinetic modeling have thus yielded important insights in the kinetics of production and interconversion of lipoproteins *in vivo* and how these vary in diseased states. Such changes will be reflected, albeit in an indirect and complex manner, in lipoprotein profiles. Thus, modeling directed at extracting flux-related information from lipoprotein profiles may help to reveal and diagnose disease phenotypes.

The relation between Particle Profiler and lipoprotein kinetics models is two-fold. First of all, Particle Profiler has been developed and validated using results from this type of models. Secondly, Particle Profiler can itself be seen as a kinetic model, but in the current implementation it only models particle subfraction concentrations at steady state. However, in principle the Particle Profiler framework could be extended to include the analysis of stable isotope kinetic data. With such an extension, the model would be able to analyze data from experiments where stable isotope enrichments are measured in many subfractions. This would in turn give information for further refinement of the model, as discussed above under 'Methodological Considerations Related to Particle Profiler'. So Particle Profiler at once builds forward on the work of earlier kinetic models, and could also become a kinetic model itself if extended further.

Modeling to understand and predict lipoprotein metabolism

Knowing from kinetic as well as from molecular studies which processes are involved in the metabolism of lipoproteins, new, mechanistically driven approaches are emerging in cholesterol and lipoprotein modeling. Such approaches hope to predict what lipoprotein profile results from a given set of parameters characterizing the different biological processes in which lipoproteins are involved. Once the prediction is correct, the model can be subjected to various analyses. For example, sensitivity analysis can be used to predict which interventions should be made to bring about a desired change in the lipoprotein profile, such as an optimization of the LDL/HDL cholesterol ratio. One could also predict the effect of genetic mutations on the lipoprotein profile. The mechanistic models to understand and predict lipoprotein metabolism may further be linked to computational mechanistic models of a specific disease process (for example atherosclerosis, (229)) to generate predictions on how the lipoprotein profile will change depending on disease parameters, provided that the lipoprotein metabolic processes are represented in sufficient detail in the integrated model. This offers the possibility to effectively predict candidate lipoprotein-derived causal disease biomarkers from *in silico* studies.

Below we describe several predictive models that have recently been published.

Plasma LDL-cholesterol levels are in part determined by the rate at which LDL particles are removed from the bloodstream by hepatic uptake. The uptake of LDL by mammalian liver cells occurs mainly *via* receptor-mediated endocytosis, a process which entails the binding of these particles to specific receptors in specialized areas of the cell surface, the subsequent internalization of the receptor-lipoprotein complex, and ultimately the degradation and release of the ingested lipoproteins' constituent parts. Receptors are then partly recycled to the cell surface, and there is *de novo* cholesterol dependent receptor formation by the cell. These processes are key determinants for the principal mechanism of action of cholesterol-lowering statins. These drugs decrease intracellular hepatic cholesterol, leading to an increase of LDL receptor formation that subsequently causes increased extraction of cholesterol from LDL and VLDL lipoproteins, lowering plasma cholesterol as a consequence (230). Three deterministic models for lipoprotein endocytosis and associated events have been published and their properties analyzed. August et al (231) focused on the effect of model parameters on plasma cholesterol levels, predicting distinct high- and low cholesterol states. Tindall et al (232) predicted the effect of variation in binding, unbinding and internalization rates, the fraction of receptors recycled and the rate at which the cholesterol dependent free receptors are created by the cell on the overall uptake dynamics of either VLDL or LDL particles and subsequent intracellular

cholesterol concentration. Pearson et al (233) focused particularly on how the competition between the LDL and VLDL particles for binding to the pits on the cell surface affects the intracellular cholesterol concentration.

Lipoproteins are influenced in an indirect way by many more processes than those studied in the three mechanistic models mentioned above. Considering this fact, Van de Pas et al(234,235) have developed a physiologically-based kinetic (PBK) model for plasma cholesterol that includes all processes in the mouse that are key determinants of plasma cholesterol. The choice for processes to include or not was made based on plasma cholesterol values reported for knockout mouse strains included in the Mouse Genome Database¹⁸ (236). The authors identified no less than 36 key genes for plasma cholesterol, and used 12 of these to build a conceptual model of 20 metabolic and transport processes that directly involve cholesterol (234). Since kinetic parameters for all these processes are hardly available, the authors took the approach of building an ensemble of 64k submodels each having a unique combination of zero-order and first order apparent kinetics for 16 of the 20 processes included, calibrate each with *in vivo* data on pool sizes and fluxes, and selecting the best predictive models to generate an average prediction (235). This model is in the process of being translated to a human model.

In addition to the conclusions on the behavior of cholesterol metabolism as a whole, the dynamics of LDL and VLDL uptake and binding competition, and the effects thereof on intracellular cholesterol, the described models also show that mechanistic characteristics of lipoprotein metabolism are reflected indirectly in the lipoprotein profile. While the submodels lack detail in the plasma lipoprotein compartments (only 2 are included), especially the PBK approach has created the unique possibility to link processes and genes affecting cholesterol transport in the body as a whole, to changes in the plasma lipoprotein profile. The latter may be subject to analysis using more refined models. All in all, these models either provide a larger whole-body context than Particle Profiler, or zoom in on specific details of processes that are relevant for Particle Profiler. They therefore form a valuable addition to the quantitative analysis of lipoprotein metabolism.

Modeling to quantify physiological parameters that underlie different subclass distribution phenotypes

Using the knowledge gained from molecular and kinetic studies, a reverse procedure may be followed: from a measured lipoprotein profile, derive the parameter values of the underlying biological processes. This is the approach Particle Profiler has followed. We have shown that the results of such an analysis are especially useful for diagnostic purposes in chapter [6]. It is of

¹⁸ available via <http://www.informatics.jax.org>

interest that computational modeling for diagnostic purposes poses specific demands for the design, construction and evaluation phases of model development and can benefit from techniques for efficient model formulation, model calculation, workflow structuring and quality control as described in the chapter [7](126).

Next to particle profiler, only Hübner et al (61) have published a model with the intention of analyzing lipoprotein subclass profiles.

Hübner et al (61) have developed a particle-based model solely of plasma lipoproteins that considers the entire protein and lipid composition spectrum of individual lipoprotein complexes, of both ApoB-100- and ApoA-I- containing lipoproteins. Subsequently, their distribution over density (which equals the lipoprotein profile) was calculated and it was shown that cholesterol plasma levels can be simulated as resulting from a steady state of a particle distribution. Their model was able to (i) successfully reproduce clinically measured lipoprotein profiles of healthy subjects; (ii) assign lipoproteins to narrow density classes, named high-resolution density sub-fractions (hrDS), revealing heterogeneous lipoprotein distributions within the major lipoprotein classes; and (iii) generate predictions of changes in the lipoprotein distribution elicited by disorders in underlying molecular processes. Formulated as a stochastic particle population model, a large number of individual lipoprotein particles must be modeled for a simulation, and considerable computational power is required. A potential use of the model for diagnostic purposes was indicated but not demonstrated. The estimation of the 20 parameters of the model will require the measurement of a very highly resolved lipoprotein profile indeed, along with compositional analysis of each subfraction.

In constructing Particle Profiler, we have taken much care to avoid overparametrization of our model, essentially by limiting ourselves to ApoB-100 containing lipoproteins and by implementing particle size-dependent mass action kinetics as opposed to the non size-dependent kinetics used in Hübner et al (61). This left only 5 parameters to be estimated from a lipoprotein subclass profile in the final model (chapter [3]). By defining and simulating a large number of lipoprotein compartments that contain lipoproteins in a small size range (as small as 0.1 nm has been used), the computational efficiency was greatly improved compared to a stochastic particle population model. It was shown (237) that the variables calculated by the model, can be combined to calculate (sub)class specific lipoprotein flux ratios that reflect disease physiology of liver and extrahepatic tissue. These ratios can be used as novel lipoprotein profile-based diagnostic markers (Chapter [6]. Hübner *et al.* have not yet published any clinical application of their model.

Statistical modeling for the construction of biomarkers that have diagnostic potential

The availability of detailed lipoprotein subclass profiles may lead to new biomarkers that spur the development of new disease concepts (see e.g. (238)). However, rather than affecting specific individual (sub)classes, disease may be associated with more complex patterns of distortion in the lipoprotein profile. Thus, there is a role for yet another type of modeling used in the analysis of lipoproteins, i.e. multivariate statistics. Of course multivariate statistics approaches are not specific to lipoproteins but we nevertheless mention a few interesting applications. Musunuru et al (57) used principal component analysis (PCA) of ion-mobility detected lipoprotein subfraction concentrations to identify 3 major orthogonal (i.e., zero correlation) components of CVD risk, one representing LDL-associated risk, a second representing HDL-associated protection, and the third (the “atherogenic lipoprotein phenotype”) representing a pattern of decreased large HDL, increased small/medium LDL, and increased triglycerides. The patterns appear to be associated with SNPs in specific genes, suggesting independent mechanistic pathways for development of CVD.

Self-organizing maps (SOMs) is a data visualization technique that reduces the dimensions of data through the use of self-organizing neural networks. The way SOMs go about reducing dimensions is by producing a map of usually 1 or 2 dimensions which plot the similarities of the data by grouping similar data items together. SOMs were used by Suna et al (239) and Kumpula *et al.* (240) to cluster lipoprotein profile phenotypes, leading to the emergence of complex lipoprotein associations. In Kumpula *et al.* (240) a metabolic subgrouping of the associations between plasma LDL cholesterol concentrations and the structural subtypes of LDL particles was discovered. This type of clustering can for example provide clues as to the correlation of compositional variations within the lipoprotein particles with metabolic and clinical parameters.

Clustering in general is a helpful method to assign patients to specific subgroups based on lipoprotein profiles. Correlating characteristics of the new subgroups with treatment outcomes may identify larger groups of patients that would benefit from the treatment than current clinical practice allows, as we (241) made clear in the case of fibrate therapy.

Particle Profiler can contribute to statistical analyses in two ways. First, as we showed in chapter [5], it can be used to interpret the outcome of lipoprotein clustering. But more importantly, to relate the parameters that result from Particle Profiler analysis to a disease endpoint, as we did in chapter [6], multivariate statistics is needed. In this last case, by generating new variables with specific metabolic information, Particle Profiler has improved the capacity of statistical analyses to relate relevant information from the lipoprotein profile to the disease endpoint.

Future Research Recommendations

In the above discussion, several recommendations for future research have emerged. Briefly, on the fundamental side these include further kinetics studies with stable isotope tracers, in which isotope enrichment is followed in a detailed lipoprotein profile. The Particle Profiler model framework needs to be extended to include kinetics data for these analyses.

Another possible study would be a comparison of the Particle Profiler outcomes using different lipoprotein profile analysis methods on the same samples. For best results, the methods should measure as many lipoprotein constituents in each of the subfractions as possible.

The area of cardiovascular disease prediction could be carried further by looking at specific endpoints, or by further validating the model for other populations. It would also be interesting to relate the relevant Particle Profiler parameters to genetic variants. Other possible domains of application include diabetes type II prediction and obesity research.

Concluding Remarks

In this PhD thesis, the Particle Profiler model has been developed, calibrated, corroborated, validated and applied. Particle Profiler has shown its usefulness for indicating different specific types of dyslipidemia, for understanding population-wide dyslipidemia, and for examining the effects of a nutritional intervention. Particle Profiler has also led to new risk biomarkers that are able to improve the diagnosis of cardiovascular disease, which have been identified using data from the Framingham cohort.