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**CAUSAL PROBABILISTIC NETWORK MODELLING
OF LIPID AND LIPOPROTEIN METABOLISM**

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**Thesis submitted in fulfilment of the requirements for the Degree of Doctor of
Philosophy in Measurement and Information in Medicine.**

**DEPARTMENT OF SYSTEMS SCIENCE
CITY UNIVERSITY**

September 1994

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Acknowledgments

I would like to express my appreciation for the work of Professor Ewart Carson and Professor Derek Cramp in supervising this work. Their guidance and advice has been most valuable both in the course of this study and during the preparation of this thesis.

Thanks also to Dr. Gerald Watts and Professor Derek Cramp for sharing their clinical and experimental expertise in the field of lipid and lipoprotein metabolism.

I am also grateful to St. Thomas' Hospital for providing a CASE studentship award for the duration of this work.

Finally, many thanks to all the research students and staff at the Department of Systems Science for making my time at the Department so enjoyable.

Abstract

This thesis has described, and illustrated the use of, causal probabilistic network (CPN) techniques in the modelling of lipid and lipoprotein metabolism. The models constructed enable prediction of the health outcome associated with disorders of lipid and lipoprotein metabolism, i.e. an individual's "potential for atheroma", and as such could be used as part of a strategy for decision support in the management of hyperlipidaemia.

CPN techniques are shown to be appropriate in modelling lipid and lipoprotein metabolism where relatively few data exist to support hypotheses concerning metabolic pathways. These techniques enable uncertainty and conditional independence assumptions to be included in model formulation, limiting the amount of data necessary to construct a model; a high level of uncertainty being represented where few data exist.

The modelling process has highlighted numerous areas of controversy concerning the physiology of lipid and lipoprotein metabolism. In particular questions have been raised concerning the mechanisms involved in reverse cholesterol transport and in the metabolism of very low density lipoproteins (VLDL) and low density lipoproteins (LDL). In addressing these issues, via the modelling process, it has been possible to illustrate consistency between data from numerous studies, both experimental and epidemiological. In effect, the models provide a summary of current knowledge which can be used to direct further research toward areas of controversy or areas where few data exist describing lipid and lipoprotein metabolism, and in the interpretation of the results of new research describing metabolic pathways.

Chapter 1 - Introduction and Thesis Structure

1.1 Background and Motivation

The management of patients in a specialised clinic consists of an iterative process of testing, diagnosis and treatment. Diagnosis involves interpretation of the results of previous tests and treatment, the clinician forming a mental picture of the patient's state. Then, by predicting the outcome of different treatments, for an individual with a particular diagnostic state, the clinician can choose a treatment which is most likely to benefit that individual.

Selecting treatment in this way implies that the clinician has a measure of outcome, and preference toward different values of this outcome, so that a treatment can be selected which "increases the likelihood of achieving desired health outcomes" (Lohr, 1990). This, according to Lohr (1990), is the definition of a "quality decision". It follows then that clinicians making quality decisions have methods of classifying patients, predicting likely treatment results, and assessing preferences toward these results.

In formulating these methods the clinician will have interpreted information from many sources. Broadly speaking, we might define these information sources as:-

- 1) Experience and training - Throughout his or her career the clinician will acquire knowledge of, and develop opinions on, the efficacy of drugs and other treatments, and on physiological and pathological mechanisms.
- 2) Quantitative studies - The medical literature includes a wealth of information, from multi-centre trials describing the efficacy of drug treatment, through to studies of individuals examining the underlying pathology associated with rare disorders.

In general, the clinician must interpret the results of these studies along with their

training and experience, and incorporate them into their decision making process. It is not easy to visualise how a clinician interprets the results of studies, checks to see if they are consistent with their beliefs on physiological mechanisms and their clinical experience, and updates their knowledge accordingly. However, if systems are to be constructed which support quality treatment decision making, then there is a need to consider how to interpret both expert opinion and literature data in a structured manner so that neither valuable source of information is excluded.

1.2 Objectives

The objectives of this thesis are to illustrate how expert opinion and literature data can be incorporated into a model describing lipid and lipoprotein metabolism; and to show how (given sufficient data) such a model can be used to predict an individual's health outcome, as part of a strategy for clinical decision support.

Both clinical and experimental advantages occur because of these objectives:-

- Predicting the health outcome of an individual enables clinical decisions regarding treatment to be directed toward improving this health outcome, i.e. the definition of a "quality" treatment decision given by Lohr (1990).
- In combining expert opinion and literature data in a more traceable way, a more formal checking of consistency between these two information sources can be performed, and areas identified where current opinion cannot be quantifiably validated, and hence further experimental research is required.

1.3 Thesis structure

In order to attain these objectives this thesis is structured in the following way:-

Chapter 2 describes the application area of this study, that of managing disorders of lipid and lipoprotein metabolism. It discusses the goal ("desired health outcome") of management (i.e. minimising the risk of coronary heart disease, CHD), strategies for achieving this goal, current management practice, and decision support currently available. This chapter notes several formulae for estimating CHD risk, derived from population data, describing why use of these formulae is inappropriate when managing individuals. In this situation a more detailed representation of CHD risk is required.

Chapter 3 describes the knowledge representation methodology necessary to include both clinical expertise and clinical literature data into a "quality" treatment decision making process. In this case, for managing individuals at high risk of CHD relative to the population.

The chapter describes the selection of a knowledge representation technique, concluding that the causal probabilistic network method (CPN), an intensional modelling approach, is best suited to problems such as this where diagnosis is uncertain and no clear management protocols exist. A description of the CPN technique is given.

Further, this chapter looks at the type of CPN model required i.e. static or dynamic, and describes methods of pooling data (meta-statistical analysis) from studies so as to construct relationships in this model. The chapter concludes by describing a strategy for model construction using both the CPN and meta-analytic techniques, combining both: a) expert opinion on the structure of metabolic processes and b) pooled data from literature studies describing these processes. This strategy illustrates how this model would be used in the patient management process. Examples of model construction using these techniques are taken from chapters 5 and 6, without

considering issues of lipid and lipoprotein metabolism.

Chapter 4 provides the physiological and pathophysiological basis for modelling atheroma (and hence CHD risk), as the difference between cholesterol transport to and from the peripheral tissues. The chapter describes whole body cholesterol metabolism and its regulation, reviewing those studies which describe this metabolism, and suggesting how information in these studies might indicate an individuals susceptibility to atheroma.

Chapters 5 and 6 describe model construction. Chapter 5 details the modelling of cholesterol fluxes to and from the peripheral tissues, the difference between these fluxes representing an individuals susceptibility to atheroma. Models are constructed for the non-physiological uptake of cholesterol at the peripheral tissues, and the cholesterol flux from the tissues to the plasma, a process known as "reverse cholesterol transport".

Chapter 6 describes how the model of cholesterol fluxes to and from peripheral tissues, constructed in chapter 5, could be extended to incorporate the effects of genetic disorders, obesity and treatment. Model development is limited to the effects of obesity and genetic disorders the aim being to reduce the complexity of the model and provide a core structure for future development.

Chapter 7 illustrates the validity of the models constructed in chapters 5 and 6 in qualitative and quantitative terms. This chapter shows how the model of "potential for atheroma", constructed in chapter 5, is consistent with a population on a western style diet, studies of whole body cholesterol metabolism, the assumptions used in model construction, and the results of a study not used in model construction. Validation in qualitative terms was performed on the model of apoprotein B and triglyceride production constructed in chapter 6, illustrating that the model obeys the assumptions made during its construction.

Chapter 8 reviews this thesis describing: a) the original objectives, b) how the work

described in this thesis addresses these objectives and c) the contributions of this thesis, in particular highlighting consistency between different information sources and areas of contention within lipid and lipoprotein metabolism.

Chapter 9 briefly summarises the conclusions of this thesis in terms of: a) the original objectives, and whether these were met, b) the contributions of this thesis and, c) future research required.

Chapter 2 The Application Domain:- Lipid and Lipoprotein Metabolism.

2.1 Introduction

In chapter 1 the objectives of this thesis were outlined. Briefly, these were to illustrate how expert opinion and literature data can be incorporated into models which have potential for use in supporting quality treatment decisions.

This chapter introduces the application domain in which this hypothesis is being considered, namely that of lipid and lipoprotein metabolism, disorders of which cause hyperlipidaemia. It describes the desired health outcome (that of minimising the risk of coronary heart disease (CHD)), and suggests why disorders or modifications of lipid and lipoprotein metabolism are relevant to this outcome.

Ideal strategies for reducing the CHD risk are discussed, both population and individual "high-risk", and compared with current practice. The chapter looks at available decision support tools, i.e. management guidelines, computer systems, and CHD risk formulae, describing why these formulae are inappropriate for use in the individual "high-risk" strategy. The chapter concludes by outlining requirements for decision support when managing patients using the individual "high-risk" strategy.

2.2 Reduction in CHD Risk - A Desirable Health Outcome.

The goal of treating hyperlipidaemia is primarily to "minimise the risk of CHD" (The Study Group European Atherosclerosis Society (EAS), 1987; 1988). This is the ultimate desirable outcome, where it is possible to distinguish between intermediate outcomes and health outcomes (Schachter, 1989). In the case of hyperlipidaemia intermediate outcomes would be the commonly measured variables of lipid metabolism such as low density lipoprotein cholesterol, high density lipoprotein

cholesterol and very low density lipoprotein triglyceride. The goal of management is not to modify these variables but to use modifications in them to reduce an individual's risk of CHD.

The progression of CHD is multifactorial, the three greatest risk factors being smoking, high levels of serum total cholesterol, and high blood pressure (Lewis et al., 1989). These factors modify physiological processes in different ways so as to increase the likelihood of blockage in the coronary artery and hence myocardial infarction (the acute event occurring as a result of CHD). Abnormalities in the lipid and lipoprotein metabolism increase the risk of CHD via cholesterol deposition at the periphery which causes furring of the arteries, known as atherosclerosis.

2.3 Strategy for Reducing CHD Risk.

The current strategy for reducing CHD risk is twofold i.e. there are distinct strategies both for the population and for the individual at high risk relative to the population (Lewis et al., 1989). The following section including figure 2.1, taken from Lewis et al. (1989), illustrates the ideas behind these two, complementary strategies.

"It is widely accepted that the control of CHD depends on reduction of the levels of its major known risk factors. Two strategies for risk factor reduction have been proposed, the population strategy and the individual high-risk strategy ... They are complementary and reduction of CHD is likely to be most successful where both are pursued simultaneously."

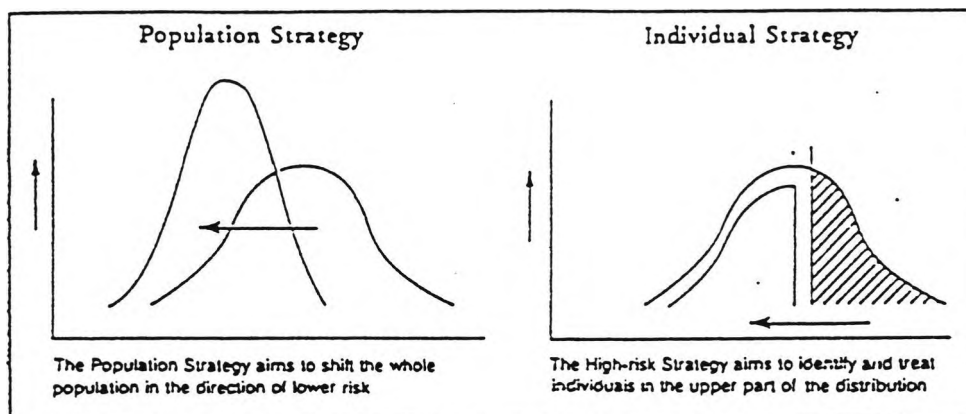


Figure 2.1 - The population and individual risk reduction strategies

"Although different in their approach, the population and individual (high-risk) strategies provide similar preventative measures. Both are necessary to achieve overall success in reducing CHD. The goal of the population strategy is to shift the entire population's risk factor levels in the direction of lower risk; that of the individual strategy is to identify and treat persons with higher levels of risk factors in a clinical setting."

"Mass education via a population strategy seeks to promote a healthier lifestyle for all individuals, through improved diet, reduction of smoking and increased exercise. This strategy is based on the recognition that the majority of people in European countries have a moderately high level of one or more risk factors for CHD largely due to unhealthy diet, smoking and lack of exercise."

When managing disorders of lipid and lipoprotein metabolism in a clinical setting, the individual high-risk strategy is being followed. Once having identified those patients at high risk of CHD, and having ascertained that a significant part of this elevation is due to disorders of lipid and lipoprotein metabolism, the aim is to regulate the patient's metabolism so as to lower their risk of CHD.

2.4 Current Practice

When managing patients at high risk, current practice does not explicitly weight different treatment options in terms of their potential for risk reduction.

First line treatment begins with modification of diet. If diet does not achieve satisfactory control of the hyperlipidaemia (i.e normalising the phenotypic expression) then selection of an appropriate drug occurs. More often than not once drug contra-indicators have been examined and the severity of the phenotype looked at, only one or two drugs are appropriate. One of these drugs is then tried and if it does not reduce the patient's levels to that which the clinician believes appropriate, then either the patient is switched to another type of drug within the same subclass (i.e. same metabolic point of action), or a different type of drug is tried.

First line management is relatively simple and may not require treatment selection based upon the potential for risk reduction of each treatment. During follow-up however, the treatment selection process is far more complex. For example, it is possible for a patient to achieve a good level of phenotypic expression after their first visit to the clinic but subsequently - due to either obesity, increased alcohol intake or non-insulin dependent diabetes (NIDDM) - to once again present with hyperlipidaemia. In this situation should the response be to a) increase the dosage of the drug, b) change to a new drug, c) try a combination of drugs, or d) decide that the new situation is within the appropriate CHD risk limit? This is a common clinical situation where control is initially good, but over a period of time it is eroded due

to non-compliance with treatment or the presentation of secondary causes of disorders in the lipoprotein metabolism.

In the above example the complexity of the decision is increased by the presentation of secondary disorders. Management guidelines suggest that these should always be treated prior to managing underlying disorders of lipid metabolism. However, this is not always possible, with secondary disorder reoccurring or new ones presenting during management.

2.5 Decision Support Currently Available

Current decision support is in the form of management guidelines (Lewis et al., 1989; Thompson et al., 1990; The Working Group, 1991; The Study Group EAS, 1987; The Expert Panel, 1988; J Shepherd et al., 1987), computer-based tools automating these guidelines (Anon, 1991; Rucker et.al., 1990), and CHD risk formulae, which are often built into calculation devices (Tunstall-Pedoe, 1991; Shaper et al., 1986; Assman, 1989).

Many reports have been produced detailing guidelines for the management of hyperlipidaemia. These provide advice on appropriate first-line treatment, suggesting different diets for severity of phenotypic presentation and the appropriate use of drugs. They do not deal with the complex situations arising from coexistent primary and secondary disorders and justification of treatment options in these situations.

Computer-based tools automating guidelines are appropriate for first line management, but are somewhat limited after that. They deal with the situation where a non-specialist is managing patients under the responsibility of a specialist. Their role is therefore to fix levels of referral to the specialist clinic, formalising communication links between the general practitioner and the hospital's clinical specialists and laboratories; and to define first line treatment strategies to be followed

by non-specialists. One of these systems (Anon, 1991), which automates the British National Guidelines (Shepherd et al, 1987; Thompson et al., 1990), is a system which provides advice for first line treatment across a number of disorders, along with colourful pictures illustrating well known symptoms of metabolic defects such as tendon xanthoma.

Numerous formulae are available for estimating CHD risk, several of which have been automated in hand held risk calculation devices. These formulae are extremely useful in screening patients. One of them, developed from the British Regional Heart study (Shaper et al., 1986), has the potential to relieve the burden of large quantities of cholesterol testing involved in the screening process by developing two formulae one of which does not include total cholesterol as a variable. In this way total cholesterol need only be measured if the patient is at risk due to other factors, resulting in good screening practice without over-burdening hospital laboratories with unnecessary cholesterol testing. However, these formulae are inappropriate for use when managing individuals using the "high-risk" strategy. This is because:-

a) The population of interest when managing the lipid and lipoprotein metabolism of individuals in the "high risk" group is different from the population in which CHD formulae were developed. Individuals in the "high-risk" group are more likely to have primary and secondary disorders of lipid and lipoprotein metabolism which cannot be distinguished from measurements of age, sex, "father died before the age of 50 from myocardial infarction", total cholesterol level etc, i.e. the variables included in CHD risk formulae. When managing these individuals more detailed measurements are required to determine the individuals' abnormalities and hence the most appropriate treatment to reduce their risk of CHD.

b) Current formulae for estimating risk generally include factors which are non-modifiable, for example, age, sex, "father died before the age of 50 from myocardial infarction" etc (Assman, 1989; Tunstall-Pedoe, 1991; Shaper et al., 1986). By including fixed variables it is assumed that there exists a baseline level beyond which further CHD risk reduction is impossible.

This has several implications for management strategy:-

- i) It might prevent treatment in the elderly who would have a high baseline level of CHD risk due to age.
- ii) It may suggest that it is more profitable to treat women at high risk as greater risk reduction can be achieved (their baseline level of CHD risk being lower than that of men).

However, when examining the physiological reasons as to why these variables might be related to atherosclerosis it could be argued that there is no such baseline of risk. For example, when including age as a fixed risk factor it is assumed that atherosclerosis is a natural aging process. However, this may depend upon dietary intake since in populations not consuming a western style diet atheroma progression due to old age may be less pronounced. Dietary modification could therefore reduce the risk of CHD normally associated with age, particularly if atherosclerosis is reversible with dietary modification.

Using the current risk formulae for managing patients could therefore, hide potential reductions in CHD risk. Perhaps a more physiological representation of CHD risk is required, reflecting an individual's metabolic state. For example, the difference between cholesterol flux to and from the peripheral tissues could be a potential indicator of cholesterol deposition and hence atherosclerosis. In fairness, few of the current CHD risk formulae claim to be management tools, their use in the main being limited to screening as part of the population strategy, to identify those individuals at increased CHD risk relative to the population as a whole.

In summary, this work assumes that long term risk due to disorders of lipid and lipoprotein metabolism is modifiable in all patients in whom metabolic control can be improved, and that metabolic control and coronary risk can be estimated by measuring those variables describing lipoprotein metabolism, described in chapters 5 and 6.

2.6 Decision Support Required

Current decision support has focused on the implementation of the population strategy and first line treatment of an individual. To support more complex situations a system is required which predicts changes in CHD risk for treatment options in cases where primary and secondary disorders of lipid metabolism co-exist. Potential CHD risk reduction for each treatment option can then be weighed against the cost of treatment, and a decision made whereby cost is traded-off against potential benefit. As management proceeds, the system should provide a clearer picture of the patient's underlying metabolic defects from their response to treatment, enabling a) greater accuracy in future predictions of CHD risk, and b) identification of secondary disorders present.

When considering cost, both the financial cost of treatment and the cost to the patient in terms of lifestyle modification and potential side-effects need to be addressed. The first of these issues concerns resource utilisation so that the scarce resource available for treatment may be used in the most appropriate circumstances. The latter issue concerns patient preference toward treatment, the side-effects of treatment modifying patient lifestyle to a greater or lesser extent. An ideal strategy for managing the individual at high risk is therefore one in which treatment costs are weighed against potential CHD risk reduction, a process involving the cooperation of both clinician and patient.

Current guidelines do not deal with the appropriate use of resources, suggesting when it is relevant to allocate scarce resource to reduce an individual's level of CHD risk. This issue is illustrated below in a letter to the Lancet (Olbright, 1991), where current standards were unable to provide guidelines on weighing risk against benefit for an individual.

"Should we treat hypercholesterolaemia in the elderly?

Sir,-- A total serum cholesterol of 270 mg/dl with a low density

lipoprotein (LDL) cholesterol of 190 mg/dl were detected in a 66 year-old man who has no signs of coronary heart disease (CHD). A cholesterol-lowering diet did not sufficiently reduce the serum cholesterol and a cholesterol lowering drug was prescribed. He asked me about the risk-benefit of cholesterol-lowering treatment at his age. To my surprise, expert panels and consensus conferences have not addressed this question in detail. While US statements admit that there is "little direct clinical trial evidence" on the benefit of cholesterol lowering to be expected in the elderly they continue to recommend such treatment when the cholesterol exceeds 240 mg/dl. The European Atherosclerosis Society offers just two sentences, indicating that there is "no basis for withholding treatment of hyperlipidaemia in older patients."

2.7 Summary

This chapter has described the management of CHD risk in the population and in individuals at elevated risk relative to the population; the goal of treating hyperlipidaemia being to minimise CHD risk.

Current decision support for managing CHD risk has been examined, this chapter concluding that current risk formulae, computer-based advice and management guidelines are appropriate for screening and first line management, but that subsequently, decision support should be provided which enables treatment decisions to be taken based on the complex trade-offs defining the benefit of treatment to an individual. More specifically this requires a measure of CHD risk which is more detailed, does not include fixed baseline factors, and enables consideration of the cost of treatment, both financial and to the patient.

Chapter 3 describes knowledge representation techniques appropriate to the more

complex decision making processes involved in the long term management of individuals at elevated risk relative to the population.

Chapter 3 Methodology

3.1 Introduction

This chapter examines the methodology necessary to construct physiological models which form part of a strategy for quality decision support in the management of hyperlipidaemia. It describes why a physiological model-based approach to knowledge representation is most appropriate when considering the complex treatment decisions of second line management, and how both quantitative and qualitative knowledge can be included in these models.

The chapter comments on other studies where a physiological model-based approach to knowledge representation was required, enabling consideration of the complete patient context in situations where diagnosis is uncertain and no clear management protocol exists. The Causal Probabilistic Network (CPN) technique, an intensional approach to knowledge representation (i.e. context sensitive) is then described. This technique allows uncertainty to be represented in the model and provides a framework for the incorporation of qualitative and quantitative knowledge into model construction.

Further, this chapter considers the type of model required i.e. static or dynamic, and methods of pooling data from numerous studies so as to construct relationships in this model i.e. meta-statistical analysis.

The chapter concludes by describing a strategy for model construction using both the CPN and meta-analytic techniques, illustrating how these models would be used in the patient management process. Examples of model construction using these techniques are given. These are taken from chapters 5 and 6 and illustrate the modelling techniques without considering issues of lipid and lipoprotein metabolism.

3.2 Choosing a Knowledge Based Representation Technique

3.2.1 The Different Stages in Patient Management

In chapter 2 several management strategies were discussed for the treatment of lipid disorders so as to minimise the risk of coronary heart disease (CHD). That chapter concluded that up to referral to the clinical expert, sufficient guidelines existed to enable management of patients by fixed protocols, i.e. a rule-based automation of these guidelines.

However, subsequent to referral, a more detailed management process is required, allowing decisions to be made based on the likely effects of treatment on an individual (in terms of CHD risk), trading this off against any adverse reactions likely from that particular treatment, and patient preferences toward treatment.

The need for a more complex representation of knowledge when managing individual patients expertly (i.e. in a hospital clinic) has been recognised by other authors (Tu et al., 1988; Langlotz et al., 1987; Quaglini et al., 1989). These authors have split the management process, providing decision support at two different levels. For example, in the development of a system for managing patients with cancer, two sub-systems have been created, namely ONCOCIN and ONYX:-

ONCOCIN (Tu et al., 1988), has been developed for routine clinical care. It uses the skeletal plan refinement technique, where a skeleton protocol is automated and adjusted during the management process. This was possible because, in the majority of cases, agreed protocols for chemotherapy were available. When a patient was too complex to manage using standard protocols, ONCOCIN referred them to experts who "recommend therapy using his or her knowledge about the physiology of the human body, the disease process, strategies for oncology chemotherapy and the goals for the patient" (Langlotz et al., 1987).

A second system, ONYX (Langlotz et al., 1987), simulates the expert's management

of individuals, providing advice in the situation where:

- Expert guidelines for plan selection are not available,
- Current state is not known with certainty,
- Consequences of action are not known with certainty and,
- Planning goals cannot be satisfied completely (because they are inherently contradictory).

The necessity for a two level approach to management has been described by Quaglini et al. (1989) in the management of anaemic patients, as illustrated below:-

"Depending upon the diagnosis, one of two approaches to the decision is selected. For most anaemic states a well agreed on therapeutic plan can be pointed out. In such cases the task does not involve basic strategic choices, but rather consists in fixing details such as dosage and route of administration in order to meet the specific clinical conditions of the patient. To this aim a traditional approach based upon "condition ---> action" rules may be appropriate. Other anaemic states involve trade-offs between conflicting goals, in presence of uncertainty about therapy effects. The latter class of problem poses difficulties."

This two level strategy corresponds closely with the management of hyperlipidaemia as illustrated in chapter 2. Management of an individual, as part of the population, can be performed by protocols documented in guidelines and automated in rule-based systems. In the management of an individual at high-risk relative to the population, no clear guidelines exist, and diagnosis is uncertain. This situation requires representation of the patient's state and the likely effects of treatment on that state, treatment selection being a trade-off between potential CHD risk reduction offset against costs and patient preferences.

3.2.2 Representing patient state and perturbations of that state using an intensional model.

Planning strategies using intensional models and decision theory have been developed to tackle the complex management situations described in section 3.2.1 (Haddawy and Rendel, 1990). These authors note that to plan therapy an "autonomous agent" must have some "knowledge about the state of the world, knowledge of possible actions he can perform, knowledge of how these actions affect the world, and a set of desires or preferences." To perform therapy planning in complex situations it is necessary then: a) to represent patient state i.e. a patient specific model, b) to be able to predict the results of treatment from this model in terms of CHD risk, and c) to have a method of estimating an individual's preference toward the most likely results of treatment.

Rule-based or extensional systems are inappropriate for representing complex situations where therapeutic decisions must be made from an analysis of the patient's underlying state, the uncertainty in this state, the most likely effects of treatment, and patient preferences toward treatment. This is due to their inability to update findings in the context in which they occur. In particular, the principle of "modularity" applies (Pearl, 1988b), such that "if you see A anywhere in the knowledge base then regardless of what other things the knowledge base contains, and regardless of how A was derived, you are given the licence to assert B and add it to the database". In intensional systems the conditional statement $P(B/A)=p$ means that (Pearl, 1988b) "if A is true and A is the only information that you know, then you can attach to B a probability p". Intensional updates are performed conditionally based on the context of the whole knowledge base. If C was observed as well as A, then the extensional approach would still conclude "If A then B", whilst intensionally the probability of B would now be represented as $P(B/A,C)$.

The lack of contextual representation in extensional systems introduces inconsistencies into their reasoning (an example of which is given in section 3.3.2). This makes it an inappropriate representation for complex situations where a

consistent model of patient state is required, so that diagnosis and prognosis can be performed from the same model.

Several groups of workers have shown that causal probabilistic networks (also belief networks, influence diagrams etc.) can provide an intensional representation of patient state, allowing prognosis and diagnosis to occur from the same model. When linked to a model of utility, decision theory can be used to represent patient preference toward the likely outcomes of treatment predicted by the model. The basic principles of the CPN modelling techniques are described in the next section. In that section the work of Pearl (1988b) is referenced extensively; where possible examples illustrating modelling issues are taken from the field of lipidology.

3.3 The Causal Probabilistic Network (CPN) Technique

3.3.1 Introduction

This technique represents variables by nodes, and causal relationships between these variables by directed arcs between the nodes. Uncertainty is represented by probability distributions on the states of each node, with the distribution on the root nodes (those with no incoming arcs) representing the current diagnostic state. The initial distribution on the root nodes is known as the *a priori* distribution, and represents our belief as to the diagnostic state in the population.

Relationships between nodes are represented by conditional probability tables, and model inference is performed using Bayes' theorem.

3.3.2 Probability as a reasoning structure

Pearl (1988b) quotes Schafer as saying that "probability is not really about numbers; it is about the structure of reasoning". This is due to the potential of causal probabilistic networks (CPNs): 1) to update the knowledge base contextually, preventing inconsistencies inherent in rule-based systems, and so removing the need for truth maintenance, and 2) to allow representation of relevance and irrelevance through the causal structure.

These ideas are best illustrated through the following examples.

3.3.2.1 Example 1 - The Consistency of knowledge representation

The causal diagram in figure 3.1 illustrates an extreme simplification of the effects of dietary saturated fat and the genetic disorder familial hypercholesterolaemia (FH) on the concentration of total plasma cholesterol. It is a simplistic example but serves to illustrate the principle issues.

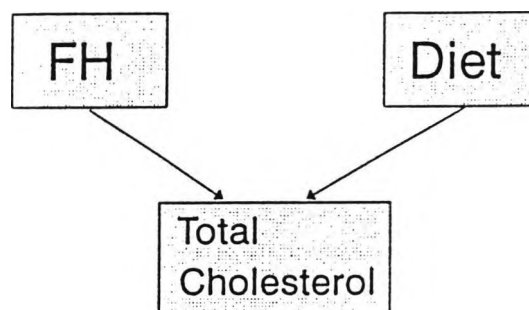


Figure 3.1 - A simple causal network

Figure 3.2 extends this representation including: 1) discrete states for each of the nodes, 2) *a priori* distributions on the root nodes representing the population distribution of dietary fat and genetic disorder, and 3) a distribution on the total cholesterol node derived from: a) conditional probabilities describing the relationship between the three nodes, and b) the *a priori* values of the root nodes. The resulting

distribution is calculated using a probabilistic inference mechanism, as described in section 3.3.3.

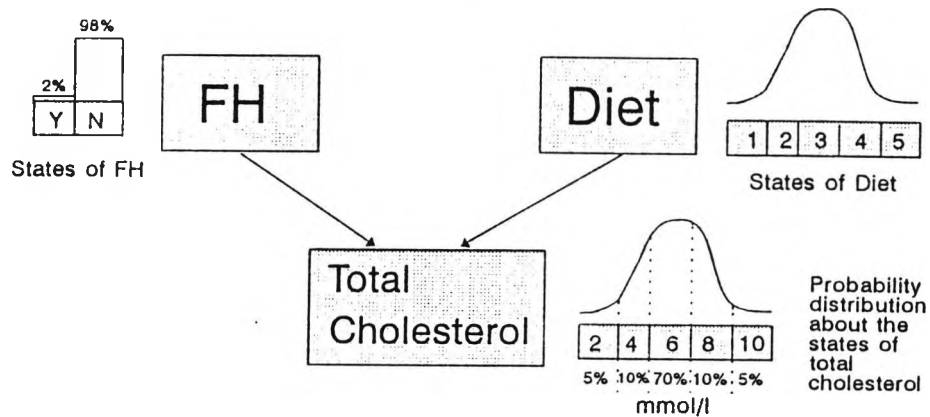


Figure 3.2 -A simple causal network illustrating states and distributions

Possible purposes of this model might be: a) to prognose changes in the total cholesterol level for different genetic disorders and dietary regimes, and b) to diagnose whether the genetic disorder was present in an individual, given their dietary regime and their total cholesterol concentration. To represent this knowledge using a rule-based system would require rules for prognosis and diagnosis of the form:-

Prognosis

If FH=yes, then total cholesterol = high

If Diet = moderate, then total cholesterol = moderate

Diagnosis

If total cholesterol = high, then FH =yes

If total cholesterol = moderate, then diet= moderate

Inherent in this structure are inconsistencies of representation. For example, if the dietary cholesterol is high, then the rule based approach would follow a line of reasoning such that:-

1) Prognosis rules would fire, i.e.

Dietary cholesterol = high, then total cholesterol= high

2) Diagnostic rules would fire, i.e.

Total cholesterol= high, then FH = yes

This is an erroneous inference since the high dietary cholesterol should provide a reason for the high total cholesterol concentration and hence make the presence of the genetic disorder less likely. This is a feature of probabilistic reasoning is known as "explaining away" (Pearl, 1988b). Causal probabilistic techniques prevent inconsistencies in reasoning by conditioning all inferences on the context in which they occur. Potentially, this can result in a large number of computations for each update, however, statistical inference techniques have been developed to limit this computational complexity. These are discussed in section 3.3.3.

A CPN representation of the above problem would result in prognosis and diagnosis as follows:- If we knew that the patient had FH and was on a very low saturated fat diet, then it would be possible to predict the total cholesterol concentration as shown in figure 3.3.

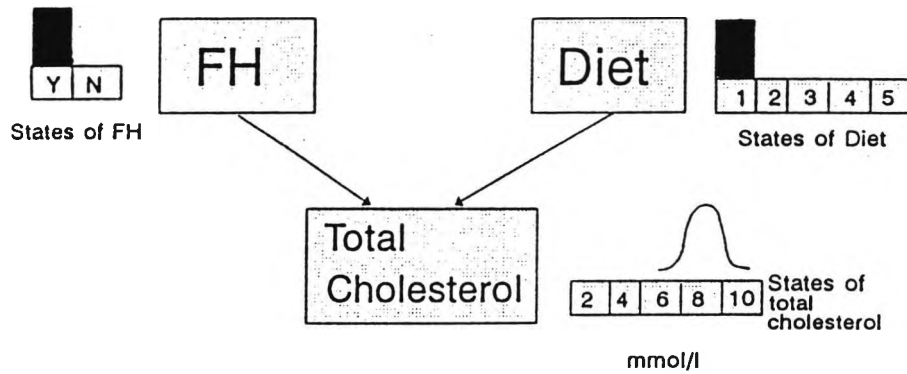


Figure 3.3 - A simple causal network illustrating an instantiation

If the patient's underlying genetic disorder was not known, but it was known that they were on a very low saturated fat diet and had a high cholesterol level, then the only explanation for this high cholesterol level would be that the genetic disorder was present, as indicated in figure 3.4 .

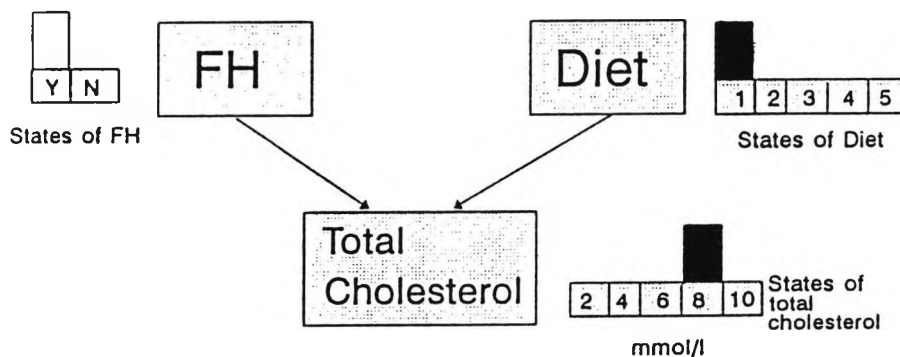


Figure 3.4- instantiating diet and total cholesterol level

In contrast, if the individual was on an increased saturated fat diet for the same high level of cholesterol, then this dietary regime would partly explain the increase in total cholesterol concentration. Hence, there would be less certainty as to the genetic state of the individual, as illustrated in figure 3.5.

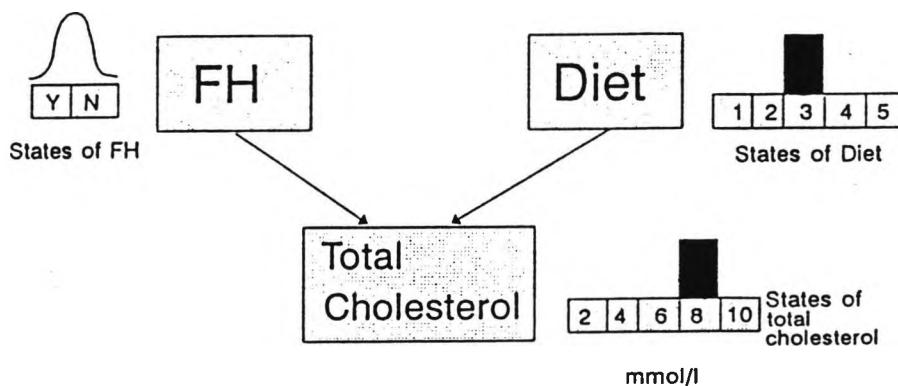


Figure 3.5 - Instantiation of diet and total cholesterol level

The above example illustrates the concept of marginal independence, i.e. the level of dietary saturated fat and the genetic disorder are independent until the value of total cholesterol concentration is known, at which point they become dependent so as to explain this value of cholesterol. Under these circumstances dietary saturated fat and genetic disorder are said to be marginally independent of each other.

3.3.2.2) Example 2- Introducing Expert Opinion into the Causal Structure by Representing Relevance and Irrelevance.

Extending the previous example, one might assume that if nothing were known about an individual's dietary regime, then a measure of their lifestyle or social class might indicate their most likely diet. This can be represented causally, as shown in figure 3.6.

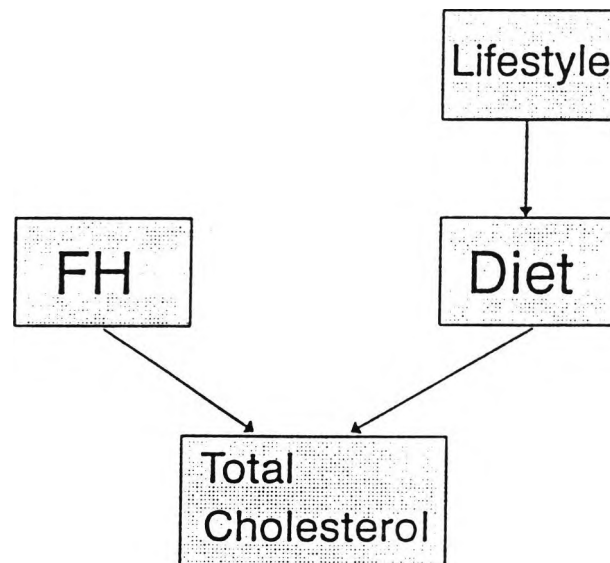


Figure 3.6 - The CPN extended to include lifestyle

The important point about this diagram is that relevance and irrelevance are explicitly encoded into the model by structuring the causal links in this way. Lifestyle becomes irrelevant to the individual's total cholesterol level once information is available on their dietary regime. In Bayesian statistics this is known as "conditional independence", i.e. an individual's total cholesterol concentration and their lifestyle are conditionally independent given their dietary regime, or more formally :-

$$P(\text{Total cholesterol} | \text{Diet, Lifestyle}) = P(\text{Total cholesterol} | \text{Diet}) \quad (3.1)$$

By using conditional independence, qualitative knowledge concerning metabolic processes is built directly into the model structure. This is particularly useful in the domain of lipid metabolism. In this field, data describing the whole metabolism are extremely limited. Instead, data are available describing small, localised areas. By representing the metabolism using a qualitative structure of metabolic processes derived from expert knowledge, i.e. utilising the irrelevance information available, data describing localised relationships can be used to full effect. As Pearl (1988b) notes "An important feature of the network representation is that it permits people to express directly the fundamental, qualitative relationships of direct influence; the

network augments these with derived relationships of indirect influence and preserves them, even if the numerical assignments are just sloppy estimates."

3.3.3 Propagation of evidence

Propagation of evidence in a CPN is via conditional probability tables, which describe the relationships between variables in the model. Using these conditional probabilities the probabilistic inference mechanism updates the model as new evidence presents. The generation of conditional probability tables is described in section 3.4.2. In this section, inference mechanisms are described, from Bayes' theorem through to more complex representations of inference which reduce some of the computational complexities associated with modelling using causal probabilistic networks.

3.3.3.1 **Bayes' inversion formula**

Propagation of probabilities between the nodes is possible because of Bayes' inversion formula which states:-

$$P(H|e) = \frac{P(e|H) P(H)}{P(e)} \quad (3.2)$$

Pearl (1988b) describes this formula as stating "that the belief we accord a hypothesis H upon obtaining evidence e can be computed by multiplying our previous belief $P(H)$ by the likelihood $P(e|H)$ that e will materialise if H is true. $P(H|e)$ is sometimes called the posterior probability (or simply posterior) and $P(H)$ is called the prior probability (or prior). The denominator $P(e)$... hardly enters into consideration because it is merely a normalising constant"

As Andreassen et al. note (1991b), in the medical domain it is often a simple process to estimate $P(e|H)$ or the probability that symptoms exist given a disorder or set of disorders, and it is these estimates which are used to construct the conditional probability tables which describe the relationships between nodes. This is one of the strengths of the technique during knowledge acquisition.

Prior probabilities are required for root nodes and may be of many different styles' uniform, improper etc. The probabilities on root nodes, when updated, can be used to represent the new belief in the underlying disorders associated with an individual. As Pearl notes (1988b) this new distribution "completely summarizes past experience", and can therefore be used to learn more about the patient's diagnostic state during management. This feature of CPNs has been used to great effect in DIAS (Andreassen et al., 1990), a probabilistic model of glucose metabolism, where individual heterogeneity of insulin sensitivity amongst the diabetic population has been represented by a sensitivity node. This node is a root node, its prior distribution being modified according to the results of insulin treatment over a period of time. The posterior distribution of insulin sensitivity can then be used as the prior for this node the next time the model is used for that patient. This is because the distribution on this node represents a summary of the evidence concerning the individual's sensitivity to insulin inferred from this patient's data collected to date. In this way insulin treatment can be adjusted to a level more specific to the individual's diagnostic state.

3.3.3.2 Computational overhead associated with Bayes' formula

Propagating evidence in intensional systems i.e. conditionally across the entire network, requires a far greater number of calculations than in extensional systems where rules are defined and used regardless of the situation in which they present. In effect the joint distribution table is required which describes the probability of every situation in the entire network.

This overhead is reduced by including conditional independence relationships in the network which automatically limit the number of possible states, due to the representation of irrelevancies. Pearl (1988b) has expressed the joint distribution in terms of the chain rule where "if we have a set of n events, $E_1, E_2, \dots, E_n$, then the probability of the joint event $(E_1, E_2, \dots, E_n)$ can be written as a product of n conditional probabilities":

$$P(E_1, E_2, \dots, E_n) = P(E_n | E_{n-1}, \dots, E_2, E_1) \dots P(E_2 | E_1) P(E_1). \quad (3.3)$$

The chain rule representation of joint probability may be simplified, removing those states which conditional independence assumptions prove redundant.

This rule represents the joint distribution as the product of local dependencies expressed in the graph. Pearl (1986) argues that representing probabilities locally is more natural to human reasoning, i.e. "the elementary building blocks which make up human knowledge are not the entries of a joint-distribution table but, rather, the low order marginal and conditional probabilities defined over small clusters of propositions". The ability to represent knowledge as local representations is particularly useful in the domain of lipid and lipoprotein metabolism where much of the clinical literature consists of correlations performed between lipid variables describing small subsections of the metabolism.

However, even with the ability to represent dependencies in causal networks, the inference problem is still computationally time-consuming. This computational problem, and further problems associated with representing loops in causal structures have led to the development of statistical techniques which aim to reduce the computational complexities of probabilistic inference. In the next section three of these techniques are described, i.e. a) Pearl's message passing algorithm, b) Stochastic simulation, and c) Lauritzen and Spiegelhalter's method of updating probabilities by local computations. Greater emphasis is placed upon the third technique since it is the basis of the inference mechanism used in HUGIN (Andersen et al., 1989), the

modelling tool used to construct the model of lipid metabolism. An understanding of the technique i.e the formation of cliques, and the density of network construction, is important when looking at model implementation in chapters 5 and 6.

3.3.3.3 Pearl's message passing inference mechanism

Pearl (1988b) describes the architecture of a probabilistic inference technique known as message passing. The technique involves passing messages between nodes along causal links, and prevents evidence instantiated in the network from being used more than once when updating belief distributions at the nodes.

Pearl (1988b) describes this inference method when implementing: causal trees, where "every node except the one called root has exactly one incoming link"; trees where each node has only one parent but may have more than one child; and causal polytrees where each node may have more than one parent and child but the network remains singularly connected i.e. there are no situations in which there is more than one path between two nodes.

In the simplest case the formula for updating the belief in any node x , $BEL(x)$, when new evidence has been found on y is

$$BEL(x) = \alpha P(x) \lambda(x) \quad (3.4)$$

Where α is a normalising constant adjusting the sum of the distribution to 1, $P(x)$ is the prior probability and

$$\lambda(x) = M_{y/x} \quad (3.5)$$

i.e. the row of the conditional probability table M consistent with the instantiated value of y . Pearl generalises this to the situation of a series of nodes linked as a chain, i.e.

If

$$\rightarrow U \rightarrow X \rightarrow Y \rightarrow$$

is a causal chain of linked nodes, then

$$\lambda(x) = M_{y/x} \cdot \lambda(y) \quad (3.6)$$

Such that the update of the belief on x becomes dependent on the prior and conditional probabilities of y and its parent.

Pearl introduces the notion of bidirectionality of propagation, allowing evidence propagation from either end of the chain. In this case the belief function changes such that the prior probability $P(x)$ is represented as a matrix $\pi(u)$, similar to that of $\lambda(y)$. Inference is performed as in the unidirectional case.

The overall belief function, BEL, in a causal chain with bidirectional update is represented as

$$BEL(x) = \alpha \lambda(x) \pi(x) \quad (3.7)$$

For trees, Pearl further expands this theory to include cases where more than one π message needs to be generated from each parent, so that it may propagate evidence to its many children. Pearl also expands this theory to the situation of causal polytrees. This is, however, outside the scope of this section which aims to illustrate the principles of several probabilistic inference mechanisms.

Pearl (1988b) describes methods for representing loops which otherwise cause erroneous inference due to circling of evidence in the network. These methods are clustering, conditioning and stochastic simulation.

Clustering is a method whereby the nodes in the loop are collapsed to form one

supernode, where a belief table and forward and reverse message passing matrices are stored for the supernode. Beinlich et al. (1989) comment on the performance of this method of loop removal noting that "As all possible combinations of propositions from individual nodes must be represented in the supernode, only very small local loops can be handled by this method."

Stochastic simulation (Pearl, 1988b), is "a method of computing probabilities by counting how frequently events occur in a series of simulation runs".

Conditioning (Pearl, 1988b) "breaks the message-passing cycle by assuming one node in a loop as being observed". "In a network with multiple loops each loop must be interpreted in such a fashion. The result is a set of nodes, the loop cutset, that renders a network singly connected when assuming the cutset-nodes are observed". According to Beinlich et al. (1989) this technique "works moderately quickly with a small number of loops".

A specific form of probabilistic inference using clustering, in this case combining nodes into groups called cliques, has been developed by Lauritzen and Spiegelhalter (1988) (see section 3.3.3.5). This inference mechanism has been used by Jensen et al. (1990) as the basis for probabilistic inference in the Hugin tool (Andersen et al., 1989), a tool which has been used to develop the models of lipid transport described in chapters 5 and 6.

3.3.3.4 Stochastic simulation

Stochastic simulation can be used as a probabilistic inference method, where probability distributions are generated from simulation runs. Unlike inference mechanisms based upon clustering, no cliques or supernodes are formed. This makes it a more appropriate inference mechanism for use when the network is tightly connected or when a large number of loops are present, since when this occurs, cliques will be large and hence the network will propagate evidence more slowly.

Pearl (1986) has commented upon the use of sparse networks (i.e. those with few links between nodes) as a natural representation for human knowledge. This assumption has however, been questioned by Hand (Comment in Lauritzen Spiegelhalter, 1988) who notes that "many problems match a broad shallow network with many parents for each child". Shwe and Cooper (1991) have used a stochastic simulation algorithm when implementing a two level diagnostic model which is tightly connected.

3.3.3.5 The Lauritzen-Spiegelhalter method

In the landmark paper from Lauritzen and Spiegelhalter (1988) the authors describe an inference mechanism for causal probabilistic networks which "exploit(s) a number of different representations of a joint probability distribution, each representation being *local* in the sense defined by the topology of the graph. Fast algorithms for transferring between the representations allow efficient means of calculating conditional and marginal distributions for specified parts of large sparse networks"

As discussed in section 3.3.3.2 local representations of conditional probability are useful as it is relatively easy to estimate the conditional probability tables of local events compared with joint distributions. Lauritzen and Spiegelhalter use these local representations of the joint distribution to full effect limiting propagation in the network to that between groups of nodes known as cliques.

The authors describe the process of converting a causal structure and estimated conditional probability tables into a "simple function of ... individual marginal distributions on.. cliques", the reason for this being that "storage of clique marginals makes retrieval of probabilities of single nodes trivial, after each batch of evidence has been processed". This procedure requires three local representations of the joint distribution function in terms of: evidence potentials on the cliques, potentials from set chains, and individual marginal distributions on each of the cliques.

The conversion of the joint probability distribution into local representations is in four steps as described in Lauritzen and Spiegelhalter (1988).

1) The joint probability distribution is converted into a series of conditional probability statements using the chain rule (see equation 3.3) which is reduced by including irrelevance represented in the network.

2) These conditional probability statements are converted into evidence potentials by removing the conditionality of the statement

e.g. $P(a|b) = \mathcal{U}(a,b)$ (3.8)

This is possible because $P(a|b)$ is proportional to $\mathcal{U}(a,b)$. In converting conditional probability statements into evidence potentials, the directionality of the causal links must be dropped, and the parent nodes married, see for example figure 3.7

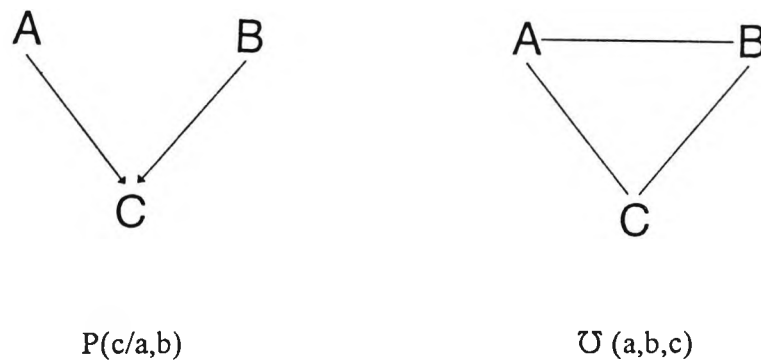


Figure 3.7 - A moralised network

The resulting graph is referred to as moralised.

3) The moralised graph may however, still have more than one pathway between two nodes and this can result in a lack of coordination in evidence propagation (Jensen et al., 1990). The graph is therefore "triangulated" so that (Lauritzen and

Spiegelhalter, 1988) "there are no cycles of length 4 or more without a *chord* or short-cut". Methods for this are known as filling in, and Lauritzen and Spiegelhalter (1988) describe the maximum cardinality search method.

By calculating evidence potentials from conditional probability tables, and structuring the triangulated groups of nodes correctly, inference mechanisms become simpler, as shown in step 4, below.

4) The groups of nodes collected in each of the triangulated evidence potentials are known as cliques. Lauritzen and Spiegelhalter structure these into a tree using the "running intersection property", such that cliques that are linked share common nodes, or more formally (Lauritzen and Spiegelhalter, 1988) "nodes of a clique (C_i) also contained in previous cliques ($C_1, \dots, C_{i-1}$) are all members of one previous clique".

This produces yet another local representation of joint distribution. That is the $P(\text{Residual}/\text{Separator})$ for each clique, the separator nodes being those common amongst the cliques (from the running intersection property). In this way the marginal distribution on each clique can be calculated from

$$P(\text{Clique}) = P(\text{Clique Residual}|\text{Clique Separator}) * P(\text{Clique Separator}) \quad (3.9)$$

where $P(\text{Clique Separator})$ is derived from updating the parent clique's marginal distribution and then summing the clique marginal across the separator node.

Using these local representations Lauritzen and Spiegelhalter (1988) describe how each of the following is performed, i.e. *initialisation* of the network, *absorption of evidence*, *global propagation* of the evidence absorbed, *hypothesising and propagating single items of evidence* (i.e. performing what if type queries), *planning*, and *influential findings*, i.e. by retracting certain evidence we may judge its influence, can be performed on the network.

The performance of this inference mechanism is dependent upon the complexity of

the graph. For example, Beinlich et al. (1989) comments that "the complexity of evidence propagation using this algorithm is linear in the number of cliques and in the size of the largest clique used in the network". The effects of clique size on propagation time are illustrated section 5.6.3.1 where the performance of the network constructed in chapter 5 was greatly enhance by reducing the number of states in the largest clique, and hence the size of this clique.

3.4 Model Development Using CPNs

3.4.1 Representing lipid and lipoprotein metabolism

Section 3.2 described why it is appropriate to use an intensional modelling approach when supporting complex treatment decisions, that is, so as to represent an individual's state and the effects of treatment upon this state. In section 3.3 one approach to intensional modelling, i.e. causal probabilistic networks, was presented.

It would be preferable, when modelling the effects of treatment on patient state, to construct a dynamic model of the whole of lipid and lipoprotein metabolism. It would then be possible to examine the changing patterns of flow in the system after perturbation. Dynamic systems have been modelled using CPNs, feedback being included in the model by using repeated time slices of the same CPN, thus avoiding loops (Andreassen et al., 1990). However, the construction of a dynamic model of the whole of lipid and lipoprotein metabolism is an enormous task. This is illustrated in chapter 6 where studies are referenced describing the modelling of apo B metabolism. Often 10-15 compartments are required to describe fully the metabolism of this single, albeit significant, lipoprotein.

At the present time the author knows of no composite compartmental model describing the whole of lipid and lipoprotein metabolism. Indeed the literature in the

lipid field is characterised by numerous studies examining small subsections of the lipid and lipoprotein metabolism, either modelling compartmentally individual apolipoprotein and lipid fluxes in the plasma, lipid excretion in the bile etc., or correlating concentrations of lipid, enzyme, etc with metabolic fluxes.

The problem presents, how can a wealth of information describing small subsections of lipid and lipoprotein metabolism be combined with expert opinion on physiological mechanisms to construct models representing patient context, these models having potential for use in decision support. The approach taken in this thesis is to use expert opinion in the definition of a CPN causal structure describing the lipid and lipoprotein metabolism, and literature data to represent the relationships within this causal structure. These relationships are statistical correlations between adjacent variables in the causal structure, and are then converted into conditional probability tables for use in CPN models, as described in the next section.

Obviously, such a model is unable to describe the dynamics of lipid and lipoprotein metabolism. However, in the management of lipid and lipoprotein disorders, one is not concerned with short term perturbation in a patient's lipid levels. Unless severe pancreatitis exists, the problem being addressed is the long term shift in metabolic control from one steady state to the next, and the assessment of CHD risk at this new state, CHD being a long term progressive disorder. This is in contrast to the case of diabetes where apart from long term control, good management requires control of short term perturbations in glucose metabolism which otherwise cause hypo or hyperglycaemia.

3.4.2 Techniques to Estimate Conditional Probability Tables for the Representation of Steady State models.

3.4.2.1 Introduction

Much of the literature describing CPNs has been concerned with inference

mechanisms, addressing the problems of computational complexity associated with Bayesian probabilistic inference (see section 3.3.3.1). The literature has been concerned to a much lesser extent with the estimation of the conditional probability tables.

In many cases expert opinion is the only source of probability estimates. Pearl (1988b) argues that this may not be a problem due to the robustness of the methodology as a reasoning tool, and that "in many applications the exact values of the parameters play only a minor role; most of the knowledge needed for reasoning plausibly about a domain lies in its structure". Indeed Pearl (1988b) argues that one of the necessary attributes of the Bayesian approach is the "willingness to accept subjective opinions as an expedient substitute for raw data". Recent work by Spiegelhalter and Lauritzen (1989) should enable the adaptation of conditional probability table estimates during the management process, meaning that the accurate estimate of conditional probabilities initially is less urgent.

In many situations, however, information describing relationships in a model might be available not only from the clinical expert but also from the clinical literature. The accuracy of conditional probability estimates could potentially be improved by the incorporation of the results of literature studies. Cheesman (Comment in Lauritzen and Spiegelhalter 1988) questions the use of expert opinion as a good source of estimates for conditional probability tables. He asks the questions "where did the expert get the numbers?", and "does the expert have sufficient experience to justify all those numbers, or is he making them up?". Certainly when a new drug is released clinicians will often base their initial assessment of its usefulness on literature reports of its efficacy, rather than clinical experience of its use, i.e. expert interpretation of literature results.

As outlined in Chapter 1, the problem lies in incorporating the clinician's opinion on the structure of metabolic process and the clinical effectiveness of treatment, with literature data. It has been suggested by Havranek (Comment in Lauritzen and Spiegelhalter, 1988) that "In general, linking expert knowledge with knowledge

obtained from data can be very dangerous". However, expert knowledge consists of both experience and that knowledge obtained from experimental data, as illustrated above in the example of a new drug, where the clinician's opinion as to its efficacy will be based to a large extent on literature reports. An expert system which relies solely on expert judgement, either to define rules or estimate conditional probability tables, could then, be seen as including information from literature data implicitly. Having a traceable method by which expert opinion and clinical data can be combined and tested for consistency must therefore be an advantage. As Lauritzen and Spiegelhalter (1988) note "we agree that linking expert knowledge with knowledge obtained from data *can* be very dangerous, we also feel that developing appropriate methodology to do so is one of the most vital challenges for future statistical/artificial intelligence research. *Not* combining knowledge from both sources would be even more dangerous".

In a CPN representation, the clinician's expert opinion can be included in the structure as relevance and irrelevance assumptions (see section 3.3.2.2), with local relationships generated from literature data. Apart from the more general benefits to clinical decision making of constructing an intensional model for use in patient management, modelling in this way can have the following advantages :-

- it can illustrate consistency within the literature data across different studies, in different areas of metabolism, and between different research teams. (For an illustration of consistency checking of the models constructed see chapter 7 and the contributions of this thesis described in chapter 8).
- it enables identification of research areas which require further study, i.e. model relationships for which little data exist.
- it incorporates clinical and experimental data in the patient management process.

To use data from different studies in order to formulate relationships requires that the data are comparable. However, it is often the case that data reported in the literature cannot be immediately compared, due to bias in experiments, sample size, etc. It is necessary then, to have techniques for : a) adjusting the data from different studies to make them comparable and b) generating relationships between variables for these adjusted data.

Meta-statistical analysis is a branch of statistics which has addressed the issues of combining data from different studies, e.g, adjusting these data for bias in the individual studies, so that they are comparable. Section 3.4.2.2 describes meta-statistical analysis, whilst section 3.4.2.3 describes a particular method of meta-statistical analysis known as "meta-statistical analysis using the confidence profile method" (Eddy et al., 1992). This method represents the results of meta-statistical analysis using confidence profiles which can then be automated using CPNs (called influence diagrams in the methodology).

3.4.2.2 Meta-statistical analysis

Meta-statistical analysis is the technique whereby the results of similar studies can be pooled to infer an average effect or outcome for the group of studies as a whole. It can be performed in three stages: a) Problem definition, where the scope of the analysis is set, b) examination of heterogeneity within the data, where explanations are sought for differences in the data, and 3) modelling of the studies, where a meta-analytic technique is chosen and applied. The following discussion illustrates these stages in more detail.

1) Problem definition - i.e. the scope of the meta-analysis, and the selection of the most appropriate level for exploration of heterogeneity in the data.

Before embarking on a meta-analysis the reviewer should be clear as to the question

of interest, as this will determine the most appropriate level for exploration of heterogeneity within the available data (Light, 1987). Light (1987) illustrates how different reviewers will be interested in different questions and hence, different analysis of the same data. He notes that "policy makers" will be interested in questions of "average effect", for example, 'is the lowering of cholesterol beneficial in the population?', whereas "Researchers", will be more interested in "where and with whom is a particular treatment particularly effective or ineffective", for example, 'in whom is cholesterol reduction most beneficial?'. Clearly the researcher is interested in a far more detailed analysis of the available data, finding the effects of treatment in homogenous groups within the whole population rather than the average effect of treatment.

This thesis deals with the management of patients at high-risk of CHD, and how differences in subgroups of this high-risk population make treatment options more or less applicable. The differences between patients which are relevant to treatment selection are, therefore, modifications in lipid and lipoprotein metabolism. Modelling this metabolism should therefore be at a level which distinguishes between patient subgroups within the high-risk band, this being the most appropriate level of heterogeneity to represent.

The goals of the meta-analysis will, in part, determine the studies selected for use in the analysis. Pocock (1993) describes the three most important factors when selecting studies for use as "breadth, quality and representativeness". Breadth refers to the selection of studies that are appropriate to the question of interest. Quality refers to selection of studies that adhere "to recognised criteria of acceptability (e.g. randomisation, appropriate blinding, analysis by intention to treat)". Pocock (1993) notes that "any relaxation of such standards requires careful consideration as to whether the consequent precision of more data is counter productive given the increased potential for bias and loss of credibility". Good representation requires study selection which accurately reflects those studies performed. Publication bias, which occurs when only published studies are included in the meta-analysis, has been reported to increase inaccuracies in the analysis because of poor representation (Begg,

1985). Published work may not reflect the truth as it often contains only those studies which report significant results.

Model construction in chapters 5 and 6 has been based upon expert opinion and data from published studies. Due to the detailed level of modelling very few studies exist for each of the local relationships modelled. It is inappropriate, therefore, to use rigid selection criteria omitting studies. Similarly there is unlikely to be any publication bias as, when only a few studies exist in a high profile field, such as lipid metabolism, it is likely that even studies with rather poor results would be published.

2) Exploration of heterogeneity by graphical or statistical techniques.

As noted previously, the most appropriate level of data exploration depends upon the purpose of the study. For example, if the aim of the meta-analysis is to define policy, then heterogeneity within the data is of little interest. In this situation a random effects model might be used to pool the data. This model includes uncertainty due to heterogeneity as a random effect which is included in the confidence interval describing the pooled outcome. One of several outcome measures can be used to represent the pooled results including effect size, relative risk, sample difference etc.

If the heterogeneity in the data is relevant to the question at hand then the data need to be explored for such differences. Heterogeneity can be due to either physiological or clinical differences between individuals in the studies, or bias in the studies (for example, methodological differences). Meta-statistical analysis using the confidence profile method (Eddy et al., 1992) (see section 3.4.2.3) provides formulae for the explicit representation of bias between studies. To test for heterogeneity due to differences in study populations both graphical and statistical methods are available.

Methods for graphical examination of heterogeneity are given by L'Abbe (1987), Whitehead et al. (1991) and Light (1987). Light (1987) notes that if study outcomes are graphed, plotting outcome on the X axis and their frequency on the Y axis, then

"if treatments in several studies are really similar, the graph should be well behaved. It should not be especially skewed or asymmetrical. Ideally, it should look approximately like a normal distribution, suggesting that differences amongst findings are basically due to sampling error. If outcomes look grossly irregular, a reviewer must question whether all studies come from the same population. For example, a bimodal distribution would be a first indication that a group of studies should not be combined in too facile a way; there might be two underlying populations. The challenge for a reviewer is then to identify the factors that divide the studies into two groups".

An illustration of graphical examination of heterogeneity can be seen in section 6.4.2.4. In that section the relationship between the VLDL apolipoprotein B cascade synthesis and the LDL apo B cascade synthesis was examined for different classes of genetic disorder within the study groups. This example illustrates how raw data can be very useful when exploring heterogeneity, allowing division of patients by groups which the reviewer thinks might provide further information. Thompson (1993) "in the context of clinical trials" investigated the heterogeneity in reducing CHD risk by cholesterol reduction, by using logistic regression and information on the individual's "extent and duration of cholesterol reduction".

Fleiss (1993) and Whitehead (1991) describe a statistic for testing the heterogeneity of study outcomes when combining data using the fixed-effects model (see below), i.e.

$$Q = \sum_{c=1}^c W_c (Y_c - \hat{Y})^2 \quad (3.9)$$

where

Q = weighted sum of squares of deviations

C = total number of studies

Y = outcome measure

W = reciprocal of variance

However as L'Abbe notes (1987) "the power of these statistical tests for homogeneity

is often low because most meta analyses pool the results of a very limited number of individual studies; investigators should resort to informed subjective judgement and examine a graphic display of study outcomes for homogeneity when formal tests fail to reject the homogeneity assumption".

Further heterogeneity might be due to bias in study methodology, study selection etc. If possible it is beneficial to represent biases as one would other forms of heterogeneity, as cofactors in a model describing the outcome. Begg (1985) has illustrated a formal method of calculating publication bias based upon the reviewer's perception of the number of studies performed which remain unpublished in the literature, i.e. the "source population". The confidence profile method (see section 3.4.2.3) describes formulae for the representation of different biases, as part of influence diagrams relating the health technology to the outcome.

3) Modelling Using Meta-analytic Techniques.

Two standard techniques are available for pooling data across a summary outcome measure. These are the fixed and random effects models. The fixed effect model pools data based on the assumption that homogeneity exists within the study population and that any variation between studies is due to the effects of sampling. The random effects model assumes that heterogeneity exists and this is represented in a confidence profile which is wide enough to explain it in terms of random variation. The assumption taken when using the random effects model is that the question being asked is broad enough so that random variation need not be explained.

The fixed effect model usually produces confidence limits for the pooled outcome that are narrower than that of the random method, although when the results of both methods produce similar confidence limits then it is likely that sampling is the only cause of heterogeneity.

Fliess (1993) illustrates the formula for pooling data using the fixed effect method

i.e.

$$\hat{Y} = \frac{\sum W_c Y_c}{\sum W_c} \quad (3.10)$$

Where $\hat{Y}$ = mean effect produced by the meta-analysis
C = total number of studies
Y = "generic measure of experimental intervention"
W = reciprocal of variance

where the standard error is

$$SE(\hat{Y}) = (\sum W_c)^{-1/2}$$

3.4.2.3 The confidence profile method

In this Confidence Profile methodology Eddy et.al. (1992) have combined the meta-statistical analysis and influence diagram techniques into a single approach (and a computer package).

The confidence-profile method enables the results of meta-analysis to be represented in an influence diagram. This is done by representing the results of each experiment as a likelihood function and then combining these likelihood functions. A different likelihood function is derived for each experiment depending upon the nature of the experiment, i.e. the outcome measure used -dichotomous, categorical, count, or continuous; and the experimental design -one-arm prospective, two-arm prospective, n-arm prospective, 2*2 case control, 2*n case control, matched case-control and cross sectional. Different likelihood functions are required because various types of experiments and outcome measures will produce different shapes of confidence profile distributions. For example, in an experiment with a dichotomous outcome Eddy (1992) illustrates that "if the outcomes are independent and identically

distributed, the likelihood function is derived from the binomial distribution"

$$L(y|\theta) \propto \theta^s (1-\theta)^f \quad (3.11)$$

where

s = number of successes

f = number of failures

θ = outcome parameter, in this case the "true probability of success"

y = evidence

The overall likelihood function for the combined effect of different studies (**L**) can then be defined as the product of the separate likelihood functions.

The confidence profile method allows these likelihood functions to be redefined so that heterogeneity can be represented as cofactors in the function. It also provides formulae for the explicit representation of numerous types of study bias in model formulation.

By linking separate meta-analytic studies as "chains", Eddy et al. (1992) illustrate that studies relating health technologies to intermediate outcomes can be combined with those relating intermediate outcomes to the health outcome of interest. In this way the health outcome of interest can be modelled in terms of a combination of studies at different points in the chain and health policy can be defined from the effects of health technologies on the final health outcomes.

3.5 Strategy for model creation using CPNs and the application of meta analytic techniques.

The literature describing the field of hyperlipidaemia and its effects on CHD is split into two groups:-

- a) Large studies describing the whole population and,
- b) Small studies describing the effects of metabolic perturbation in individuals with specific disorders.

Meta-statistical analysis on population studies has been performed to test the lipid hypothesis, i.e. that cholesterol reduction is beneficial to a reduction in overall CHD risk in the population (Thompson, 1993). Testing this hypothesis aims to define policy on cholesterol reduction in the population, which is useful in screening. However, this population is not the same as that entering a specialised lipid clinic. Patients in the lipid clinic will have already been classified as high-risk relative to the population, and as such are much more likely to have genetic disorders of their lipid and lipoprotein metabolism. These disorders mean that their response to treatment and their progression of CHD will not be the same as the normal population, and are therefore likely to be outliers in the population studies.

In managing the individual at high-risk of CHD relative to the population we are very much concerned with the question "what treatment is appropriate for this individual". The level of heterogeneity studied should therefore be that which allows us to distinguish between individuals in the high-risk group, who often have complex patterns of primary and secondary disorders of the lipid metabolism, increasing their risk of CHD. Meta-analysis of large population-based studies do not provide the level of patient classification necessary to identify this level of heterogeneity.

Smaller, more detailed studies provide information on the action of treatments in very precise circumstances. For example, in reviewing effects of probucol, it was found that the effects of this drug have been reported during heterozygous and homozygous

FH, and unclassified hypercholesterolaemia, with or without plasma exchange, and on differing diets. The effects of this drug in these situations have been reported in terms of changes in the levels of total cholesterol, HDL cholesterol, apoprotein AI, triglyceride, LDL cholesterol, and LDL apoprotein B (Le Lorier et al., 1977; Baker et al., 1982; Yamamoto et al., 1986; Mordasini et al., 1980; Yamamoto et al., 1983; Salel et al., 1976; Reisen et al., 1980; Hunninghake et al., 1980; Yashiro et al., 1990; Durrington et al., 1986., Helve et al., 1988; Meittinen et al., 1986; Felin et al., 1986). Modelling the action of this drug in these numerous situations, in order to predict all of these outcomes, would be extremely complex, and result in a very dense, flat, network as illustrated in figure 3.8.

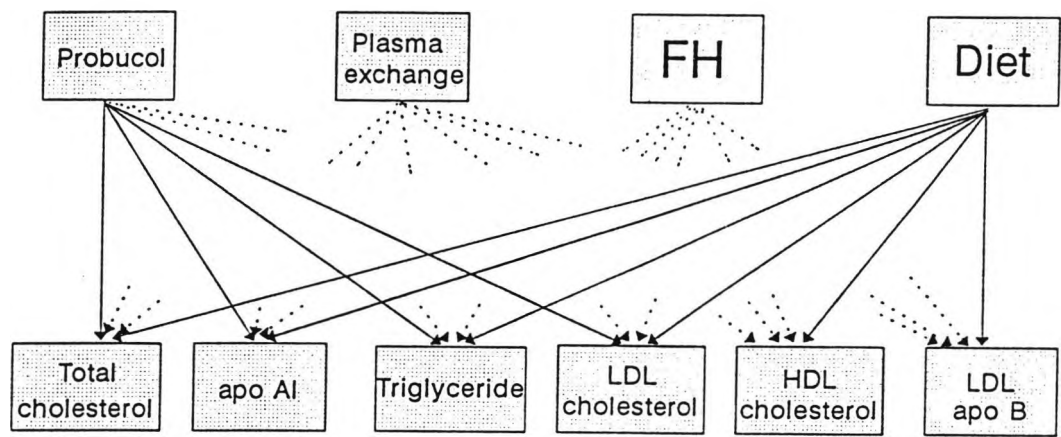


Figure 3.8 - A flat network representation of the action of probucol

Figure 3.8 represents the effects of only one, seldom used drug, without the complicating factors caused by concomitant secondary disorders or other treatment of lipid metabolism. A network describing the complete range of treatments in all these situations would therefore be highly connected.

Many of the outcome nodes illustrated in figure 3.8 are dependent on each other, and result from the overall perturbation of the lipid and lipoprotein metabolism caused by the drug, which in reality only has a few points of action. By modelling the lipid metabolism as a whole, i.e. representing the dependencies between intermediate outcome variables, many advantages occur :- Firstly, the network becomes sparse, and as such more amenable to the probabilistic inference mechanisms available (see

section 3.3.3.5). Secondly, and more importantly, the action of drugs and disorders can be modelled on individual points of action, the network then being used to modify the metabolic picture accordingly. A new sparse network might then be constructed as illustrated in figure 3.9.

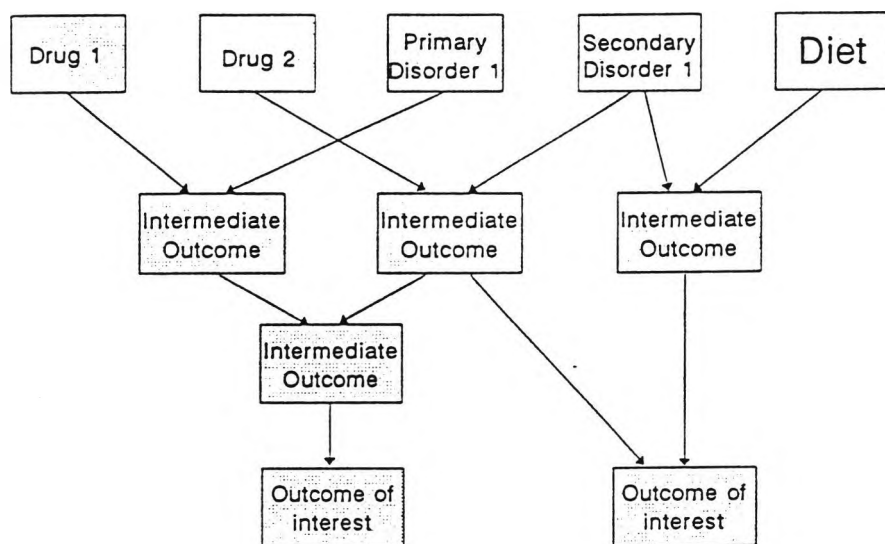


Figure 3.9 A sparse network illustrating the combined action of treatment and disorders

In order to use a model of lipid and lipoprotein metabolism for decision support the model must include variables which describe CHD risk, as illustrated in figure 3.10. By linking these intermediary outcome measures to an estimator of CHD risk, it is possible to predict CHD risk for modification of treatment.

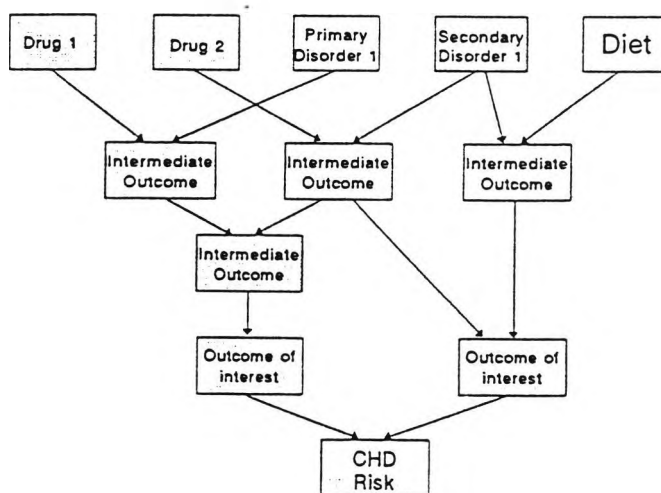


Figure 3.10-Representing CHD risk in the causal network

By linking the CHD risk to a utility node which formulates an individual's utility in terms of : a) potential reduction in CHD risk, b) the most likely side effects of treatment, and c) individual patient preferences, as illustrated in figure 3.11, the "best" treatment can be determined.

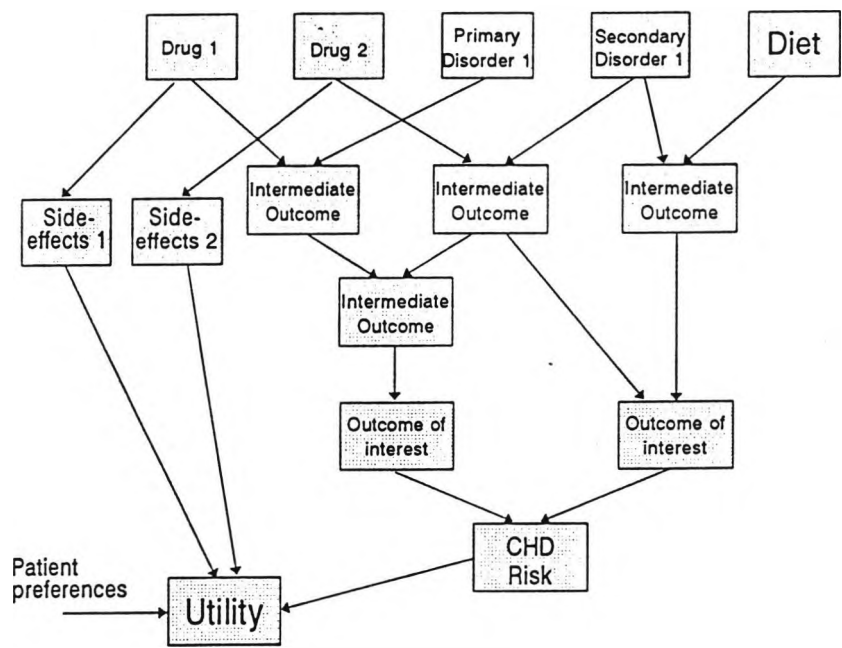


Figure 3.11 - Representing utility in the causal network

The estimation of an appropriate utility function for this circumstance is beyond the scope of this thesis. The issues concerning patient preference toward lifestyle modification are extremely complex, and dependant upon the doctor - patient consultation. However, a decision support tool which enables patient specific prediction of CHD risk and likely treatment side effects should facilitate this consultation and make the eventual decision easier. For a description of the use of utility functions in influence diagram (CPN) applications see Smith (1987), and for a practical application of their use see Andreassen et al. (1990).

Using this strategy enables future predictions to become more patient specific. As extra information is obtained during a patient's visit to the clinic, and instantiated in the model, then the distributions about root nodes will change indicating a new probability distribution for the underlining genetic disorders. By fixing this distribution the model used in the next visit becomes specific to that individual.

3.6 Use of meta-analytic techniques and CPN's to implement strategy

3.6.1 Introduction

In sections 3.1-3.5 methodological techniques have been described which are appropriate to the problem in hand, and a strategy developed for model construction using these techniques. Other techniques for both knowledge representation and model construction are inappropriate for the following reasons:

Rule bases - The use of rule bases is generally extensional. Representing metabolic processes in terms of rules would require a truth maintenance system to preserve the consistency of the inference, as discussed previously. Representation of uncertainty is through truth values bolted on to the rules rather than as a part of the underlying philosophy.

Compartmental modelling - The lack of detailed kinetic data across the whole of the lipid and lipoprotein metabolism prevents the use of these techniques for the construction of a composite model.

Neural networks - These are generally used in situations where large amounts of complete data are available, combined with very little structural information. In these areas modelling the structure of metabolic processes using expert opinion is not required, since sufficient data are available to make the problem one of finding the best relationship between input and output variables. Often this is done by the automatic creation of a node structure which, in the medical domain, may be physiologically meaningless.

Clearly then, the most appropriate techniques for modelling metabolic processes in order to represent patient state, in this situation, are those which :-

- 1) Enable incorporation of both expert opinion on the metabolic processes and literature data describing these processes in model

construction.

2) Enable construction of a model which is capable of predicting CHD risk for different treatments in individuals with different diagnosis.

3) Enable construction of intensional models, preserving the consistency of inference necessary in such a complex domain, and allowing the representation of uncertainty. The necessity to represent uncertainty reflects the limited availability of data, the lack of diagnostic precision in this field, and the necessity to predict treatment results in terms of CHD risk, where risk itself is an uncertainty measure.

4) Enable the construction of models which are usable within a decision making context so that trade offs between potential CHD risk reduction, side effects of treatment and patient preferences can all be built into the decision process.

These conditions can be met using both the causal probabilistic network and some meta-analytic techniques. Section 3.6.2 describes the practical use of these techniques in this thesis, including description of the implementation tools used and practical problems encountered when constructing those models described in chapters 5 and 6.

3.6.2 Implementation of Model Structure and Relationships Using HUGIN and HMM

Having selected the CPN technique as that which is most appropriate for the problems tackled in this thesis, this section illustrates how the modelling tool HUGIN (Andersen et al., 1989), a CPN development environment, can be used to construct models of lipid and lipoprotein metabolism. In doing so examples of models

representing lipid and lipoprotein metabolism are taken from chapters 5 and 6 without considering issues of lipid and lipoprotein metabolism.

HUGIN uses a simplification of the Lauritzen and Spiegelhalter inference method (Lauritzen and Spiegelhalter, 1988) and as such constructs cliques to simplify probabilistic inference. The main differences in inference mechanism between the Lauritzen and Spiegelhalter method and HUGIN are:

1) In HUGIN the tree of cliques, now known as the "junction tree", remains fixed on absorption of evidence, i.e. the graph is not simplified by a re-triangulation process (Oleson et al., 1989).

2) In HUGIN (Comments in Lauritzen and Spiegelhalter, 1988) "each parent clique in the set chain passes evidence to its children through multiplying each term in the marginal distribution of the child by the ratio of new to old probability on the appropriate intersection term". As shown in Jensen et al. (1990) the new belief matrices on a clique can be calculated as the product of the current belief in the clique and the ratio of the new belief in the separator node: the old belief in the separator node.

Jensen et al. (1990) call this "calibration". This calibration algorithm is used in the scheduling algorithms "DistributeEvidence" and "CollectEvidence" as described in Jensen et al. (1990).

HUGIN enables CPN development both interactively and by the generation of a specification file. Both these methods require a description of the node structure, causal links between nodes, conditional probability tables describing these links and, for root nodes, *a priori* distributions.

Models created in this way can be tested using HUGIN's graphical interface, examples of which can be seen in figures 3.11 and 3.12. When the value of a node is "instantiated" i.e. fixed at a state, and the network propagated, the tool

automatically updates the probability distribution associated with the remaining nodes.

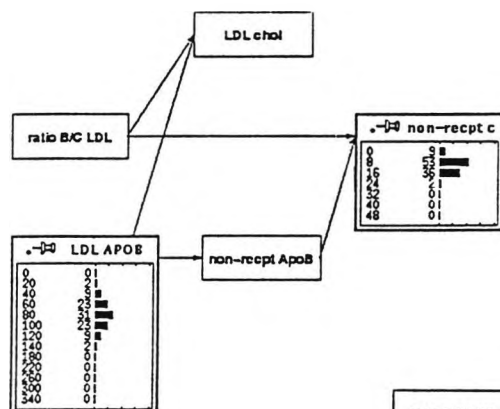


Figure 3.12a

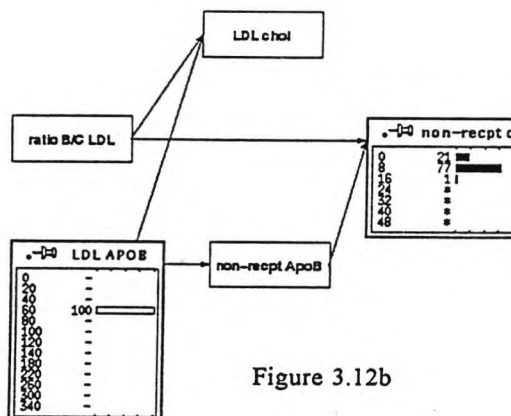


Figure 3.12b

Figures 3.12a and 3.12b illustrate the same network before and after instantiation of node "LDL APO B" (LDL apoprotein B concentration) at state 60 (mg/dl), and propagation. Several of the nodes are "exposed" to illustrate their states and probability distributions about these states. Nodes may either be instantiated to a single value or given a likelihood distribution about a series of states, this representing uncertainty about the instantiated finding.

Recently, an extension to the HUGIN package, HMM (HUGIN Model Module), has increased the simplicity with which specification files might be generated for implementation of CPN models. More specifically, this tool allows definition of simple relationships using "add" and "multiply" functions, plus the definition of more complex relationships using the "tabulated-normal" model. This tool solves the problem of specifying all the elements of large conditional probability tables, for example: if 3 nodes each with 7 states were causally related (two parents one child) then the conditional probability table would include $7*7*7=343$ elements.

The following section gives examples of model construction using HMM and HUGIN using the three types of HMM model.

HMM "ADD" and "MULT" models

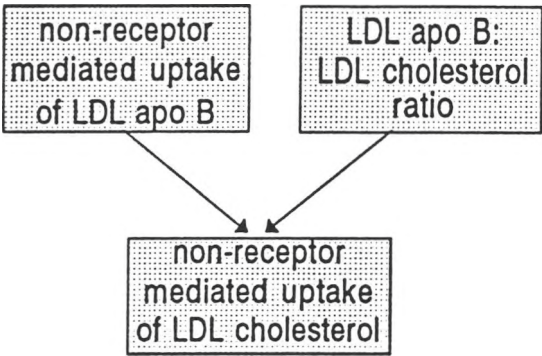


Figure 3.13 - Causal model and code illustrating a "mult" model in HMM for the non-receptor mediated uptake of cholesterol

```

states NONRECB
'0' '4' '8' '12' '16' '20'
states LDLB/C
'0.42' '0.47' '0.52' '0.62' '0.67' '0.72' '0.77' '0.82'
states NONRECHOL
'0' '8' '12' '16' '24' '32' '40' '48'

table LDLB/C
'0.42' '0.47' '0.52' '0.62' '0.67' '0.72' '0.77' '0.82'
'4.7' '9.3' '8.9' '4.7' '18.6' '23.3' '16.3' '9.3'

model NONRECHOL mult 1.0
NONRECB
'0' 0
'4' 4
'8' 8
'16' 16
'20' 20
etc.
LDLB/C
'0.42' 2.38
'0.47' 2.13
'0.52' 1.92
'0.57' 1.75
'0.62' 1.61
etc.
NONRECHOL
'0' 0
'4' 4
'8' 8
'16' 16
'20' 20
'24' 24
etc.

cpn frdtrans
label 'forward trans'
node-width 100
node-height 40
scale 1

node NONRECHOL
label 'non-recpt chol'
coordinates 110 300
states NONRECHOL
parents NONRECB LDLB/C
model NONRECHOL
  
```

The example in figure 3.13, taken from the model constructed in section 5.3.2, illustrates the specification of a multiplication model using the HMM front end to HUGIN. The code illustrated in figure 3.13 is structured as follows: Node states are defined along with tables of *a priori* distributions for root nodes. This is standard regardless of the type of model constructed. A "mult" model is then represented where the states of the first parent node are listed in a column along with the values that these states represent; in the above case these are equivalent. This is then repeated for the second parent and the child as illustrated. To represent division, the

values representing the states of the second parent node are inverted. The remaining code then describes the structure of the CPN linking each node to its states, parents and appropriate model.

The add model "add" uses the same format as the "mult" model with the "model NAME mult 1" being replaced with "model NAME add 0". The constant at the end of these model definitions can be used to alter the relationship by some fixed amount or proportion. Subtraction can be performed by negating the values representing the states of the second parent node.

The "Tabulated-normal" model.

If the relationship is more complex and requires specification of uncertainty then the tabulated-normal model may be used, as illustrated in figure 3.14.

This figure represents the relationship described by the graph in figure 3.15. This graph was sampled for each combination of possible parent states, and a standard deviation estimated for that sample value. The tabulated-normal table was constructed as illustrated in figure 3.14.

The standard deviation included for each sample can be estimated from the plotted relationships. The theoretical basis of the HMM tool is in the representation of extended linear models, a good description of which can be found in both Andreassen (1991), and Egeberg and Schröter (1993).

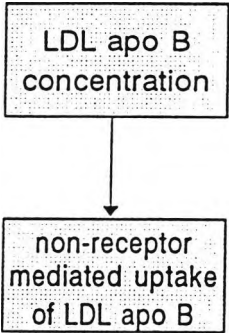


Figure 3.14 - Causal model and code illustrating a "tabulated normal" model in HMM for the non-receptor mediated uptake of LDL apo B

```

states LDLAPOB
'0' '20' '40' '60' '80' '100' '120' '140' '180' '220' '260' '300'
'340'
states NONRECB
'0' '4' '8' '12' '16' '20'

table LDLAPOB
'0' '20' '40' '60' '80' '100' '120' '140' '180' '220' '260' '300'
'340'
0.0019 0.0202 0.0923 0.2295 0.3108 0.2295 0.0923 0.0202
0.0019 0.0001 0.0001 0.0001 0.0001 0.0001 0.0001
model NONRECB
tabulated-normal
(<0 0.1> <1.62 0.1> <3.41 0.2> <4.61 0.4> <6.64 0.5>
<7.28 0.8> <8.5 1.0> <9.63 1.5> <11.66 1.77>
<13.35 2.0> <14.73 3> <15.78 3.5> <16.51 4.0>)

NONRECB
'0' 0 4
'4' 4 4
'8' 8 4
'16' 16 4
'20' 20 4

cpn frdtrans
label "forward trans"
node-width 100
node-height 40
scale 1

node NONRECB
label "non-recpt ApoB"
coordinates 200 100
states NONRECB
parents LDLAPB
model NONRECB
  
```

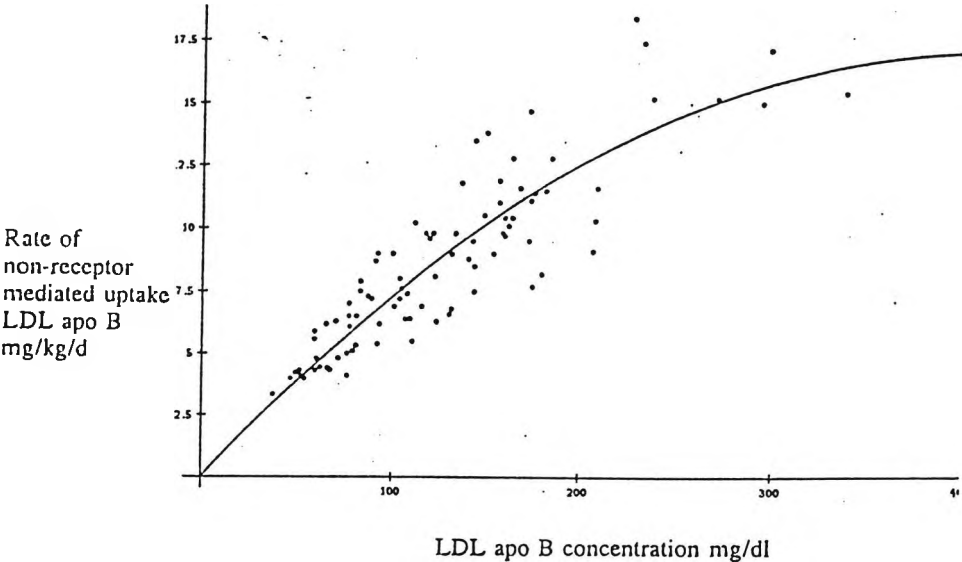


Figure 3.15 - Variation in non-receptor mediated uptake LDL with LDL apo B concentration

3.6.3 Practical application of meta-analytic techniques to this problem

Creating a structural model of the lipid and lipoprotein metabolism explicitly encodes irrelevance into the model structure (see section 3.3.3.2). The problem becomes one of pooling available literature data and generating those relationships described in the causal network. In the field of lipid and lipoprotein metabolism studies describing individual metabolic pathways and their relationship to measurable variables are limited, such that only a few studies exist which describe each pathway. However, this thesis uses the same philosophy as Eddy et al. (1992) toward meta-analysis. This philosophy is "*to do the best possible with whatever evidence is available*" (Italics added). Using this philosophy meta-analytic techniques are combined with expert opinion on lipid and lipoprotein metabolism to create a network structure and relationships which are consistent with current knowledge.

The relationships used in model construction have therefore been defined by pooling and analysing the available literature data. Data analysis from these studies has differed from classical meta-analysis in several ways:-

- 1) The data collected from the different studies are raw data not the mean and standard deviation or odds ratio etc, used normally in meta analysis.
- 2) Since raw data are available and both input and output variables are continuous, it has been possible to perform statistical correlation on the data from all studies, inspecting for heterogeneity within these data by visual plots, and representing uncertainty in these relationships not explained by heterogeneity as distributions about outcome measures. Examples of the correlations can be seen in chapters 5 and 6.
- 3) The rigid study selection criteria used in meta-analytic studies, i.e. double-blind etc. cannot be used here as only a few studies exist describing each pathway.
- 4) Publication bias is unlikely to exist as the number of studies examining each area is limited, such that any work done, regardless of results, is likely to be published.

3.7 Summary

This chapter has described the methodology appropriate to constructing models of lipid and lipoprotein metabolism, and illustrated a framework for decision support within which these models can be used. Both the CPN and meta-statistical analysis techniques have been shown to be appropriate in this problem domain where: an intentional, physiological model based, approach to knowledge representation is required, both expert opinion and literature data need to be incorporated into the same model, and few data exist for each model relationship such that uncertainty needs to be represented. Examples of model construction using these techniques are given in the text.

The following chapters (4, 5 and 6) illustrate the practical use of the techniques described in this chapter to the field of lipid and lipoprotein metabolism. Chapter 4 sets the scene, whilst chapters 5 and 6 describe the modelling of cholesterol transport and triglyceride/ apoprotein B transport respectively, these models being within the decision support framework described in this chapter.

Chapter 4 - Whole Body Cholesterol Metabolism

4.1 Introduction

Chapter 2 discussed CHD risk formulae generated from large epidemiological studies. It concluded that whilst these formulae are suitable for screening purposes (in order to identify patients at elevated risk relative to the population), they are not suitable for managing individuals.

In order to manage individuals, a new representation of CHD risk is required which depends upon the variation in the lipid and lipoprotein metabolism causing atherosclerosis. Atherosclerosis occurs as a result of cholesterol deposition at the peripheral tissues, so that modelling cholesterol fluxes to and from the periphery can provide a useful estimate of an individual's CHD risk due to abnormalities in lipid and lipoprotein metabolism. When the model represents how different disorders and treatments modify cholesterol fluxes, it is possible to predict changes in CHD risk for different treatment options, i.e. part of the strategy outlined in section 3.5.

Chapters 5 and 6 will describe the modelling of both cholesterol fluxes to and from the periphery, and how disorders modify these fluxes. To provide the physiological and pathophysiological basis for that detailed modelling this chapter gives an account of whole body cholesterol metabolism. In doing so the results of studies describing whole body cholesterol metabolism can be compared for consistency with the models, relationships and hypotheses generated in chapters 5 and 6 (see section 7.2.1.2 for this comparison). Any consistency illustrated by this comparison provides valuable evidence for model validation, since the studies of whole body cholesterol metabolism and those used in chapters 5 and 6 to build models of lipid and lipoprotein metabolism involve different methodology, and are performed over different lengths of time.

The chapter is divided into two sections. Section 4.2 describes current opinion on

whole body cholesterol metabolism, its regulation, and how disorders can result in increased cholesterol deposition in peripheral tissues. Section 4.3 reviews the studies which examine whole body cholesterol metabolism, suggesting how information in these studies might describe an individual's susceptibility to atheroma, and how this can be compared with the results of Chapter 5.

4.2 Whole Body Cholesterol Metabolism - Cholesterol Fluxes

The model used to describe the pathways of whole body cholesterol metabolism throughout this work is illustrated in figure 4.1.

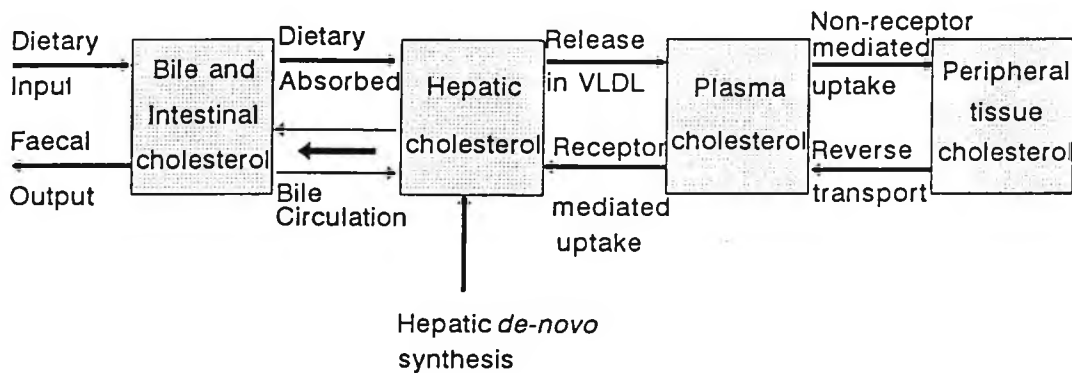


Figure 4.1 - Cholesterol metabolism

Regulation of the cholesterol pathways illustrated in figure 4.1 is discussed in the following section.

4.2.1 The regulation of cholesterol fluxes

As noted by Grundy et al. (1990), the key to determining an individual's regulation of whole body cholesterol might be a "metabolically active" cholesterol pool in the liver. Preservation of equilibrium in cholesterol fluxes to and from this pool would then, be responsible for disturbances in whole body cholesterol metabolism. For example, in figure 4.1, a disorder causing decreased hepatic receptor-mediated uptake

of cholesterol could result in an increased *de novo* synthesis of cholesterol by the liver. Control of whole body cholesterol metabolism by a "metabolically active" hepatic cholesterol pool is consistent with the following experimental results and clinical observations.

1) Cholesterol excretion occurs via the formation of bile acids which are excreted in the faeces. Bile acid sequestering drugs (BAS) block the bile-acid circulation, resulting in greater amounts of cholesterol being used in their formation, and hence an increased cholesterol output from the hepatic pool.

Equilibrium in the hepatic cholesterol pool is consistent with the effects of bile acid sequestrants. Increased cholesterol use in the bile results in a greater expression of Apo B₁₀₀/E receptors as a result of an increased hepatic receptor-mediated cholesterol uptake. However, BAS do not have optimal efficacy unless combined with a cholesterol lowering diet as the increased use of hepatic cholesterol in bile acid formation could increase dietary cholesterol absorption. Similarly, the efficacy of the drug is limited due to increased hepatic *de novo* synthesis of cholesterol, which may also be elevated so as to preserve the equilibrium of the hepatic cholesterol pool. HMG CoA reductase drugs are a class of drugs which reduce *de novo* cholesterol synthesis. Using both BAS and HMG CoA reductase drugs on the same patient (on lipid lowering diet) has been shown to have a synergistic effect in elevating Apo B₁₀₀/E receptor activity (Mabuchi et al., 1983; Illingworth, 1984)

2) Meittinen et al. (1989) found that obese patients have lower dietary cholesterol absorption combined with increased cholesterol synthesis. Again this is consistent with the preservation of equilibrium in the "metabolically active" hepatic cholesterol pool.

3) Meittinen et al. (1989) also found that "the lower the mean and absolute dietary cholesterol absorption the higher the cholesterol synthesis and the higher the biliary secretion, absorption, and faecal output of cholesterol."

Overall hepatic regulation of cholesterol metabolism is disturbed by dietary factors, and by primary and secondary disorders of the lipid and lipoprotein metabolism. For example: a) Familial hypercholesterolaemia (FH), a disorder which results in reduced expression of the Apo B₁₀₀/E receptors, decreases the hepatic removal of cholesterol from the plasma, resulting in elevated plasma and LDL cholesterol concentrations; b) variation in an individual's apoprotein E phenotype has been shown to alter the dietary cholesterol absorption (Kesäniemi et al., 1987) and alters the hepatic receptor-mediated uptake of cholesterol enriched VLDL remnants in type III hyperlipidaemia; and c) disorders of both apoprotein B and triglyceride metabolism increase the VLDL formation and release into the plasma, as will be illustrated in chapter 6. Since cholesterol is a packaging lipid in the formation of VLDL, an elevated hepatic VLDL production is likely to increase hepatic cholesterol release into the plasma.

Disturbances in the regulation of cholesterol metabolism often result in an increased cholesterol concentration in atherogenic particles within the plasma, i.e. LDL and VLDL remnants. As will be illustrated in chapter 5, increased concentration of these cholesterol enriched lipoproteins results in elevated cholesterol deposition at the periphery via a non-physiological, non-receptor mediated route. It is also possible that cholesterol may be removed from the periphery in a process known as reverse cholesterol transport (see chapter 5). Where cholesterol deposition exceeds reverse cholesterol transport atherosclerosis is likely to occur, increasing the risk of CHD.

4.3 The Relationship Between an Individual's Potential for Atheroma and Whole Body Cholesterol Metabolism.

4.3.1 Introduction

This section reviews those studies which examine whole body cholesterol metabolism, suggesting how information in these studies might describe an

individual's potential for atheroma. Potential for atheroma is defined here as either the difference between cholesterol fluxes to and from atheroma plaques, or the difference between cholesterol fluxes to and from cholesterol pools which, when at elevated concentration, might cause atheroma.

In reviewing these studies, this section describes two methodological techniques used to examine whole body cholesterol metabolism, and comments on the study results, assumptions, and variation in these results when the data are analysed in different ways.

The conclusions of this section are twofold:

- a) Detailed modelling at a low level of abstraction is required to represent the relationship between potential for atheroma and lipoprotein metabolic state, i.e. the level of abstraction adopted in chapter 5, and
- b) the difference in turnover between the two and three pool model of isotopic study, or an increase in the mass of pool 3, might indicate cholesterol deposition in peripheral tissues and hence potential for atheroma. The values reported in these studies serve as a useful comparison with the model predicted potential for atheroma described in chapter 5.

4.3.2 Whole Body Cholesterol Turnover Studies

Two experimental techniques have been used to examine the parameters of whole body cholesterol metabolism. These are: measuring sterol balance, and the technique of injecting labelled isotope into the plasma pool and monitoring its decay.

In the sterol balance technique, the cholesterol excreted in the faeces is measured. Then, by fixing the dietary cholesterol input and assuming equilibrium in total body cholesterol, the cholesterol turnover rate is estimated; this being the difference

between the faecal output and dietary input.

In the isotope method, decay curves are obtained from injecting labelled isotope into the plasma. These curves are then modelled using either compartmental or input-output approaches, to estimate cholesterol turnover. Compartmental models are constructed using either one, two, or three compartments.

4.3.2.1 Estimating Cholesterol Turnover by Measuring Faecal Sterol Excretion.

An important assumption when estimating cholesterol turnover from faecal sterol excretion is that the cholesterol metabolism (see figure 4.1) is in equilibrium. This means that the only significant losses of cholesterol are via the faeces, and that over the time period associated with the study there is no significant trapping of cholesterol in any of the pools. According to Grundy and Aherns (1969) the steady state occurs when

" the following conditions are met: constant plasma cholesterol concentrations, unchanging faecal excretion of steroids, and constant body weight all coexist during long periods of study of patients who are clinically stable and free from complicating metabolic abnormalities. In the metabolic steady state the input (synthesis plus dietary intake) is balanced by outflow (excretion): in other words , synthesis equals excretion minus intake."

However, as these authors note

"it is conceivable that the tissue concentrations of cholesterol may be changing even when the concentration in the plasma is not changing. In that case the rates of synthesis and excretion will not be equal; to the extent of the difference the sterol balance method will be in error in estimating daily synthesis."

The likelihood of cholesterol trapping at the periphery increases in cases of high CHD risk, where cholesterol must accumulate in the peripheral tissues for atherosclerosis to exist. In these cases one would expect the estimation of cholesterol turnover derived from the faecal excretion method to be an underestimate of the true production rate, the error being that amount lost to the peripheral tissues.

However, the amounts of cholesterol lost in these unphysiological pathways may not be significant in terms of whole body fluxes. If not, then the estimates of cholesterol turnover given by these methods might be reasonable approximations of the true value. Indeed the cholesterol deposition in the atheroma itself, rather than that deposited in the peripheral tissues, is thought to be insignificant in terms of whole body cholesterol metabolism (Jaganathan et al., 1974), as are physiological losses to the periphery in skin (Goodman et al., 1973a), these authors noting that cholesterol excretion from the skin is "estimated at about 50-100 mg d⁻¹".

4.3.2.2 Estimating Cholesterol Turnover by Modelling Data from Experiments Injecting Labelled Isotope into the Cholesterol Pool

These studies use one, two, or three compartmental models to estimate parameters of whole body cholesterol metabolism and hence cholesterol turnover. A speculative physiological interpretation of these compartments has been given by Goodman et al. (1973a) who note that:-

"it must be recognised that the three pools in the proposed model represent mathematical constructs and do not have precise physical meaning. The finding that the long term turnover of plasma cholesterol conforms to a three pool model means that the various tissue pools of exchangeable body cholesterol fall into three groups in terms of the rates at which they equilibrate with plasma cholesterol. The first compartment consists of cholesterol in fairly rapid equilibrium with plasma cholesterol, and probably includes plasma, red blood cell, and

liver cholesterol, together with much of the cholesterol in several other viscera (e.g., intestines, pancreas, spleen, kidney, lung). A portion of the cholesterol in pool 1 is probably also located in peripheral tissues (adipose tissue, muscle skin). The second compartment (pool 2) consists of cholesterol which equilibrates at an intermediate rate with plasma cholesterol, and probably includes some of the cholesterol in viscera, together with some of the cholesterol in the peripheral tissues. *Most of the cholesterol in the peripheral tissues (particularly skeletal muscle) equilibrates more slowly with plasma cholesterol and comprises the major portion of the most slowly turning over pool, pool 3.*

In addition to the 3 pools of exchangeable cholesterol..., a complete model of body cholesterol metabolism would require the addition of a fourth pool representing non-exchangeable (or exceedingly slowly exchangeable cholesterol). The addition of such a pool of virtually non-exchangeable cholesterol to the two-pool model was proposed and discussed by Wilson.. , who indicated that in the baboon, that this pool consisted not only of the cholesterol in the central nervous system, but also much of the cholesterol in bone, and apparently some of the cholesterol in skeletal muscle, skin and other tissues" (Italics added)

Compartmental modelling of cholesterol metabolism from these studies produces different estimates of cholesterol turnover rates depending upon the number of compartments used. It is these differences which might provide us with information on the atheromic potential of an individual, as discussed in the next section.

4.3.2.3 Information on Atheromic Potential provided by studies on whole body cholesterol metabolism.

It is likely that large amounts of cholesterol at the peripheral tissues are associated

with atheroma. Indeed, if atheroma were not caused by trapping of cholesterol in the more slowly turning over pools, but by other mechanisms, e.g. redistribution of existing cholesterol to the coronary artery, then the whole lipid philosophy, i.e. that high concentration of cholesterol in serum lipoprotein subfractions causes atherosclerosis, would require re-analysis. In this thesis it is assumed that redistribution of cholesterol is not the prime mechanism of coronary atheroma, and that the lipid hypothesis is true.

Given this initial hypothesis, and the physiological interpretation of pool 3 as encompassing the majority of peripheral tissue cholesterol, it is likely that the mass of cholesterol in pool 3 and the fluxes of cholesterol to and from pool 3 will provide information as to the potential for atheroma for an individual.

Goodman et al. (1973a) have modelled the cholesterol metabolism of six patients: a) after 10 weeks using a two pool compartmental model, and b) after 32-41 weeks using a three pool compartmental model. The estimated cholesterol turnover rates for the six patients are illustrated in table 4.1.

Subject	2 pool model	3 pool model	% difference
FC	1.17	1.07	-8.5
RM	1.38	1.29	-6.5
RN	1.12	1.00	-10.7
JB	0.91	0.81	-11.0
GF	1.31	1.21	-7.6
HL	1.51	1.42	-6.0
Mean	1.23	1.13	-8.4
SEM	0.09	0.09	0.9

Table 4.1 cholesterol turnover rates (g/d) in two and three pool models

Modelling the results of this study using a three pool compartmental model provided a better fit than the two pool model. The three pool model was found to be a better fit in other studies from this group (Smith et al., 1976; Goodman et al., 1980), in which cholesterol metabolism was modelled for 54 patients with a great deal of heterogeneity of hyperlipidaemic state. Similarly, the three pool model was the best fit in a study of two patients with abetalipoproteinaemia (Goodman et al., 1983). This disorder results in a low level of VLDL release into the plasma and as such is protective against atherosclerosis. If the two pool model were appropriate in any patients it would be these where non-physiological uptake of cholesterol at the periphery is extremely unlikely, and therefore a greater proportion of cholesterol is likely to equilibrate quickly.

Modelling patient data at 10 weeks using a two pool model is inappropriate because it does not represent equilibrium in whole body cholesterol. This means that cholesterol turnover C , (as illustrated in figure 4.2), is overestimated; and this can be seen in the results of Goodman et al. (1973a) shown in table 4.1. This overestimate can be explained by lack of cholesterol equilibrium across a third pool, i.e. cholesterol loss to the peripheral tissues. As illustrated in figure 4.3 the overestimate of $C = (D-E)+F$

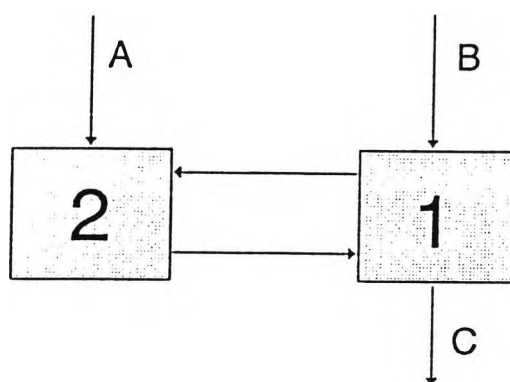


Figure 4.2 - A two pool compartmental model structure used in estimating cholesterol turnover 'C'

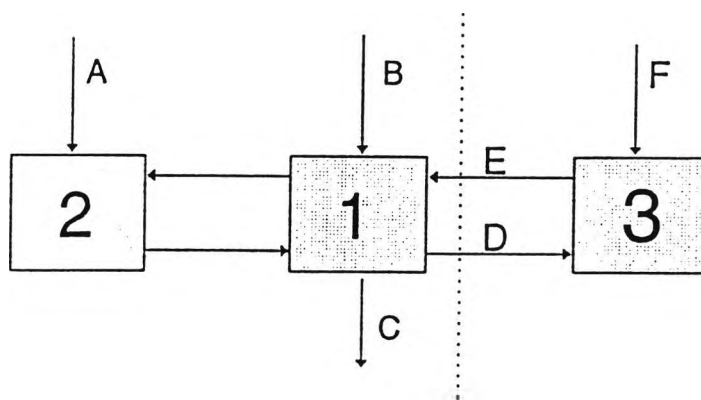


Figure 4.3 - A compartmental model structure illustrating the overestimate of cholesterol turnover "C"

The overestimate of cholesterol turnover using the two pool model might then indicate increased cholesterol flux to the periphery and hence increased potential for atheroma. Those individuals with increased potential for atheroma having a larger, more slowly turning over third pool and hence a large difference between calculated cholesterol turnover from two and three pool models.

Estimates of cholesterol turnover from faecal sterol methods have been found to be approximately 10-15% lower than those yielded from two pool compartmental modelling of isotopic data (Samuel et al., 1978; Nestel Et al., 1973; Grundy et al., 1969). Cholesterol turnover estimated from sterol balance methods might then approximate those estimated from three pool compartmental models; as both faecal sterol methods and three pool compartmental models estimate cholesterol turnover 10-15% lower than the two pool model. This is a particularly useful finding since there are numerous papers which report cholesterol turnover rates for the same patients calculated from both faecal sterol methods and two pool compartmental analysis of isotopic data (Samuel et al., 1978, Nestel Et al., 1973, Grundy et al., 1969). Peripheral tissue cholesterol loss can then be estimated as

$$\begin{array}{llll} \text{Peripheral tissue=} & \text{2 pool isotope} & - & \text{sterol balance} \\ \text{cholesterol loss} & \text{cholesterol turnover} & & \text{cholesterol turnover} \end{array} \quad (4.1)$$

There are many numerical approximations in estimating cholesterol turnover in the faecal sterol method (Grundy et al., 1969,) and it is likely that the above equation is not completely accurate. However, the range of peripheral tissue cholesterol loss across the many patients reported in these studies can provide a useful comparison with the range estimated for potential for atheroma in the model constructed in chapter 5 (where the potential for atheroma = non-receptor mediated cholesterol uptake - reverse cholesterol transport). This comparison is performed in section 7.2.1.2 as part of qualitative validation of the model constructed in chapter 5.

Smith et al. (1976) found a linear relationship between the minimum size of pool 3 (the majority of which is peripheral tissue cholesterol) and the concentration of total serum cholesterol, such that "the amount of cholesterol in slowly equilibrating tissue sites appears to particularly increase with elevation of the serum cholesterol level." These authors note that "the observed correlation between the size of pool (M_3) and serum cholesterol concentration suggests that kinetic analysis used here can provide a method for estimating the size of pathological accumulation of cholesterol in slowly equilibrating tissue sites. If this is true then it may be possible to study the effects of therapeutic intervention (e.g. lipid lowering drugs) on the amount of cholesterol in tissue pools, by repeating long-term studies of cholesterol turnover after a period of therapy in specific patients."

The correlations between clinical measurements and the size of the third compartment have been improved in subsequent papers from this group (Goodman et al., 1980). In this study the patient group was increased to 54 and a comprehensive analysis of interdependencies performed upon the parameters of whole body cholesterol metabolism and other factors. Age, weight and serum cholesterol concentration correlated significantly with the minimum size of pool 3. The authors concluded that

"The relationship between M_3 and adiposity (excess weight) is consistent with the finding that adipose tissue cholesterol appears to be an important component of pool 3... Similarly, the observed relationship with age may in part reflect the changes in body

composition, particularly the relative composition of fat tissue in the body, which occur with increasing age. The relationship of M_3 with age is also consistent with the increase in cholesterol in connective tissue (also an important part of pool 3) with age.... The finding of a significant relationship with the serum cholesterol level as well as age suggest that, with time, cholesterol deposits in slowly exchanging tissue sites and that the extent of such cholesterol deposition increases with increasing serum cholesterol level. The results also suggest... that the kinetic analysis as used here may be able to provide a method for estimating the size of pathological accumulations of cholesterol in slowly equilibrating tissue sites (presumably including arteries) in patients with hypercholesterolaemia."

It would be very useful to see if the same correlation of the size of pool 3 and age was found in patients not on a western style diet where atherosclerosis may not be a natural ageing process.

These authors found no correlation between the minimum size of pool 3 and either HDL cholesterol concentration or HDL3 subfraction size and, in a further study from this group (Blum et al., 1985), no extra information on the mass of pool 3 was given by measuring any of the lipoprotein subfractions (i.e. no association between the HDL cholesterol level, the apo E isoform, lipoprotein subfraction ratios, and the 3 pool model parameters).

Blum et al. (1985) suggest that

"This conclusion, however, does have certain limitations.... cholesterol turnover studies such as these provide a "low power" view of whole body cholesterol metabolism. Thus, arterial wall cholesterol content is a small part of pool 3 but is the locus of the atherosclerotic process. Lipoproteins certainly

have major effects on cholesterol metabolism in cells, and these effects include influences on transport into and out of the cells. Nevertheless our results suggest that the variables explored here may not be rate limiting in these processes.....It is tempting to speculate that these processes may be unpredictable from the variables studied because they are closely involved in the hour to hour and day to day regulation of cholesterol homeostasis".

4.4 Conclusions

Several conclusions can be drawn from this chapter:-

1) Whilst studies of whole body cholesterol metabolism have confirmed that elevated serum cholesterol levels result in increased peripheral tissue cholesterol (pool 3), they have been unable to illustrate any relationship between cholesterol deposition at the periphery and more detailed parameters of lipoprotein metabolism. Blum et al. (1985) note that "lipoproteins certainly have major effects on cholesterol metabolism in cells, and these effects include influences on transport into and out of the cells". It is likely then that data describing more detailed cholesterol fluxes could illustrate significant relationships between peripheral cholesterol deposition and more detailed parameters of lipoprotein metabolism. In short, a more detailed level of modelling is required describing how modifications in lipid and lipoprotein metabolism effect cholesterol deposition at the periphery, and hence atherosclerosis. Chapters 5 and 6 model lipid and lipoprotein metabolism at this more detailed level of abstraction.

2) This chapter has illustrated how a range of values for cholesterol deposition at the periphery might be estimated from the results of: a) two pool compartmental models of cholesterol metabolism and b) faecal sterol methods of estimating cholesterol turnover. In chapter 7 (section 7.2.1.2) this range is calculated and compared with the range of cholesterol deposition possible from the model constructed in chapter 5. As noted in the introduction to this chapter (section 4.1) successful comparison between

these two diverse information sources would provide significant evidence for model validation.

Chapter 5 Modelling Cholesterol Fluxes to and from the Peripheral Tissues (from Plasma)

5.1 Introduction

In chapter 4 it was suggested that an individual's risk of CHD, due to abnormalities in lipid and lipoprotein metabolism, could be described as the difference between cholesterol fluxes to and from the peripheral tissues (from the plasma). This in turn was due to the difference in cholesterol fluxes to and from the plasma (from the liver), in a process whereby whole body cholesterol metabolism was regulated.

Chapters 5 and 6 model these fluxes using: a) expert opinion to construct causal structures of dependencies which describe cholesterol flows and b) pooled literature data to generate the relationships described in these structures, i.e. use of the techniques described in chapter 3 including implementation using the package HUGIN.

These chapters describe detailed models of lipid and lipoprotein metabolism constructed in line with the overall strategy outlined in chapter 3, i.e to enable prediction of change in CHD risk for different treatment options.

Chapter 5 illustrates the modelling of cholesterol fluxes between peripheral tissues and plasma. It considers physiological and non-physiological uptake at the peripheral tissues via the low density lipoprotein (LDL) subfraction, and the cholesterol flux from these tissues, a process known as "reverse cholesterol transport. Validation of this model in qualitative and quantitative terms is described in chapter 7.

Chapter 6 extends the modelling of chapter 5, representing the effects of obesity and genetic disorders on VLDL apoprotein B and triglyceride metabolism. VLDL metabolism is described as having a great effect on those variables indicating cholesterol fluxes, as represented in chapter 6, So that in future the models

constructed in chapters 5 and 6 may be linked. Validation of this model in qualitative terms is described in chapter 7.

5.2 Background

Figure 5.1 illustrates the cholesterol fluxes of whole body cholesterol metabolism.

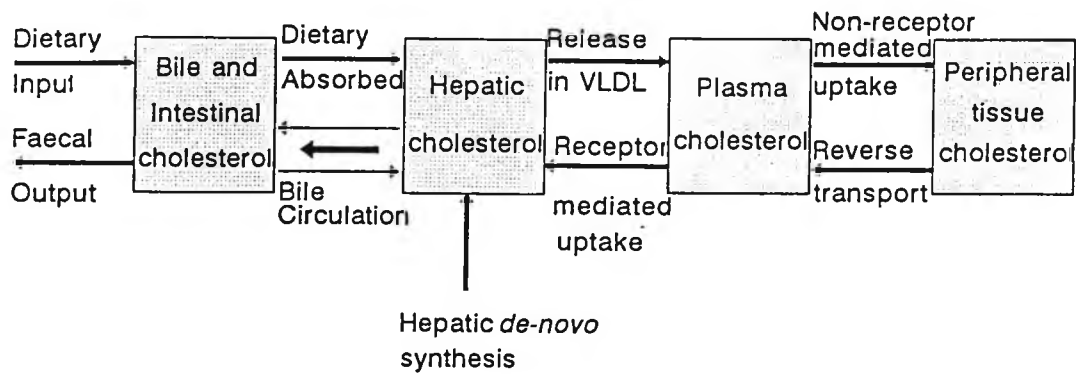


Figure 5.1 - Cholesterol metabolism

In modelling cholesterol flux between the plasma and the peripheral tissues it has been assumed that the difference between the non-physiological, non-receptor mediated uptake and the reverse cholesterol transport is significant in representing equilibrium (or lack of it) of cholesterol deposition across the peripheral tissues. This assumption is potentially flawed for two reasons: first, there is likely to be some receptor-mediated uptake at the periphery and, secondly peripheral tissues have the potential to synthesise *de novo* their own cholesterol. This results in further inputs into the peripheral tissue cholesterol pool as illustrated in figure 5.2.

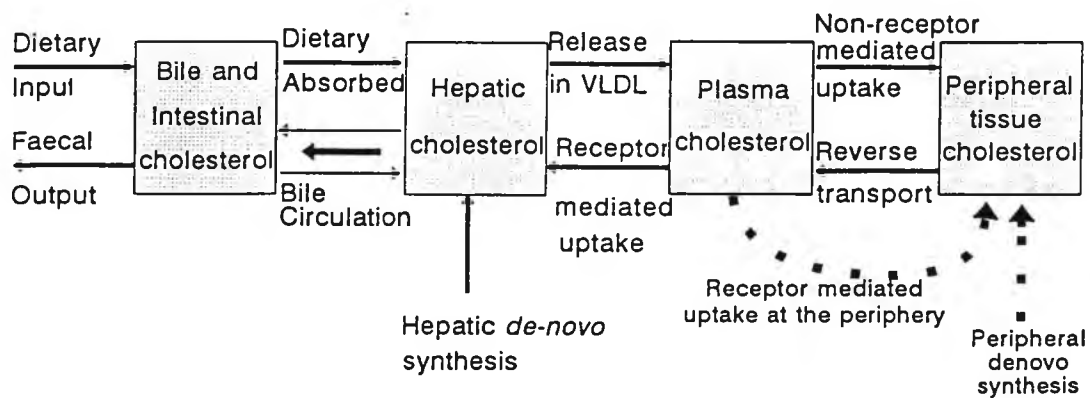


Figure 5.2 - Cholesterol metabolism

These cholesterol inputs into the periphery have been ignored, as their rates are extremely difficult to determine. Ignoring them, however, need not present us with significant errors in the model. For example, receptor mediated uptake at the peripheral tissues, if present, would be used in membrane construction. Given that tissues have the ability to synthesise their own cholesterol, receptor mediated uptake at this site is likely to be minimal. If a situation arose in which this *de novo* synthesis was down regulated and could not supply cholesterol for membrane construction, then it is just as likely that this would result in the reverse cholesterol transport being down regulated, as there being an increase in receptor mediated uptake at the periphery; since we are interested in the difference between the flux to and from the periphery this might not be significant. Nevertheless, if the model is incorrect and in the normal individual there is a large receptor mediated uptake of cholesterol at the periphery not absorbed by normal physiological processes, then this would result in an elevated reverse cholesterol transport above the level of non-receptor mediated uptake when the model is initialised to population values.

Similarly *de novo* synthesis of cholesterol at the periphery might be in excess of that usable at this site. As Fielding (1987) notes "the considerable extrahepatic cholesterol synthesis rates measured *in vivo*, and the inability of extrahepatic tissues to degrade such sterol locally, indicate that substantial amounts of peripheral cell membrane cholesterol must be esterified by LCAT in plasma" (where LCAT - Lecithin:

cholesterol acyltransferase, is the enzyme enabling cholesterol esterification). If this were significant once again the model's estimation of reverse cholesterol transport would be increased above the level of non-receptor mediated uptake when initialised to population values.

The fact that an elevated level of reverse cholesterol transport will result from an incorrect model structure in both of the above cases, can be used as qualitative validation to test the significance of these pathways. Qualitative validation of the model structure is considered in section 7.2.1.

Cholesterol fluxes to and from the periphery are now described and modelled in turn.

5.3 Cholesterol Transport to the Tissues (Non-Receptor Mediated Uptake)

5.3.1 Qualitative Structure of Model Describing Non-Receptor Mediated Uptake

The qualitative structure of the model describing non-receptor mediated uptake is illustrated in figure 5.3.

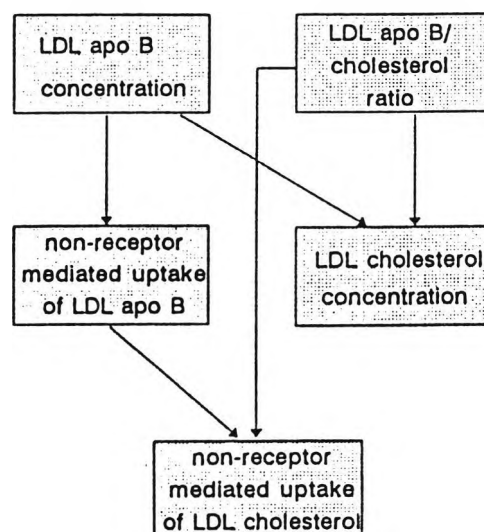


Figure 5.3 - A causal structure for the non-receptor mediated uptake of cholesterol

This is based upon the view that as the number of LDL particles increases, (as determined by the LDL apoB concentration), then normal LDL receptor-mediated pathways become saturated. This results in an increased rate of LDL uptake in the periphery via a non-physiological, non-receptor mediated route. Other nodes in this model are simply mathematical constraints, for example, the non-receptor mediated uptake of LDL cholesterol can be calculated from the non-receptor mediated uptake of LDL apoB and the LDL apoB/cholesterol ratio.

The LDL apoB concentration and the LDL cholesterol concentration are both highly dependent upon the synthesis and catabolism of VLDL and on the composition of newly synthesised VLDL. It is important then, when merging the models of chapters 5 and 6, that this dependency be represented. This problem is considered further in chapter 6.

5.3.2 Quantitative relationships

Individual patients' measurements of LDL apo B concentration and rate of LDL apoB non-receptor mediated uptake were collected from the literature, (Shepherd et al., 1979, 1980, 1982; Stewart et al., 1982; Kesaniemi et al., 1983; Packard et al., 1983; Simons, 1983) and represented on a single graph (see figure 5.4). These studies have used LDL which has been chemically modified to block its uptake by the receptor pathway, so that the catabolism of LDL via non-receptor routes can be measured.

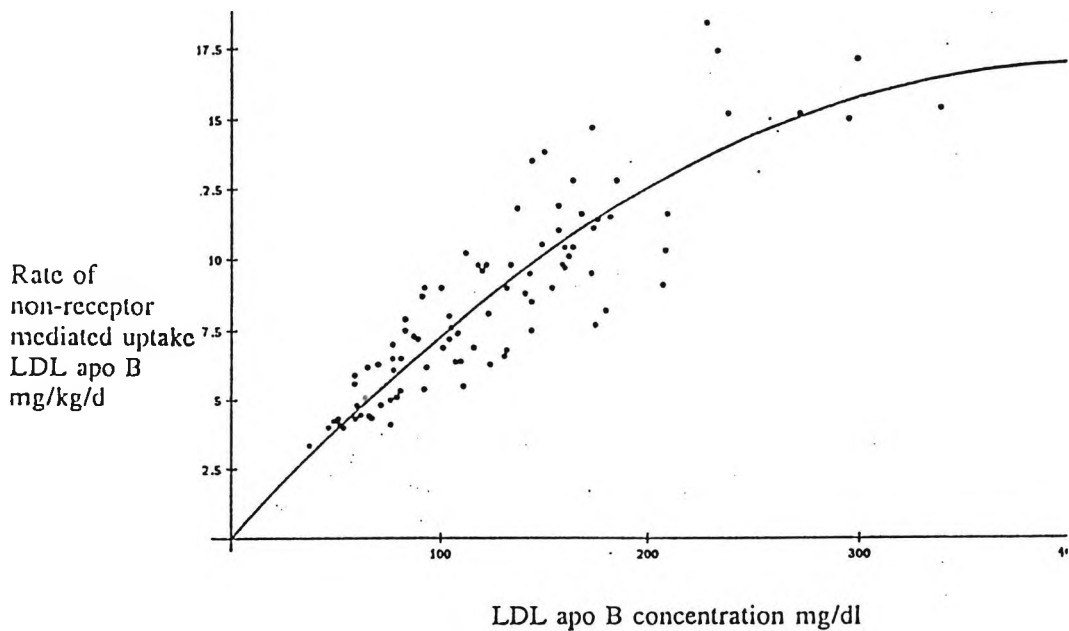


Figure 5.4 - Non-receptor mediated uptake of LDL apo B

The graph in figure 5.4 illustrates a great deal of homogeneity in this relationship despite the wide heterogeneity of the populations used in the studies, i.e. normals, patients with FH, and those on a range of different drugs.

Extremely detailed *a priori* distributions were obtained for the population values of LDL apo B concentration and the LDL apoB/ cholesterol ratio, the latter of which, although not of normal distribution, resulted in a normal distribution for LDL cholesterol concentration when propagated in the network. These distributions were obtained from the work of Vega and Grundy (1984), who determined the distributions of LDL apo B concentration and LDL apoB/cholesterol ratios in many different populations. This model includes those distributions calculated for normotensive middle aged men.

These relationships and *a priori* distributions result in a model which when initialised to population values is as shown in figure 5.5.

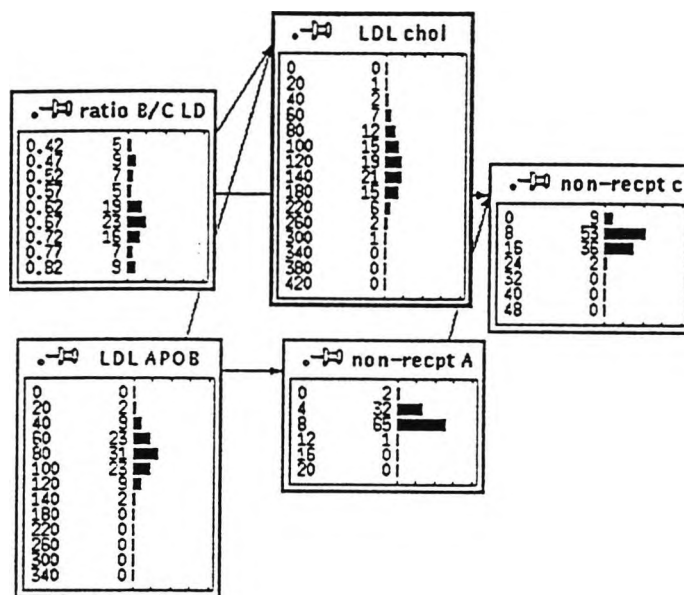


Figure 5.5 - Model of non-receptor mediated uptake of cholesterol initialised to a *priori* state

5.4 Cholesterol Transport from the Tissues (Reverse Cholesterol Transport)

Reverse cholesterol transport is the process by which cholesterol in the peripheral tissues returns to the plasma and is then removed for catabolism by the liver. Peripheral tissue cholesterol is transported initially on high density lipoproteins (HDL), the metabolism of HDL regulating the removal of cholesterol from the periphery. HDL metabolism involves a cycling of HDL particles, during which the HDL become progressively cholesterol enriched. These are then either delipidated or removed. Delipidation results in the particles returning to some earlier point in the cycle as illustrated in figure 5.6.

Measurements of: a) HDL subfraction compositions, b) transfer proteins, c) VLDL and LDL concentration, composition and synthesis, and d) lipase activities, all provide information as to cholesterol fluxes in the HDL cycle and hence reverse

cholesterol transport. The model constructed in this chapter represents relationships between these variables and the cholesterol fluxes illustrated in figure 5.6, enabling prediction of cholesterol transport from the periphery.

When analysing the data representing an individual pathway there is the need to be aware that these measurements cannot be interpreted in isolation, since a blockage or disturbance effecting an individual pathway will modify the whole cycle. It is necessary, therefore, to generate relationships consistent with expert opinion on both a) individual pathways of cholesterol transport from the tissues, and b) the effects of disorders or perturbations on the whole cycle. For this reason section 5.5 describes each of these pathways in the HDL cycle, as shown in figure 5.6, illustrating how measurements of variables can provide information on these pathways. It also discusses how disorders in a single pathway may effect the whole cycle, showing consistency with clinical presentation of these disorders.

5.5 The HDL life cycle

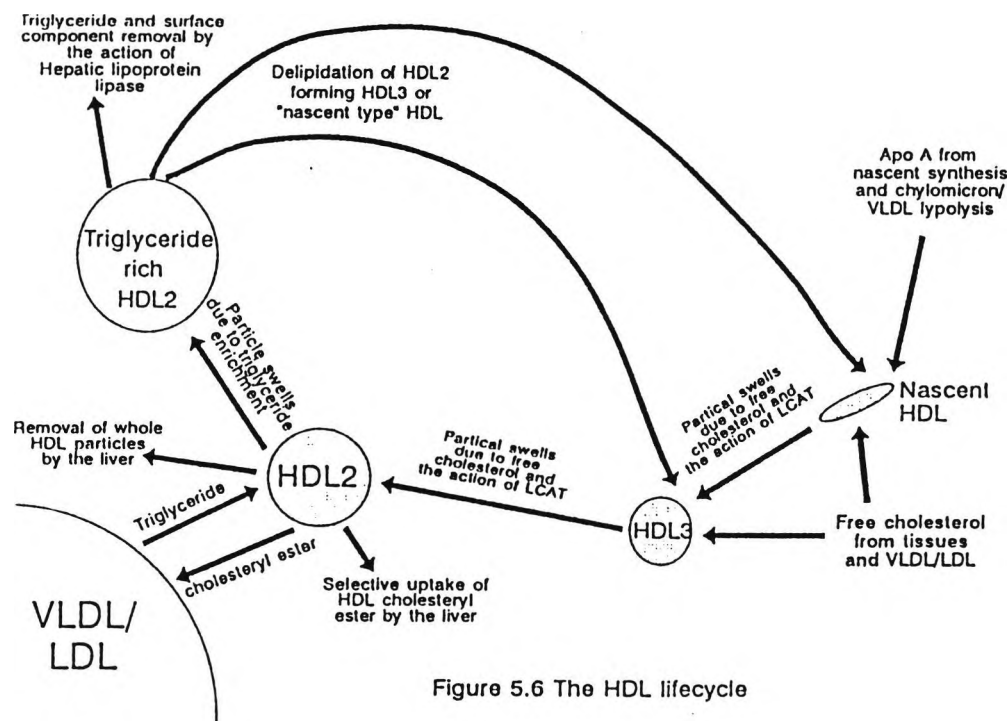


Figure 5.6 The HDL lifecycle

5.5.1 Nascent HDL Formation

HDL particles begin life as flat "nascent" particles consisting solely of surface elements. The predominant apoprotein constituent of nascent HDL is apoprotein AI (apo AI). Nascent HDL is synthesised directly by the liver, and also produced as a result of lipolysis of chylomicrons (via the action of lipoprotein lipase). Lipolysis releases apo AI and other surface components from the chylomicrons to form HDL.

Whether VLDL lipolysis provides apo AI for nascent HDL synthesis is contentious. Fidge et al. (1980) found a strong positive correlation between apo AI synthesis and VLDL apo B synthesis in varied triglyceride concentrations, whilst Magill et al. (1982) found no such correlation in a group with equal heterogeneity.

It is also possible that HDL particles similar to nascent particles are formed as a result of lipolysis of triglyceride rich HDL₂ by the action of hepatic triglyceride lipase (HTLP) (see section 5.5.6). HDL₃ formed by this process are extremely small and rich in apo AI, and as such have the potential to increase free cholesterol esterification (see section 5.5.6).

5.5.2 The conversion of nascent HDL into HDL₃

5.5.2.1 **HDL free Cholesterol Uptake**

Nascent HDL rapidly acquires free cholesterol either from peripheral tissues or from lipoproteins of lower density (Bojanovski et al., 1985; Nikkilä et al., 1987). HDL, particularly nascent HDL, is very small and therefore easily able to cross the vascular endothelium where free cholesterol is removed from peripheral tissues.

Nascent HDL has few core components and therefore has a large storage capacity for newly esterified cholesterol. LCAT, the enzyme responsible for esterification, is located on HDL, meaning that HDL is the major site of cholesterol esterification in

the plasma; free cholesterol acquired by HDL being readily esterified by the action of LCAT. Newly esterified cholesterol migrates to the core of the particle, which consequently swells and becomes HDL₃.

5.5.2.2 The Source of HDL Free Cholesterol

HDL acquires free cholesterol from two sources, the peripheral tissues and the larger lipoproteins, i.e. chylomicrons, VLDL and LDL.

The proportion of free cholesterol removed from either of these sources is likely to depend upon an individual's metabolic state. For example, if the individual has an increased release of free cholesterol into the plasma carried on large lipoproteins, then the proportion of free cholesterol transported to HDL from this source should increase.

5.5.3 HDL₂ and HDL₃ apoprotein throughput as a marker of cholesterol flux through these subfractions.

HDL consists of two major apoproteins apo AI and apo AII. Numerous studies (Magill et al., 1982; Blum et al., 1977; Fidge et al., 1980; Zech et al., 1983) have modelled both apo A metabolism and the effects of diet, drugs and sex hormones on apo AI and AII synthesis, fractional catabolic rate (FCR) and concentration (Brinton et al., 1990; Shepherd et al., 1978, 1979). It would seem reasonable, therefore, to use the predicted throughput of apo AI and AII along with cholesterol loadings on each of the HDL subfractions to determine cholesterol flux through the whole HDL subfraction, enabling us to predict changes in cholesterol flux for differing combinations of treatment.

However, synthesis and catabolism of apo AI and AII are not synonymous with those

of the HDL subfractions. As Nikkilä et al. note (1987), "it is important to realize that the synthesis and catabolism of HDL apoproteins are not equivalent to the metabolism of whole HDL particles, which undergo a continuous remodelling by uptake and removal of the lipid components before being irreversibly removed as particles".

Recent studies (Rader et al., 1991; Zech et al., 1983) suggest that there might be two classes of HDL with distinct metabolic pathways and differing potential for protection from atherosclerosis. These are Lp-AI which contains apo AI, and Lp-AI,AII which contains apo AI and AII; the Lp-AI being associated with increased protection from atheroma. Information on the physiological mechanism for the protective nature of Lp-AI is given by Fielding et al. (1991) who note that "cellular cholesterol was initially transferred to the pre-B-migrating HDL whose protein moiety contained only apo AI. LDL free cholesterol was transferred mainly to the major migrating HDL fraction, which contained both AI and AII". This suggests that Lp-AI is involved in removal of cholesterol from the tissues, whereas Lp AI-AII is involved in re-cycling large lipoprotein cholesterol. Rader et al. (1991) note a further difference between the metabolic pathways of Lp-AI and Lp-AI,AII, i.e., that particles containing Apo AII might be preferentially recycled, being lipolysed more readily by hepatic triglyceride lipase (HTGL). It is possible then, that the Lp-AI,AII particles are those lipolysed to the extremely small HDL₃ subfraction which are thought to be indicative of an atherogenic lipid and lipoprotein metabolism (see section 5.5.6).

5.5.4 The conversion of HDL₃ into HDL₂

The HDL₃ particle acquires further free cholesterol from the same sources as previously described, along with apoprotein. The cholesterol is esterified and transported to the HDL core, lowering its density to HDL₂.

5.5.5 HDL₂ cholesteryl ester transport to the liver

Two pathways have been hypothesised for the transport of cholesterol from HDL₂ to the liver. These are "*HDL₂ --> VLDL/LDL --> liver*" and "*HDL₂ --> liver*". There are conflicting opinions as to whether the "*HDL₂ --> VLDL/LDL --> liver*" pathway is atherogenic. This is due to results showing both decreased and increased fluxes via this pathway in atherogenic states (Fielding et al., 1984a; Bagdade et al., 1987, 1991).

Results suggesting that the "*HDL₂--> VLDL/LDL --> liver*" pathway is atherogenic (Bagdade et al., 1991) have led to further hypothesis concerning the direct uptake of cholesterol by the liver from the HDL₂ subfraction; this uptake being required to explain why cholesterol transport via VLDL/LDL is limited whilst atherogenicity is prevented.

This section reviews the literature relevant to this debate discussing whether or not the "*HDL₂ --> VLDL/LDL --> liver*" pathway is atherogenic. The discussion in this section and that of 5.5.6 conclude that reported data may not be as conflicting as originally thought and that the selective uptake of HDL cholesterol is not necessary to explain the results of literature studies.

5.5.5.1 Removal of HDL cholesterol, via VLDL/LDL, by the liver.

Regardless as to whether this pathway is atherogenic or not, lipid exchanges between HDL₂ and VLDL/LDL proceeds as follows:- HDL₂ is involved in an exchange of its cholesterol for triglyceride from the larger lipoproteins, predominantly VLDL. This exchange is thought to be equimolar (Mann et al., 1991), resulting in HDL particles which are further decreased in density, (as illustrated in figure 5.6), triglyceride being less dense than cholesterol. Recycling of HDL then occurs via lipolysis of triglyceride enriched HDL₂ by the action of HTGL (see 5.5.6).

Reverse cholesterol transport via VLDL in hypertriglyceridaemic states without any underlying disorder:-

Reverse cholesterol transport via VLDL has been shown to be elevated in the post prandial state (Patsch et al., 1987). As Fielding (1987) notes "it is possible that the postprandial lipaemia represents a physiologic mechanism to maintain or enhance the removal of excess peripheral cholesterol to the liver for catabolism". Since the purpose of VLDL and chylomicrons is to supply triglyceride to peripheral tissues, one might expect physiological pathways for removal of VLDL/ chylomicron packaging (i.e cholesterol) to exist, and for these pathways to be upregulated in the post-prandial state where lipoprotein formation is increased.

Numerous physiological mechanisms are involved in increasing the cholesterol flux along the "*HDL--> VLDL*" pathway. Lipolysis of chylomicrons releases protein etc for HDL formation, as discussed in 5.5.1, increasing nascent HDL production and hence cholesterol esterification. Also, the increased triglyceride concentration associated with the post prandial state might drive the equimolar exchange of HDL cholesteryl esters for triglyceride.

Mann et al. (1991) have illustrated that *in vitro* the transfer of cholesterol from HDL₂ to VLDL increases with increasing triglyceride concentration, only to be rate limited by the mass (and hence the activity) of the cholesterol ester transfer protein (CETP). Similarly, Fielding et al. (1983) found that hypertriglyceridaemic patients without CHD (vascular disease) had a significantly greater transport of HDL cholesteryl ester (CE) to VLDL than normals.

The increase in the "*HDL₂-->VLDL/LDL*" cholesterol pathway above shows the normal physiological increase in this pathway during hypertriglyceridaemia without any underlying disorder. This would lead us to the same conclusion as Fielding (1987), that is "there seems no reason to consider cholesterol ester transfer, as such, to be an atherogenic reaction. On the contrary, removal of cholesterol of peripheral origin via lipoproteins (VLDL,LDL) whose hepatic receptors are sensitively

controlled, rather than via HDL, might offer potential advantage to the normal individual in providing more effective whole-body cholesterol homeostasis".

Given this conclusion, i.e., that transport of cholesteryl ester via VLDL is a normal physiological pathway, one might expect down-regulation of this pathway when blockages in cholesterol removal from VLDL and LDL are present. Studies investigating whether removal defects in VLDL and LDL result in abnormal cholesterol transport via the "*HDL₂ --> VLDL/LDL --> liver*" pathway report conflicting results. These results have raised doubts as to whether the reverse transport pathway via VLDL is truly physiological. This is the subject of the next section.

*Transport "*HDL₂ --> VLDL/LDL --> liver*" where a removal defect exists:-*

Atherogenic particles, i.e. cholesterol loaded VLDL remnants and LDL, occur in large numbers in the plasma when the production of lipoprotein components exceeds the ability for their removal. Under such circumstances the composition of the VLDL and LDL alter such that the ratio of free cholesterol : phospholipid (FC:PL) at the surface of the particle increases. Many studies have produced data illustrating an increased free cholesterol : phospholipid (FC:PL) ratio in disorders of the lipid metabolism which are known to be atherogenic, Fielding (1984b) provides a summary table of these studies. Kuksis et al. (1982) have illustrated that an increase in the FC:PL ratio is associated with an increase in risk of CHD.

Two groups (Morton, 1988; Fielding et al., 1984a), have suggested that this FC:PL ratio indicates the potential for cholesterol transport along the "*HDL₂ --> VLDL*" pathway. Fielding et al. (1984a) found that the FC:PL ratio was negatively correlated with the cholesterol transport along this pathway, whilst Morton (1988) found a positive correlation between FC:PL and cholesterol transport via VLDL. These findings lead to two extremely different interpretations of the "*HDL₂--> VLDL*" pathway. Using Fielding et al.'s results it would be conclude that the pathway is non-

atherogenic, and that the decrease in flux along this pathway associated with disorders of the lipid metabolism reflects a blockage in VLDL/LDL removal causing an increased saturation of VLDL/LDL, as indicated by their increased FC:PL ratio. Using Morton's results it would be conclude that the increasing FC:PL ratio in lipid disorders indicates a greater transport by the VLDL/LDL pathway and therefore, increases in transport along this pathway must be atherogenic. In this situation, there must be another non-atherogenic pathway of cholesterol removal by the liver; possibly by selective uptake of HDL2 cholesterol.

Morton (1988) has justified his work as being consistent with results of Bagdade et al. (1987, 1991) who found increased cholesterol transport along the HDL₂ to VLDL pathway in patients with insulin dependant diabetes (IDDM), a disorder associated with atherosclerosis and abnormal FC:PL ratios. This is in contrast to Fielding et al. (1984a) where decreased cholesterol transport via VLDL/LDL was found in non-insulin dependent diabetic patients. However, on closer inspection it is clear that the results of both Fielding et al. (1984a) and Bagdade et al. (1987, 1991) can be explained by the same physiological pathways.

In the Fielding et al. study (1984a), no indication was given as to the size of the VLDL subfraction; in the Bagdade study (1991), however, it was found that it was the inclusion of VLDL1 to the normal VLDL which stimulated "*HDL--> VLDL*" CE transport. VLDL1 is very large and therefore most likely to approximate chylomicrons and hence the post-prandial state. In this state, the total HDL throughput of cholesterol is likely to increase, given the normal physiological processes occurring post prandially; that is, an increase in apo AI release for HDL formation and an increase in exchange of HDL cholesteryl ester for VLDL/chylomicron triglyceride.

A possible interpretation which explains the results of both these groups is, therefore, that an increasing production of large VLDL will release apoprotein for HDL formation during lipolysis, causing an increase in the total potential flux of cholesterol through HDL. However, saturation of the receiving particles, i.e. VLDL

and LDL, as indicated by an increased FC:PL ratio will reduce the amount of this potential used.

This interpretation is consistent with a further study by Fielding et al. (1983) where hypertriglyceridaemic patients without CHD (vascular disease) had a significantly greater transport of HDL CE to VLDL ($87 \pm 9 \mu\text{g chol est/ ml plasma/ h}$), than those patients with CHD; the patients with CHD having levels that were reduced to those of normals ($30 \pm 4 \mu\text{g chol est/ ml plasma/ h}$). Interpreting these results, it could be suggested that the overall potential for HDL cholesterol throughput was elevated in both hypertriglyceridaemic groups, but that a blockage in the removal of atherogenic particles in those patients with CHD would cause decrease the proportion of this potential used; the resultant transport rate being similar to that of normals.

This interpretation is also consistent with the hypertriglyceridaemia of familial hypertriglyceridaemia (FHTG) being non-atherogenic whilst the hypertriglyceridaemia often associated with familial combined hyperlipidaemia (FCHL) being atherogenic. The VLDL of FHTG are large, and are therefore most likely to simulate the post prandial state, whereas the VLDL associated with FCHL are much smaller. As Fielding notes (1987), "the influx of phospholipid into plasma postprandially is much greater than that of cholesterol, even after a cholesterol rich meal, which results in a significant decrease in the cholesterol saturation of the plasma lipoproteins". In contrast, for disorders in which an elevated number of smaller VLDL particles are released into the plasma, i.e. FCHL, a larger amount of free cholesterol production may occur per VLDL particle. This would result in both a) an elevated saturation of VLDL, due to an increased FC content, and b) a reduced proportion of the free cholesterol esterified coming from the peripheral tissues.

Treatment of subnormal levels of reverse cholesterol transport, given the above hypothesis, would therefore suggest that an attempt be made to try and increase the overall throughput of HDL cholesterol, but without increasing the input to the plasma of free cholesterol and hence saturating VLDL remnant and LDL removal pathways. Many drugs have been shown to do this by increasing the concentration of HDL

apoproteins whilst also decreasing the production of VLDL and normalising its composition (Kashyap et al., 1987; Shepherd et al., 1979; Packard et al., 1980) .

5.5.5.2 Selective uptake of HDL₂ cholesterol by the liver

There is still, however, inconsistency in the hypothesis presented in 5.5.5.1. These are:-

The hypothesis does not explain the results of Morton's (1988) experiments, i.e. that an increasing FC:PL ratio indicates increased cholesterol flux along the "*HDL₂ --> VLDL*" pathway. Neither does it explain the contradiction between the commonly observed pattern associated with hypertriglyceridaemia, i.e. low levels of HDL₂ and high levels of HDL₃ (a pattern which suggests a large cholesterol flux through HDL), and the consensus present in epidemiological studies that a high level of HDL₂ cholesterol is protective against atheroma.

The association between elevated HDL₂ cholesterol concentrations and protection from atheroma could be explained by selective uptake of cholesterol by the liver directly from HDL₂. It is necessary to postulate selective cholesterol uptake rather than removal of the whole HDL particle since, as Durrington notes (1989), insufficient apo A is synthesised per day to allow significant hepatic cholesterol uptake via removal of the whole HDL particle. The hypothesis of selective uptake of cholesterol from HDL is consistent with a "Familial deficiency of CETP activity in humans" which results in no cholesterol transport along the "*HDL --> VLDL*" pathway; this "leads to very high plasma HDL cholesterol concentrations, due to the accumulation of cholesterol ester rich HDL. No history or clinical evidence of CHD was found in a large kindred with this condition" (Miller, 1990).

5.5.6 HDL₂ Transformation to HDL₃, the Resultant Composition of HDL₃ and how the HDL Subfraction Distribution Relates to the Cholesterol Esterification Rate.

HDL₂, having been involved in exchange of its cholesterol for triglyceride with the larger lipoproteins will be, to a greater or lesser extent, triglyceride enriched. As such it is thought to be delipidated by the action of hepatic triglyceride lipase, which lipolyses triglyceride from the smaller lipoprotein subfractions.

Depending upon the triglyceride loading on the HDL₂ subfraction particles, and on the availability of apo AI in the metabolism, their delipidation results in the formation of differing patterns of HDL subfraction distribution. This has been illustrated in experiments by Hopkins and Barter (Hopkins et al., 1984, 1989).

In the first of these experiments (Hopkins et al., 1984)) incubation of HDL with Intralipid (an apoprotein deficient compound simulating VLDL) resulted in modification in HDL distribution; that is, a reduction in normal HDL₃, an increase in triglyceride enriched HDL₂, and the production of a very small subfraction of HDL₃ which these authors suggested might approximate nascent HDL. The cholesterol esterification rate was markedly increased, possibly due to increased cholesterol throughput caused by elevated triglyceride levels which promote exchange of Intralipid triglyceride for HDL CE. These authors also suggested that esterification rates might be elevated due to the increased LCAT activity associated with nascent HDL particles. Indeed, in a further study (Barter et al., 1985) these authors showed that the size distribution of the HDL₃ subfraction was inversely correlated with the potential for esterification i.e. the Vmax level of cholesterol esterification.

However, Intralipid does not include apo AI and therefore the resulting HDL distribution may not reflect the situation where hypertriglyceridaemia is due to increased concentrations of chylomicrons or very large lipoproteins which when lipolysed might release apo AI for use by HDL. By increasing the apo AI present (Hopkins et al., 1989), Hopkins and Barter found that the modification in HDL

pattern described above was prevented, whilst the cholesterol esterification rate remained elevated.

It is possible then that the cholesterol esterification rate is dependent upon two factors: a) the amount of apo AI, which might determine the carrying capacity of free cholesterol in HDL, and b) the subfraction size distribution of HDL₃. This is consistent with the mathematical model of cholesterol esterification formulated by these same authors (Barter et al., 1984).

These experiments resolve many of the contradictions discussed in section 5.5.5.2, that is to say:

1) The model produced by Barter et al. (1984) provides a useful explanation for the contradiction between Fielding and Morton as to whether FC:PL promotes or inhibits cholesteryl ester transfer from HDL to VLDL.

In this model esterification limits exist which are illustrated by the V_{max} of an esterification curve. Beyond these limits esterification would not increase even if the free cholesterol content of VLDL, and hence the free cholesterol content of HDL, increased. Therefore, *in vitro* it might be perfectly normal for an increased free cholesterol:phospholipid ratio in VLDL to cause increased free cholesterol flux from VLDL to HDL, and subsequently promote esterification and increasing cholesteryl ester transport from HDL to VLDL, as illustrated in Morton (1988). However, if the esterification of free cholesterol is saturated then it is likely that the free cholesterol transfer from HDL to VLDL will be inhibited resulting in further increases in the FC:PL ratio in large lipoproteins. Saturation of HDL with free cholesterol may occur where insufficient apo AI is released from larger lipoproteins on lipolysis.

2) The high cholesterol esterification rates associated with elevated triglyceride concentrations have been shown to result in two completely different HDL subfraction distributions depending upon the availability of apo AI (Hopkins et al., 1984, 1989). This means that the increased esterification rates and "*HDL₂-->VLDL*" cholesteryl ester transport rates associated with hypertriglyceridaemia, are not necessarily related to the atherogenic HDL phenotype (i.e. high levels of HDL_{3D} and low levels of HDL cholesterol). It follows that the selective uptake of HDL₂ cholesterol by the liver is no longer necessary to explain why high HDL₂ cholesterol concentrations are protective from atherosclerosis. This is because hypertriglyceridaemia associated with large amounts of apo AI release from larger lipoproteins could result in the following situation :-

- a) Release of apo AI increases the HDL free cholesterol carrying capacity,
- b) the esterification rate increases due to this greater free cholesterol capacity on HDL,
- c) increased apo AI release prevents the formation of HDL_{3D}, resulting in normal HDL composition, i.e. HDL₂, and
- d) high triglyceride concentrations drive cholesteryl ester transport along the "*HDL₂-->VLDL--> liver*" pathway.

This theory is consistent with the following observations :-

a) As noted by expert guidelines (The expert panel, 1988), in epidemiological studies hypertriglyceridaemia "is not an independent risk factor (for CHD), i.e., the association usually disappears when statistically adjusted for plasma total cholesterol and HDL-cholesterol levels" (brackets added). The production of two differing HDL distributions during hypertriglyceridaemia, depending upon the availability of apo AI, and the finding by Cheung et al. (1991) that only the small subfraction distribution of HDL₃ was consistently associated with risk for CHD, is consistent with this epidemiological finding.

b) Brinton et al. (1991) reported that HDL cholesterol levels correlated

negatively with the level of HTLP activity and positively with lipoprotein lipase activity. This is consistent with the above hypothesis for the following reasons: HTLP lipolyses smaller lipoproteins and hence is involved in the production of smaller HDL₃ particles which correlate positively with CHD risk (Cheung et al. 1991). Lipoprotein lipase is involved in the lipolysis of large lipoproteins which when lipolysed release apo AI to HDL, normalising HDL composition and reducing the risk of CHD.

c) Increases in plasma levels of apo AI are likely to increase the proportion of Lp-AI in the plasma above that of Lp-AI,AII. As noted in section 5.5.3 an increase in Lp A-I is thought to be protective against atheroma (Rader et al., 1991; Zech et al., 1983).

5.5.7 Conclusions.

In discussing the most likely reverse cholesterol transport pathways it is possible to simplify the model representing these pathways to that shown in figure 5.7. In this model selective uptake of HDL₂ cholesterol is no longer required to explain the modification of reverse cholesterol transport during physiological and pathological states .

The discussion also illustrates how particle composition and concentration can provide us with useful information about cholesterol transport pathways as illustrated in table 5.1.

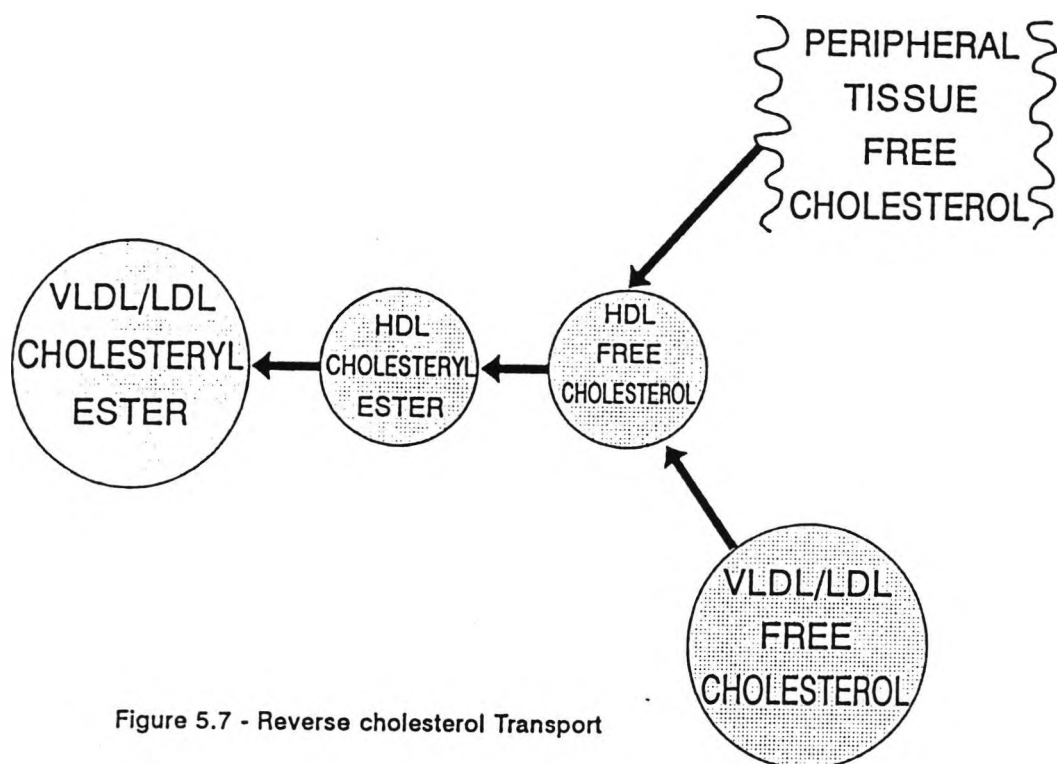


Figure 5.7 - Reverse cholesterol Transport

<i>The metabolic pathway</i>	<i>Variable describing the metabolic pathway</i>	<i>The reason why this variable provides information on this pathway</i>
HDL cholesteryl ester transport to VLDL	VLDL triglyceride concentration	High triglyceride levels drive cholesterol exchange
	CETP activity	CETP is rate limiting during hypertriglyceridaemia
	VLDL/LDL FC:PL ratio	This ratio indicates the cholesterol saturation of VLDL/LDL
HDL FC → HDL CE, i.e. HDL cholesterol esterification rate	HDL2, HDL3, FC concentration	Illustrates esterification capacity
	The size distribution of HDL3	Illustrates increased esterification in abnormal states
The source of free cholesterol, i.e. % from tissues or VLDL/LDL	The total esterification rate	Illustrates removal capacity
	VLDL FC release into the plasma	Illustrates the proportion of the removal capacity used by FC from larger lipoproteins

Table 5.1 - Table describing reverse cholesterol transport pathways

The following section will describe model construction using: (1) a causal structure based upon figure 5.7, and (2) either pooled literature data, from which model relationships are generated, or pre-existing relationships from the literature.

5.6 Construction of the reverse cholesterol transport model

5.6.1 Introduction

Section 5.5 described the cholesterol fluxes important in "reverse cholesterol transport". It concluded that the pathways illustrated in figure 5.8 were sufficient to represent current knowledge in this field, i.e. without representing selective uptake of HDL cholesterol directly by the liver.

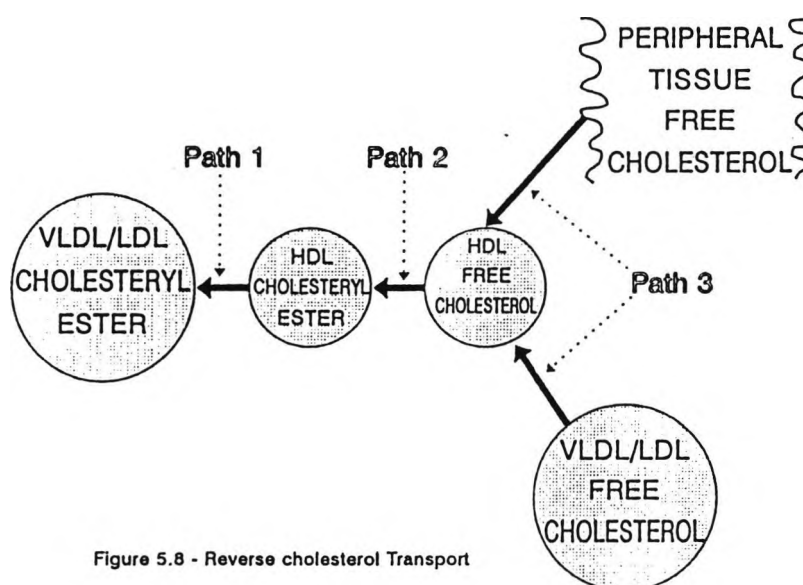


Figure 5.8 - Reverse cholesterol Transport

Section 5.5.7 tabulated these pathways, the variables which provide information as to the state of these pathways, and why these variables provide information on cholesterol fluxes along these pathways. In this section, quantitative relationships are generated between the variables and pathways described in section 5.5. In doing so, and by including that relationship described in section 5.3, a model is constructed representing forward and reverse cholesterol transport. The difference between these two fluxes is described as the potential for atheroma, i.e. CHD risk due to abnormalities in lipid and lipoprotein metabolism. Model relationships are obtained either by pooling literature data or using existing relationships derived in the literature.

5.6.2 Path 1 (figure 5.8) - HDL cholesteryl ester transport to VLDL/LDL.

5.6.2.1 Potential CE transport, and its relationship to VLDL triglyceride concentration and CETP activity.

Section 5.5.5.1 described the situation in which large lipoproteins involved in the uptake of cholesterol from HDL₂ are not saturated, i.e. the FC:PL ratio is not elevated. In this situation the CE transport from HDL₂ to VLDL is dependent upon the VLDL triglyceride concentration and the activity of CETP, the enzyme facilitating this cholesterol flux.

If it is possible, *in vitro*, to achieve a situation where the FC:PL ratio of the larger lipoproteins is normal, then, when this occurs, information about the relationship between VLDL triglyceride concentration, CETP activity and the "potential" cholesteryl ester flux from HDL to VLDL could be obtained. The "potential" CE flux is that which occurs when VLDL is not saturated.

The study carried out by Mann et al. (1991) is the only one, to the author's knowledge, which has related both CETP activity and VLDL triglyceride concentration to the "HDL --> VLDL" cholesterol flux. Mann et al. (1991) illustrate that CETP levels only become rate limiting in patients with hypertriglyceridaemia, and that at lower triglyceride concentrations any change in the transport rate is fully explained by changes in triglyceride concentration. The relationship between the VLDL triglyceride concentration and the "potential" CE transfer rate in normotriglyceridaemic states has a positive gradient and is linear. Mann et al. (1991) do not comment on the FC:PL ratio in these patients, but note that they have primary hypertriglyceridaemia which would suggest an overproduction of large triglyceride rich VLDL rather than the smaller, and hence free cholesterol enriched, VLDL associated with diabetes or familial combined hyperlipidaemia. These larger VLDL are less likely to be saturated since: (a) less FC per unit triglyceride is released per particle, and (b) on lipolysis they are likely to release surface components for HDL

formation (see section 5.5.1).

Two other studies report the VLDL triglyceride concentration and the cholesterol transport rate along the "*HDL --> VLDL*" pathway (Yen et al., 1989; Tall et al., 1986). Both these studies are on normolipidaemic patients, so that CETP is not rate limiting and the FC:PL ratio in VLDL is unlikely to be elevated.

The relationship between CETP, VLDL triglyceride concentration and the potential cholesteryl ester flux was obtained in two stages:

1) By pooling data obtained from Mann et al. (1991), Yen et al. (1989) and Tall et al. (1986) the relationship between VLDL triglyceride concentration and CE flux was obtained for normotriglyceridaemics.

2) By interpreting and extrapolating the results of Mann et al. (1989) the relationship between CETP, VLDL triglyceride, and CE flux was obtained for hypertriglyceridaemics.

Data were taken from these studies by reading values from graphs.

VLDL triglyceride concentration v CE flux during normotriglyceridaemia

The following output was obtained from the statistical package 'Minitab' (Ryan et al., 1985). Figure 5.9 illustrates the linear relationship generated when pooling data from Mann et al. (1991), Yen et al. (1989) and Tall et al. (1986).

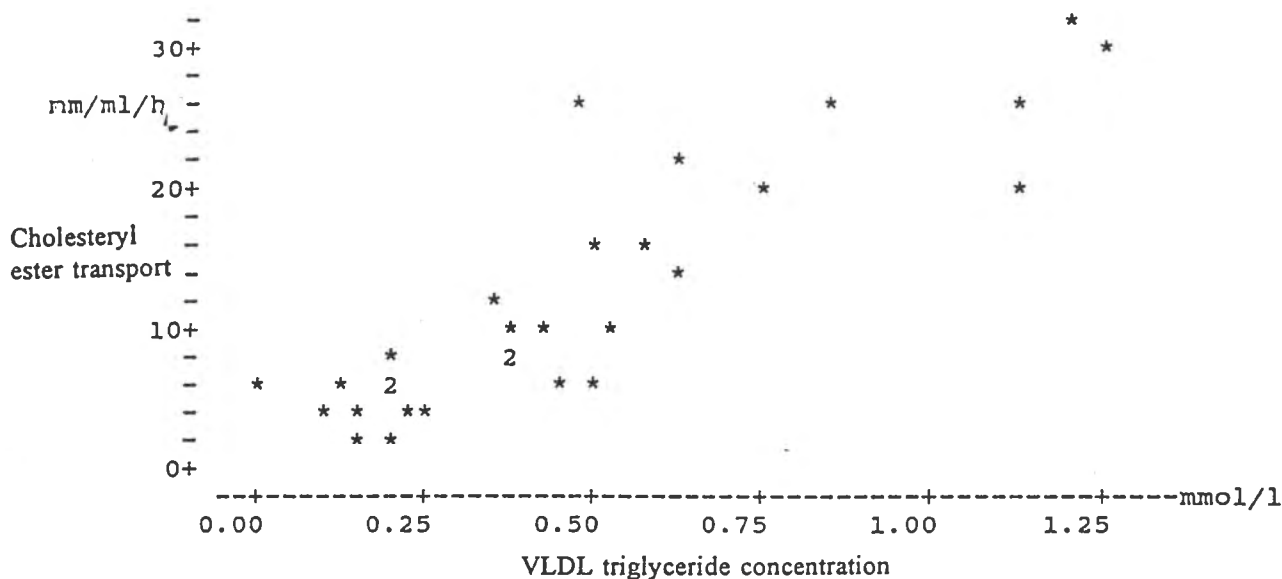


Figure 5.9 - VLDL triglyceride concentration v cholesterol ester flux in normotriglyceridaemia

where r , the correlation coefficient = 0.889

VLDL triglyceride concentration and CETP activity v CE flux during hypertriglyceridaemia

Mann illustrates this relationship with two graphs. The first is a plot of the CE flux against the triglyceride concentration for two individuals with differing CETP concentrations. In these experiments Mann shows that at high triglyceride concentrations the CE flux become saturated so that increasing the triglyceride concentration does not result in any further flux. The second graph illustrates the relationship between the CETP activity and the CE flux at a fixed high VLDL triglyceride concentration (5 mmol/l). This relationship is recreated and illustrated in figure 5.10.

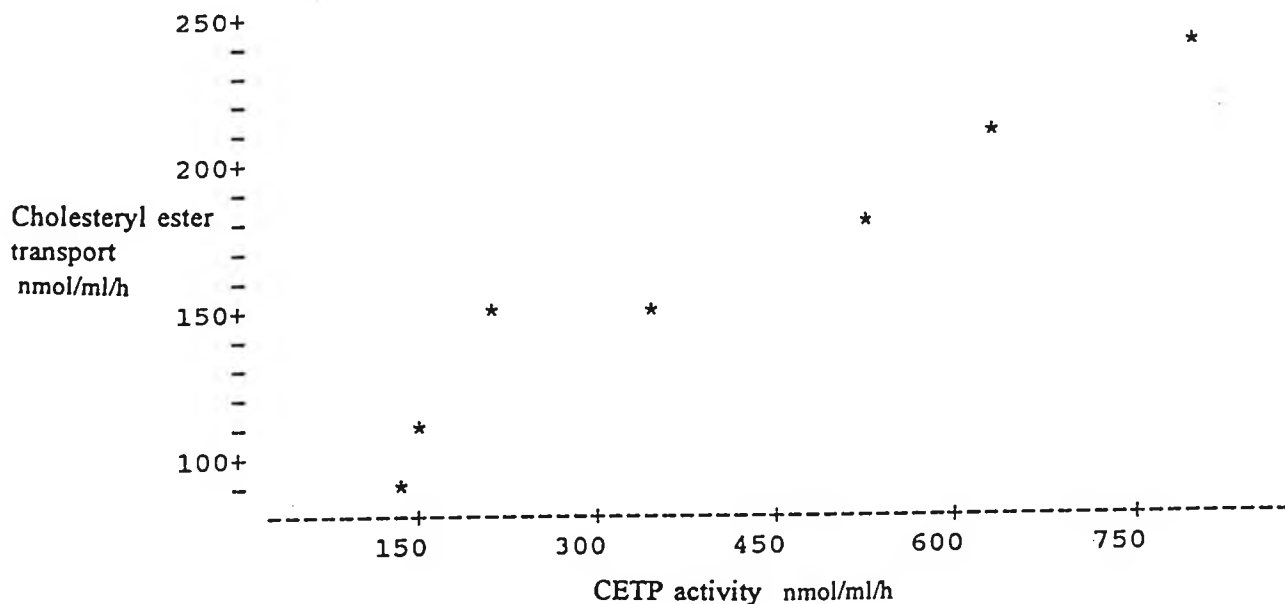


Figure 5.10 - CETP activity v cholesterol ester flux during hypertriglyceridaemia

where $r = 0.973$. Therefore at either high or low VLDL triglyceride concentrations we can be reasonably certain as to determining a useful approximation of this cholesterol flux.

Using values derived for the CE flux at triglyceride concentrations of 5 mmol/l and varying CETP activities, and the shape of the curves plotted for the two subjects in Mann et al.'s (1991) study, a series of curves approximating the relationship between CE flux, triglyceride concentration and CETP activity were generated. More specifically, the shape of these curves was determined by taking the average change from 1 mmol/l to 2mmol/l, from 2mmol/l to 3mmol/l etc. in the two patients reported, this shape being shifted for each value of CETP when the VLDL triglyceride concentration equalled 5mmol/l, i.e. from the relationship in figure 5.9. These relationships provided the most reasonable approximations given the limited information available.

Mann et al. (1991) noted that "there was no statistically significant increase in

optimum CETP activity in hypertriglyceridaemic plasma", meaning that the VLDL triglyceride concentration and the CETP activity are not causally linked, and can therefore be represented as marginally independent, depending upon the "potential" CE transfer (see section 3.3.2.1 for a discussion of marginal independence).

Composite model normo- and hyper- triglyceridaemia

Figure 5.11 illustrates the relationships used in the tabulated-normal model of "potential cholesteryl ester transfer from HDL to VLDL".

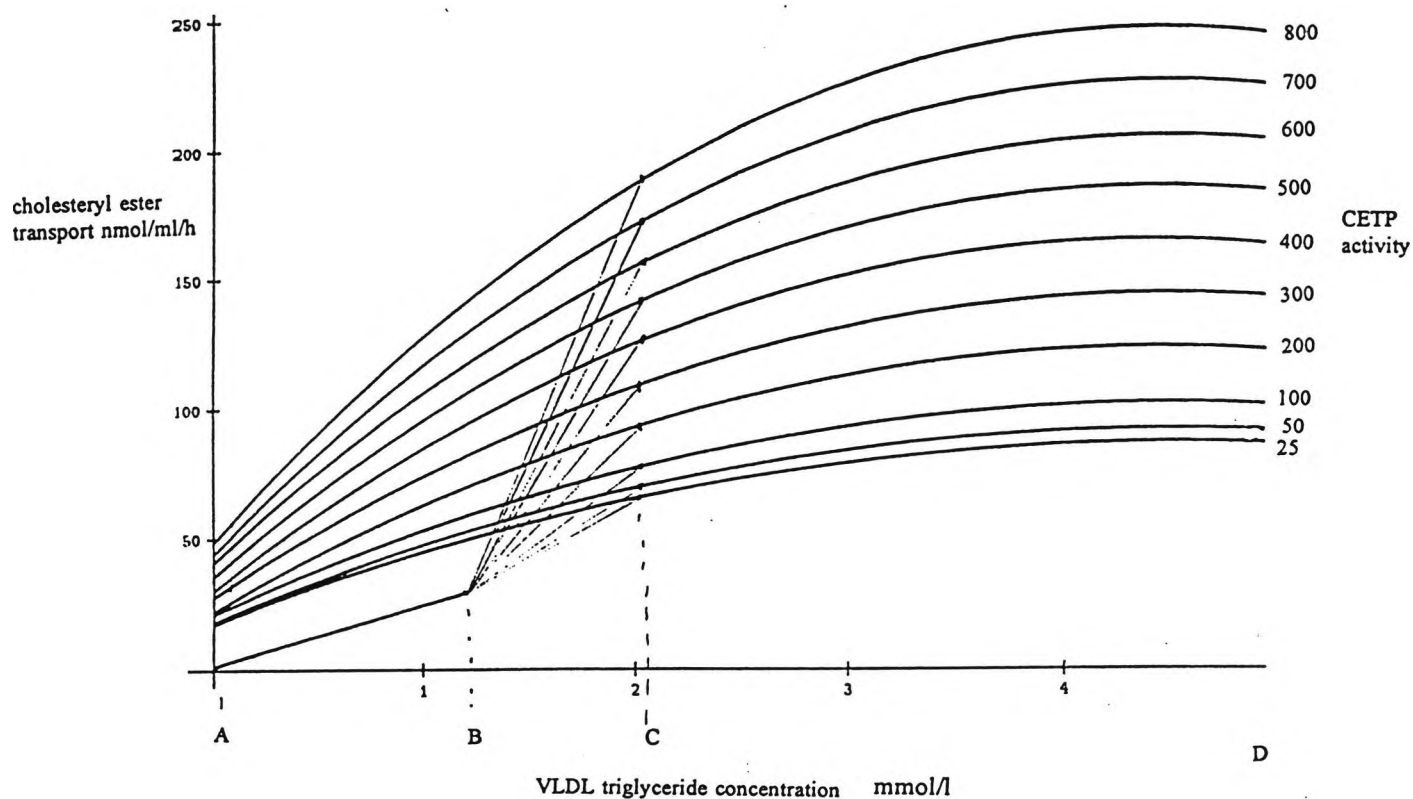


Figure 5.11 - Variation in cholesterol ester flux with different VLDL triglyceride concentrations and CETP activities

This graph is in three parts. A-B represents the linear relationship between CE flux and VLDL TG which exists up to a triglyceride concentration of approximately 1.2mmol/l. In this part of the graph uncertainty was represented by using the standard deviation generated from the least squares fit. C-D represents the relationship when the VLDL triglyceride concentration is responsible for very little variation in the CE flux, which is rate limited by CETP activity. In this section of the graph uncertainty was represented using the a rule of thumb developed by Andreassen (1991). In between VLDL triglyceride concentrations of 1.2 and 2.0 i.e. B-C approximations of cholesteryl ester transport have been made by drawing straight lines between the end of the non-hypertriglyceridaemic relationship and the 2 mmol/l point on each of the relationships for hypertriglyceridaemia, as illustrated in figure 5.11. Once again the rule of thumb was used to estimate uncertainty, however in this case the values estimated were much greater reflecting our uncertainty about this part of the graph.

5.6.2.2 The reduction in potential transport when receiving particles are saturated.

As discussed in section 5.5.5.1, the model presented here assumes that elevated FC:PL ratio indicates saturation of VLDL and LDL in receiving cholesterol esters from HDL, and hence a reduced cholesterol flux along this pathway (reduced below the potential, the flux may still be elevated above normal).

Numerous studies detail the elevation in the FC:PL ratio in different disorders (see section 5.5.5.1). However, to the author's knowledge, there is only one study which represents the relationship between the FC:PL ratio and the CE transport rate (Fielding et al., 1984a). This relationship is recreated in figure 5.12.

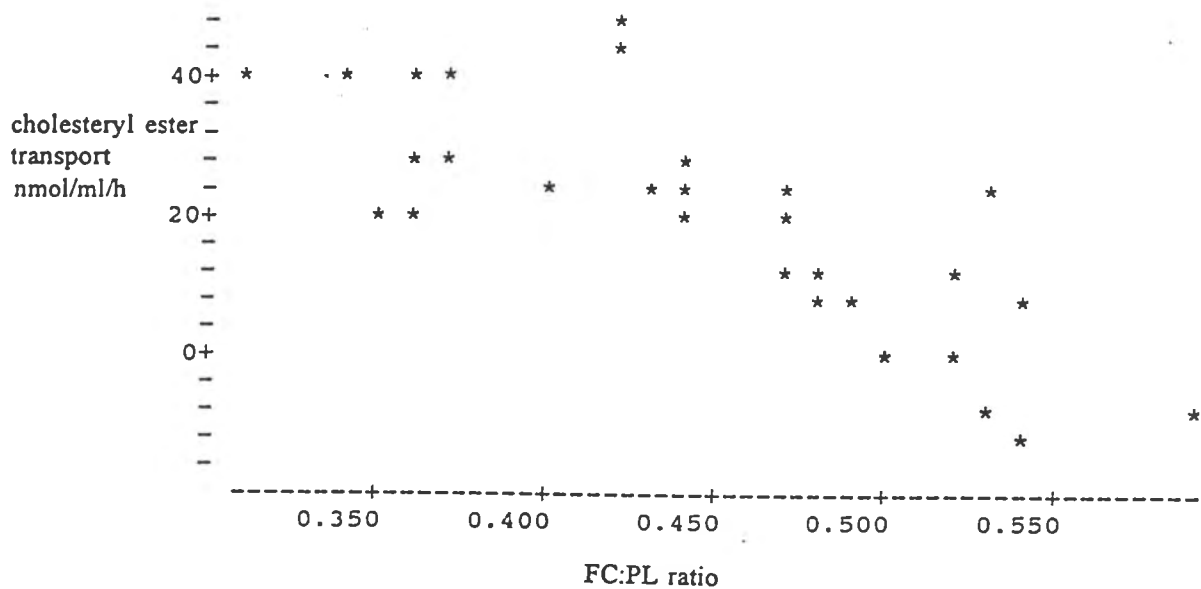


Figure 5.12 - Variation in cholesterol ester transport with saturation of VLDL (as indicated by FC:PL ratio)

In this study, Fielding et al. (1984a) examine the relationship between the FC:PL and the CE transport in both normolipidaemic and diabetics, showing the linear relationship between these two variables as depicted in figure 5.12. The diabetics have a much greater total triglyceride concentration and are therefore likely to have increased "potential" for CE transport from HDL to VLDL/LDL if no saturation occurred. Therefore, the linear relationship shown in figure 5.12 does not express the increased downregulation in diabetes above that of normals. Interestingly, if the correlation between FC:PL and CE transfer rate were performed solely for normals then no significant correlation would exist, the relationship only being significant when the diabetic data are included.

Castro et al. (1985) extended this relationship to the post prandial state where "an increase in cholesterol transport out of the cells of $+0.19 \mu\text{g h}^{-1}$ was associated with a decrease in VLDL and LDL FC:PL ratio of 0.04. This comparison supports the concept of a linear relationship between cholesterol transport and FC:PL across the

physiological range". "Secondly, just as the increased FC:PL ratio of VLDL and LDL in diabetes is associated with a greatly reduced rate of cholesteryl ester transfer to VLDL and LDL, so the decreased FC:PL ratio of postprandial lipaemia is associated with an increase in transfer to the same lipoprotein class."

In the modelling of this chapter, saturation has been represented as a switch whereby if the FC:PL ratio is greater than or equal to 0.45 then saturation occurs and the potential transport rate is downregulated. If the FC:PL ratio is less than 0.45, the VLDL are assumed not to be saturated and as such the actual transport rate would equal the potential. Partial saturation is represented as a distribution across the FC:PL node.

In cases of saturation, downregulation of the potential transport rate is automated by scaling the potential transport rate such that the full range of states possible with hypertriglyceridaemia and varying CETP levels, i.e. 0-300 nmol/ml/h is scaled down to fit into the full range of transport rate as represented in the Fielding study for saturated VLDL associated with diabetes, i.e. 0-20 nmol/ml/h.

This is an inadequate representation to be of any use in management. Further studies relating the CE transport for individuals with different CETP activities, VLDL triglyceride concentrations and VLDL/LDL FC:PL ratios are required to fully examine this relationship.

5.6.2.3 Path 1 (figure 5.8) - model structure

The final structure of the model representing path 1, in figure 5.8, is illustrated in figure 5.13.

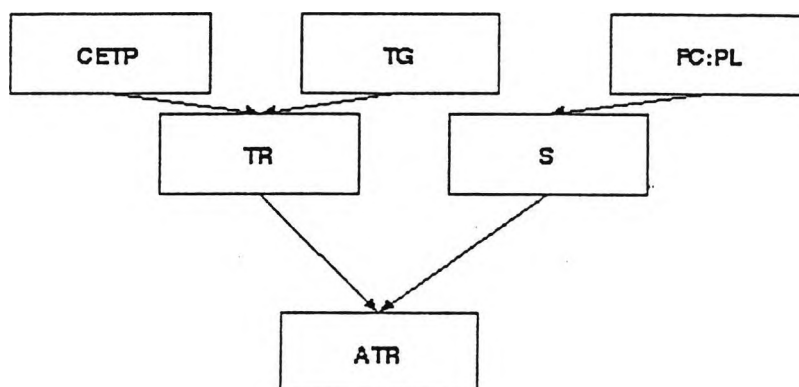


Figure 5.13 - Model illustration cholesterol ester transport from HDL to VLDL

5.6.3 Path 2 (figure 5.8) - HDL FC --> HDL CE, or the cholesterol esterification rate

5.6.3.1 Representation of esterification rate in terms of HDL free cholesterol concentration and saturation curve values of V_{max} and K_m

As discussed in section 5.5.2.1, HDL is the major site of cholesterol esterification in the plasma, such that the esterification of HDL cholesterol (i.e. path 2 figure 5.8) is synonymous with the total esterification rate.

Several papers have correlated clinical presentation of HDL with the esterification rate in the plasma. Dobiasova et al. (1991) and Karpe et al. (1990) both finding inverse linear correlations between the fraction of HDL FC esterified per unit time and the % of HDL in the HDL_{2B} subfraction.

Barter et al. (1984) have developed a fully integrated model of cholesterol esterification. In this model the esterification rate is dependent upon :- free cholesterol concentrations in HDL₂ and HDL₃ and V_{max} and K_m values for both HDL₃

and HDL₂. These values of V_{max} and K_m (for both HDL₂ and HDL₃) describe the saturation curves for cholesterol esterification when the experiment contains either HDL₃ or HDL₂ alone.

The model can be represented by the equation:-

$$\begin{aligned} \text{Esterification Rate} &= \frac{\frac{V2_{\max}[\text{HDL}_2\text{FC}]}{K_2} + \frac{V3_{\max}[\text{HDL}_3\text{FC}]}{K_3}}{1 + \frac{[\text{HDL}_2\text{FC}]}{K_2} + \frac{[\text{HDL}_3\text{FC}]}{K_3}} \quad (5.1) \end{aligned}$$

Ideally this equation could be represented as a causal structure as shown in figure 5.14.

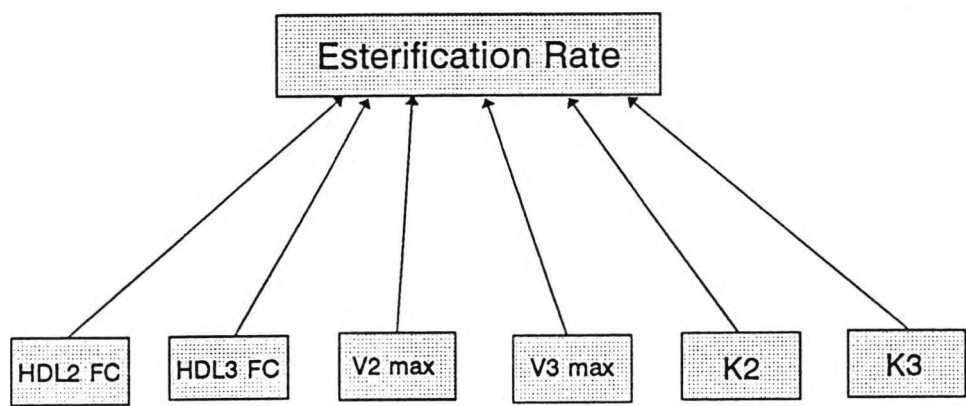


Figure 5.14- Ideal causal model of esterification equation

However, this was not possible as the facility is not available for creating a three dimensional tabulated-normal model in HMM (see section 3.6.2). Even if possible, this structure would lead to a very large clique size and poor propagation times (see section 3.3.3).

Further information was available in Barter et al. (1984) which allowed adaptation

of this causal structure, i.e. "the best fit of the model-predicted to the experimentally observed was obtained with a $V_{\max}$ for the esterification of HDL₃ free cholesterol that was 2.4-4.0 times greater than that of the $V_{\max}$ for HDL₂ free cholesterol", resulting in the model shown in figure 5.15

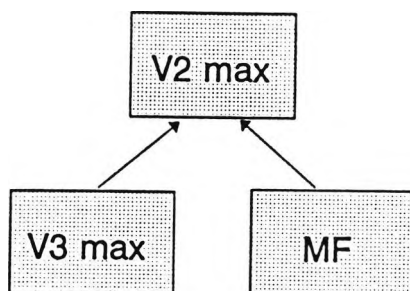


Figure 5.15 - Relationship between V3 max and V2 max

Where MF represents a multiplying factor ranging from 2.4-4 with an improper *a priori* distribution i.e. one in which all states are given equal values.

In this way it is possible to derive $V2_{\max}$ from $V3_{\max}$. As discussed in section 5.6.3.2, $V3_{\max}$ is thought to vary with the gradient gel electrophoresis profile of HDL₃ (Barter et al., 1985). Since $V2_{\max}$ has not been correlated with any measurable variable in other papers (at least to the author's knowledge), this representation between $V3_{\max}$ and $V2_{\max}$ becomes extremely valuable.

Whilst HDL₂FC and HDL₃FC are measurable experimentally, this is not true of K_2 and K_3 which are individual parameters with no physiological explanation for their variation. Automation of the full equation including these values of K would result in a complex structure and large clique sizes. Since K had no physiological explanation in the literature and simplification of the model structure was required, the sensitivity of the esterification rate to differing values of K_2 and K_3 was examined so as to determine whether fixing the values of K, and hence simplifying the model, would introduce significant error into the predicted esterification rate.

In the Barter experiments K_3 varies very little i.e. only in the range 43-58, whereas K_2 varies between 167 and 391. The largest error in fixing values of K would

therefore occur during high levels of HDL₂.

Table 5.2 illustrates the worst error possible in esterification rate for values of K₂ and K₃ at the extremes of the range reported in the study. The maximum error occurs when V3_{max} is high, the difference between V3_{max} and V2_{max} is at its greatest, HDL₂ FC is at its highest level and HDL₃ cholesterol is at its lowest level. Table 5.2 illustrates the variation in the cholesterol esterification rate in this situation, when values of K₂ and K₃ are varied across their complete range.

The greatest error possible is therefore 9.7 nmol/ml/h. However, by fixing K at its average value i.e, with K₂=300 and K₃=53.7, as shown in the last row of table 5.1, the maximum error possible is reduced to 5.2 nmol/ml/h, hardly significant.

By fixing values of K and representing V2_{max} as dependent upon V3_{max} the causal structure is simplified to that shown in figure 5.16.

V3max	V2max	HDL ₂ FC	HDL ₃ FC	K ₂	K ₃	C.E. rate nmol/ml/h
100	24	2500	100	167	43	32.3
100	24	2500	100	391	43	39.7
100	24	2500	100	167	58	30.0
100	24	2500	100	391	58	35.7
100	24	2500	100	300	53.7	34.5

Table 5.2 - The maximum error associated with fixing values of K

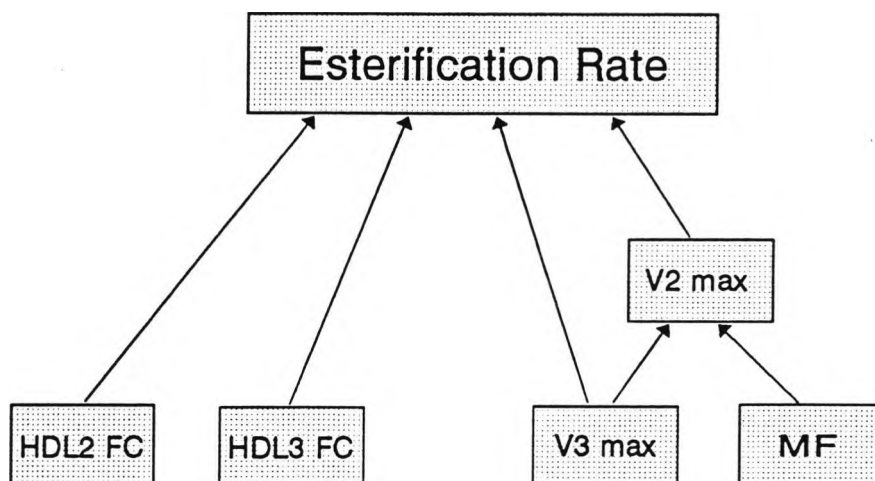


Figure 5.16 - Simplified model of esterification with Kmax fixed

A three dimensional tabulated-normal model would still be required to implement this structure. To remove this problem, intermediary nodes were included (figure 5.17) breaking the equation into components which could be represented using the "mult" and "add" facilities of HMM (see section 3.6.2).

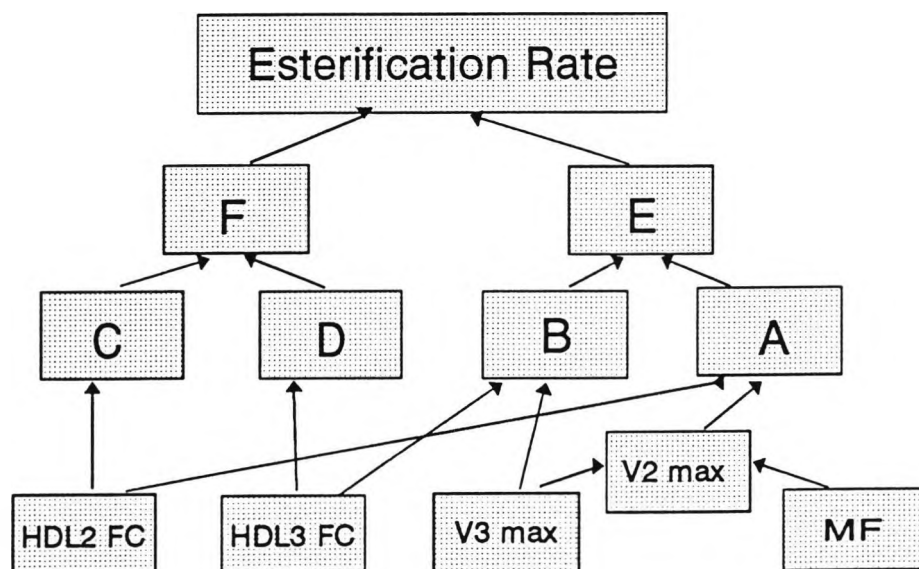


Figure 5.17 - Esterification model with intermediary nodes

In figure 5.17 the following expressions apply:

$$A = \frac{\text{HDL}_2\text{FC} * \text{V2}_{\max}}{K_2} \quad \text{or} \quad \frac{\text{HDL}_2\text{FC} * \text{V2}_{\max}}{300}$$

$$B = \frac{\text{HDL}_3\text{FC} * \text{V3}_{\max}}{K_3} \quad \text{or} \quad \frac{\text{HDL}_3\text{FC} * \text{V3}_{\max}}{53.7}$$

$$C = \frac{\text{HDL}_2\text{FC}}{K_2} \quad \text{or} \quad \frac{\text{HDL}_2\text{FC}}{300}$$

$$D = \frac{\text{HDL}_3\text{FC}}{K_3} \quad \text{or} \quad \frac{\text{HDL}_3\text{FC}}{53.7}$$

$$E = A + B \quad F = 1 + C + D \quad \text{Est} = \frac{E}{F}$$

The resolution in the intermediary node states was adjusted so as to: (a) minimise the clique size and hence reduce propagation time, whilst (b) preventing double peaks from appearing in the distributions on these nodes. A series of esterification models with differing resolutions were constructed until the optimum trade-off between resolution and propagation speed was found.

The output of the esterification model is illustrated in table 5.2. As can be seen, only single peaks are present in the distributions describing the esterification rate.

After implementation the model was tested to see if it predicted esterification rates which corresponded to those predicted by the equation (which was represented in a spreadsheet). The overall results (illustrated in the "Mean of distribution" and "calculated value from spreadsheet" in table 5.3) yielded good agreement within 4%.

Distribution about esterification rate									Mean	Instantiated values				Calculated esterification rate from spreadsheet
0	10	20	30	40	50	60	70	80	i.e. peak	V ₃ max	MF	HDL ₂ FC	HDL ₃ FC	
80	8	11							3	5	2.4	100	100	3.13
24	30	42	3	1					12.7	20	2.4	100	100	12.5
		3	35	26	23	10	2		40.4	65	2.4	100	100	40.7
		3	38	27	22	9	1		39.9	65	4	100	100	39.6
77	23	1							2.5	5	2.4	2500	100	2.38
1	36	55	8						17	35	2.4	2500	100	16.7
		2	26	55	15	3			39.5	80	2.4	2500	100	38.1
				9	41	35	11	4	56	80	2.4	2500	500	54.9
						41	36	23	68.2	80	2.4	2500	1700	68.6
						6	52	42	73.6	80	2.4	2500	2500	71.6
						9	31	60	75.1	80	2.4	1700	2500	75.6
48	52								5.2	5	4	900	2500	4.68

Table 5.3 - Comparison of model and equation predicted esterification rates.

5.6.3.2 Using the HDL₃ GGE profile to determine V3_{max}.

Barter et al. (1985) have studied the effects of the HDL₃ distribution on V3_{max} illustrating that smaller HDL₃, that is HDL_{3D}, are associated with increasing levels of V3_{max}.

Also, recent programs developed by Verdery et al. (1989, 1993) allow us to estimate the proportion of HDL in each subfraction directly from gradient gel electrophoresis curves. This is done interactively, by fitting gaussian curves to the peaks of the gradient gel electrophoresis profiles, these curves then being automatically integrated, the percentage area under each curve representing the proportion of HDL in that subfraction.

In an attempt to illustrate how the GGE profile measurement might be used to estimate V3_{max}, the areas under the curves in the Barter paper (1985) have been estimated and a weighted volume calculated for each GGE profile where:-

$$\text{weighted volume} = \sum_{\text{each HDL}_3 \text{ subfraction}} (\text{radius}^3 * \% \text{area under that subfraction}) \quad (5.2)$$

In doing so a correlation between the weighted volume and V3_{max} was obtained for each of the three studies in the Barter et al. paper (1985). All studies showed a significant negative correlation between the weighted volume and the V3_{max} individually. When two of these studies were pooled (2 & 3) the following relationship was obtained:-

$$V3_{\text{max}} = 273 - 0.032 \text{ weighted volume} \quad (5.3)$$

where adjusted $R^2 = 72.9\%$. Including the data from the first study reduced the significance of this relationship greatly. This equation was then used in a tabulated-normal model to build the model structure illustrated in figure 5.18. The nodes r4.55, r4.2, etc. represent the percentage of total HDL₃ in the subfraction with the respective radius (i.e. 4.55, 4.2 etc.) as its mean. The constraint node (CONST2) is an addition of r4.55, r4.2 etc., which needs to be instantiated to 100 before the model is run. This normalises the percentages at each of the root nodes so that they sum to 100. r³% represents the weighted volume.

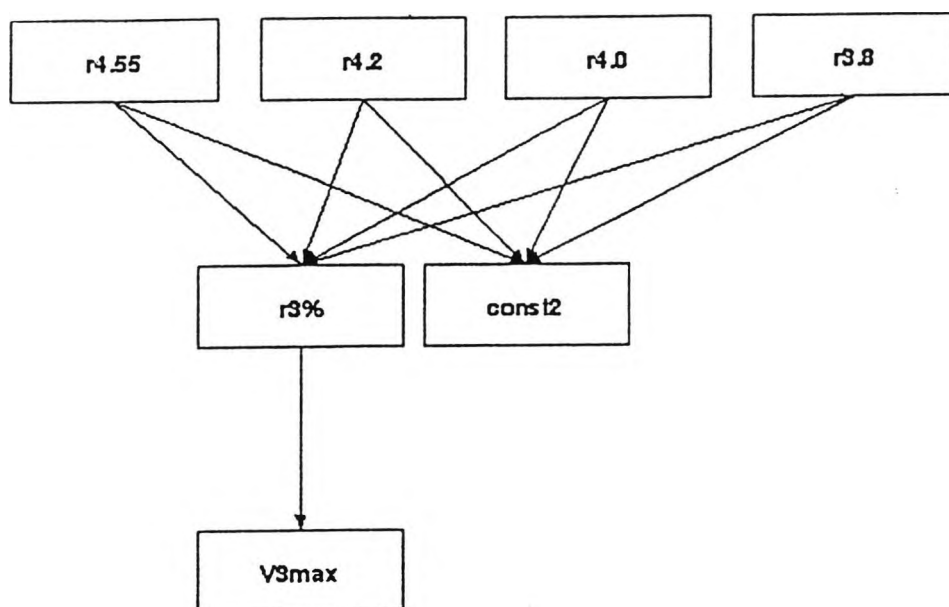


Figure 5.18 - Variation in $V3_{\max}$ with the distribution of radius in HDL

5.6.4. Combining "HDL CE to VLDL/LDL" (path 1, figure 5.8) and "cholesterol esterification rate" (path 2 figure 5.8) using a model constraint.

In the steady state condition the transport rate of cholesterol out of HDL must equal that input, that is the cholesterol flux along path 1 = cholesterol flux along path 2 (see figure 5.8).

Links between the HDL CE transport rate and the esterification rate are strong. As Castro et al. (1985) note "In human plasma, the free and ester cholesterol composition of the circulating lipoproteins is determined in large part by the activity of three coupled reactions. These are the transport of free cholesterol for esterification from either cell membranes or plasma lipoproteins; the esterification of cholesterol of either origin by the action of lecithin:cholesterol acyltransferase (LCAT); and the transfer of cholesteryl esters so formed to acceptor lipoproteins."

This constraint can be implemented by subtracting the esterification rate from the transport rate in a new node "CONST" which is instantiated to zero before the model

is used, thus fixing the flows along these two pathways to be equal.

To facilitate the linking of the transport and esterification models the resolution on both submodel states needed to be altered. However, increasing the number of states on the esterification side of the model re-introduced the large clique sizes seen in section 5.6.3.1 and the slow propagation times. Once again the trade off between resolution and propagation speed was performed. Unfortunately, no adequate compromise could be obtained, and with a reasonable propagation speed a double peak would occasionally present in the esterification rate node.

Combining the models of these two pathways produced the following causal structure (figure 5.19).

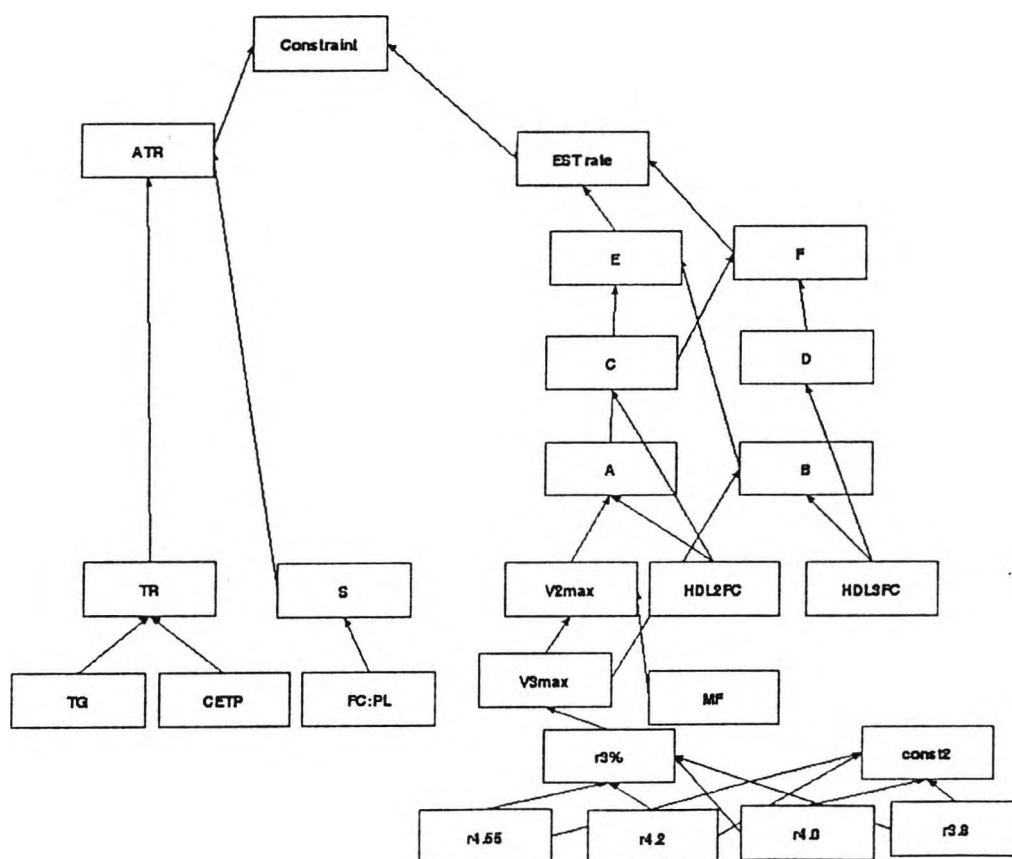


Figure 5.19 - A model combining cholesterol ester transport and esterification

5.6.5 Path 3 (figure 5.8) - The ratio of free cholesterol obtained for esterification from either tissues or VLDL/LDL

The esterification rate alone provides very little information about the removal of cholesterol from the periphery. As noted in section 5.5.6, increase esterification rates due to increased triglyceride concentration can indicate either increased or decreased atherogenicity, depending upon the amount of surface component of VLDL released during lipolysis for the formation of HDL

In summary, the quantity of cholesterol removed from the tissues is likely to depend upon the VLDL triglyceride concentration driving CE transport, and hence cholesterol esterification, and the composition of VLDL such that the release of smaller VLDL with larger surface:core ratios will have more free cholesterol per unit of triglyceride. Therefore any increase in esterification rate due to increased triglyceride concentration will be used by the proportionally increased FC concentration in the larger lipoproteins.

To the author's knowledge only two studies have estimated the ratio of cholesterol for esterification either being removed from the peripheral tissues or from the larger lipoproteins. These studies, Francome et al. (1989) and Castro et al. (1985), estimated this ratio in the fasting and postprandial state respectively. A further study (Fielding, 1987) reports estimated FC fluxes required for esterification from both plasma and the tissues in the fasting state, the postprandial state, and during uncontrolled NIDDM, controlled NIDDM and during poor renal function. This last study does not report the number of patients involved, neither does it give estimates of the standard deviation for the measurements.

Castro et al. (1985) report the cell-plasma ratio to be 4.5 ± 1.5 ($n=5$) in the postprandial state, commenting that "LCAT uses cell derived cholesterol even more efficiently (in relation to plasma lipoprotein cholesterol) in postprandial lipaemia than it does in fasting". This would lead to the conclusion that in fasting the ratio should be lower than 4.5. This is consistent with Fielding (1987), the ratios from this study

being calculated as:- Fasting 3.5, PP 5.75, uncontrolled NIDDM 0.024, controlled NIDDM 1.28.

However a ratio of less than 4.5 in the fasting state is inconsistent with the findings of Francome et al. (1989) who report the fasting ratio to be 8.2 ± 2.1 (n=7).

No studies have been found in the literature that have tackled the relationship between the concentration and composition of VLDL and this ratio. In an attempt to use the limited information available the following structure, shown in figure 5.20, has been automated.

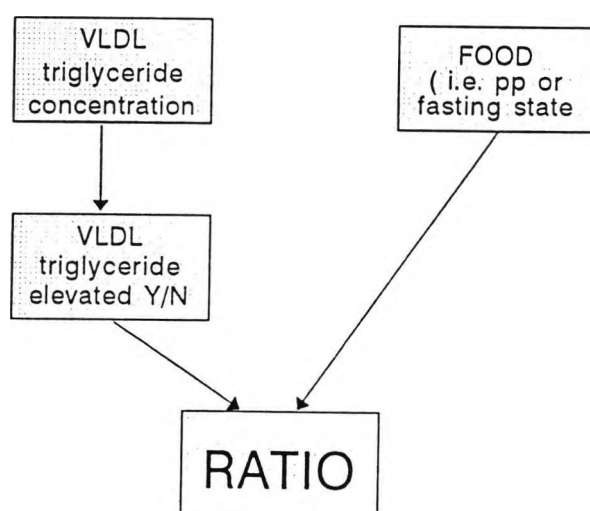


Figure 5.20 - Model describing the source of cholesterol for esterification as a proportion of the esterification rate

In this model the following assumptions have been adopted:

- a) An increased VLDL triglyceride concentration indicates more VLDL in the plasma and hence a greater amount of free cholesterol requiring esterification from this source. Increases in the VLDL triglyceride concentration therefore reduce the ratio of tissue: plasma cholesterol as a source of esterification.
- b) The fasting or postprandial state is a surrogate measure for the size of the VLDL particle released. In the fasting state smaller VLDL results in an increase in FC per unit triglyceride and hence a decrease in the ratio of tissue: plasma cholesterol as a

source of esterification. The converse is true in the postprandial state.

It is also possible that the GGE profile of HDL or HDL₃ could provide information on the source of free cholesterol for the tissues (see section 5.5.6). The HDL₃ subfraction distribution not only provides information on the overall esterification rate, but also on the source of free cholesterol for this esterification. A study to investigate these relationships would be extremely useful.

Given the current situation, the structure is limited to that represented in figure 5.20. The ratio is represented as a function of fasting/postprandial state and whether the triglyceride is elevated or not (y/n). This reduces the tabulated normal model to a 2*2 matrix, table 5.4, which is appropriate given the extremely limited information available.

	Fasting state	Postprandial state
Elevated triglyceride	1.5	4.5 $\pm$ 1.5 (from Castro)
Normal triglyceride	8.2 $\pm$ 2.1 (from Francome)	12

Table 5.4 - Variation in cholesterol source ratio with modification of VLDL composition

In table 5.4, the figures 1.5 and 12 are estimates taken from Fielding (1987).

5.6.6 Total reverse cholesterol transport

Reverse cholesterol transport can now be estimated as the percentage of the free

cholesterol from the peripheral tissues multiplied by the total esterification rate. The full reverse cholesterol transport model is illustrated in figure 5.21.

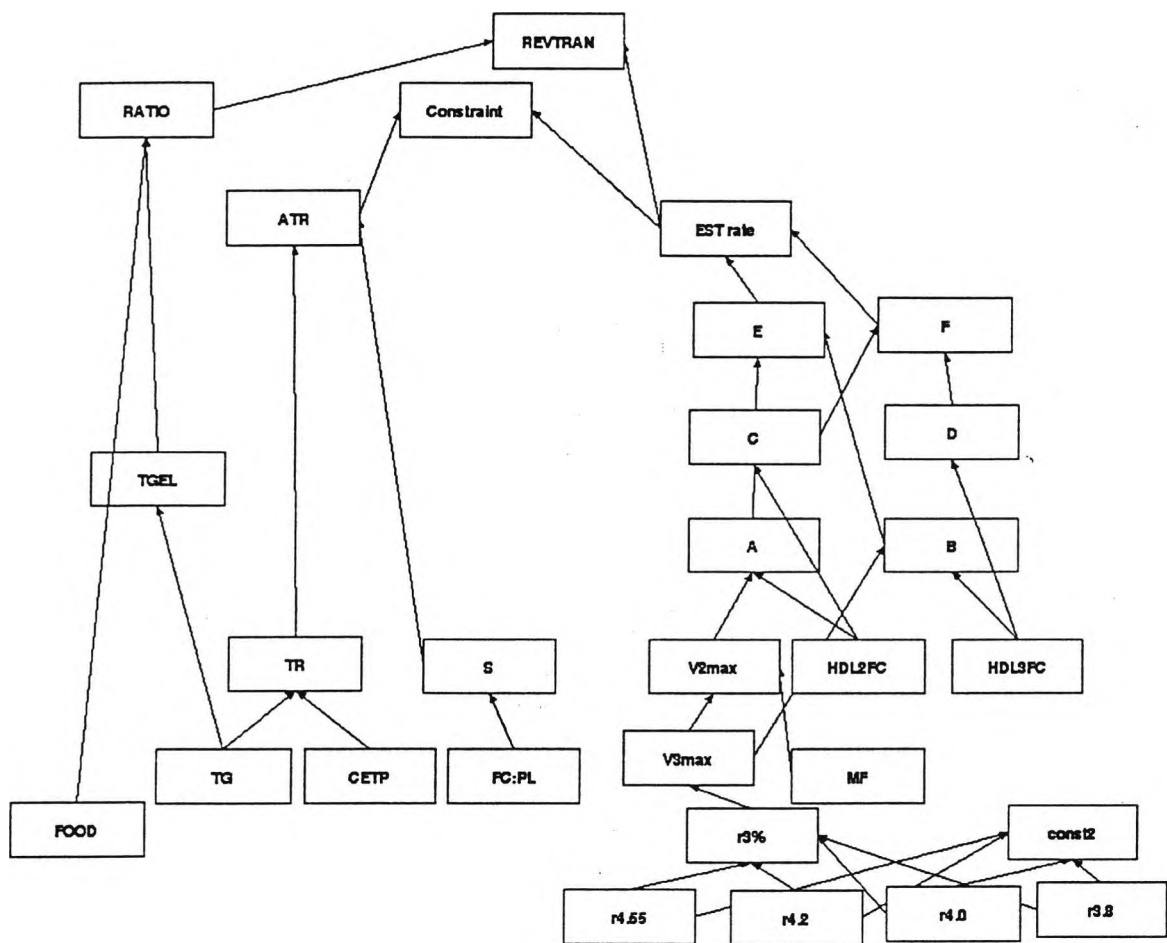


Figure 5.21 - A model of reverse cholesterol transport

5.7 Incorporating Forward and Reverse Cholesterol Transport into a Single Model

The risk of CHD due to modification in the lipid and lipoprotein metabolism can now be represented as the difference between non-receptor mediated cholesterol uptake at the peripheral tissues and reverse cholesterol transport as modelled in sections 5.3 and 5.6 respectively. The final model structure, which includes "POTATHERO" (potential for atheroma node), is shown in figure 5.22.

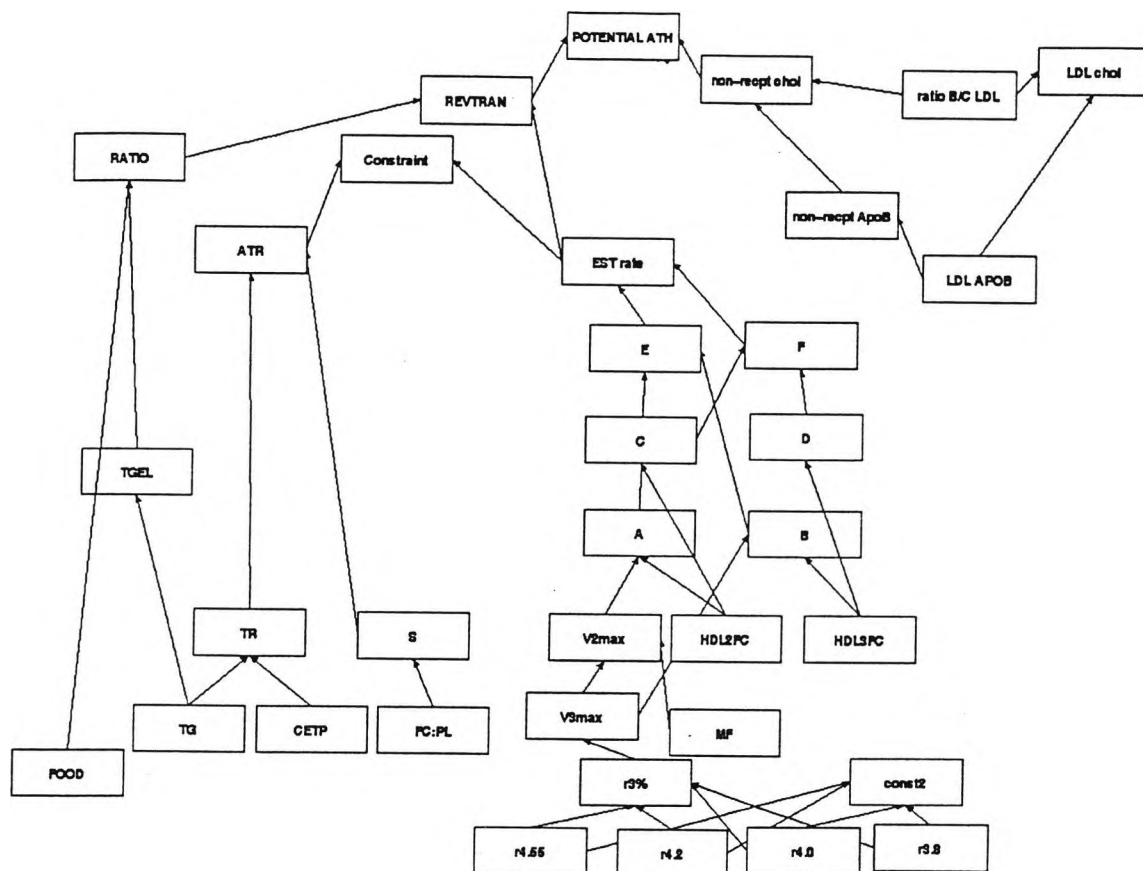


Figure 5.22 - A model of forward and reverse cholesterol transport

5.8 Summary

In this chapter a model of an individualised measure of CHD risk due to abnormalities in lipid and lipoprotein metabolism has been described. This measure, defined as an individual's potential for atheroma, is due to cholesterol deposition and is therefore modelled as the difference between cholesterol transport to and from the peripheral tissues.

A model of cholesterol transport to and from the periphery has been constructed as a CPN using both expert opinion describing physiological pathways, and literature data describing the quantitative nature of relationships on these pathways.

This chapter has addressed many controversial issues concerning reverse cholesterol transport; for example, whether cholesterol transport to the liver via larger lipoproteins is normal physiological pathway, and how larger lipoproteins saturated with cholesterol effect cholesterol transport through HDL. In considering these issues it has been possible to construct a model of cholesterol transport to and from the periphery which is consistent with expert opinion and experimental data.

In chapter 7 validation of this model is performed in qualitative and quantitative terms. In doing so consistency is illustrated between this model and data at other levels of abstraction. Chapter 8 (section 8.4) draws all of these findings together, and concludes on the nature of the model.

Chapter 6 - Modelling Triglyceride and Apoprotein B Metabolism

6.1 Introduction

Chapter 5 described and modelled the flow of cholesterol to and from the peripheral tissues, representing an individual's potential for atheroma as the difference between these two fluxes.

To enable prediction of an individual's potential for atheroma, due to genetic disorders, modification of obesity, and treatment, it is necessary to extend this model, incorporating the effects of these perturbations. Then, by predicting changes in atheroma potential for different treatment options, treatment can be selected so as to minimise the cholesterol deposition at the periphery, and hence CHD risk.

In this chapter extension of the model developed in chapter 5 is limited to the effects of obesity and genetic disorders such as familial hypertriglyceridaemia (FHTG), familial combined hyperlipidaemia (FCHL) and familial hypercholesterolaemia. This reduces the complexity of the model, providing a core structure for future development to include the action of treatment. As illustrated in section 3.5 modelling the structure of metabolic pathways enables the effects of treatments to be represented on a few variables which update the whole model, rather than on all the variables on which the treatment is known to have an effect, thus reducing the density of network construction.

The effects of obesity and genetic disorders are to modify the production and catabolism of lipoproteins. In terms of cholesterol fluxes, these perturbations modify both the cholesterol release into the plasma and the receptor mediated uptake of cholesterol from the plasma as illustrated in figure 6.1.

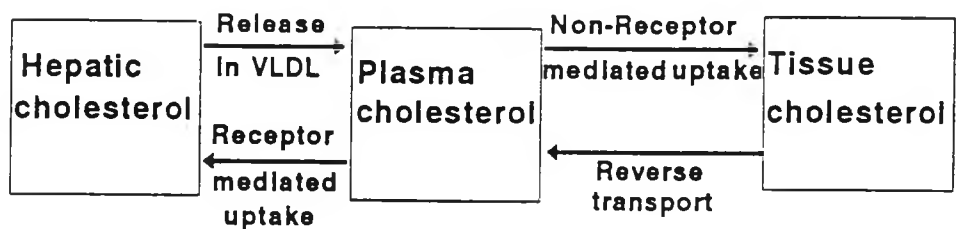


Figure 6.1 Cholesterol fluxes to and from the plasma

Figure 6.1 illustrates the cholesterol fluxes to and from the plasma pool, including those to and from the peripheral tissues modelled in chapter 5. At a steady state the cholesterol fluxes to the plasma pool equal those output from it, enabling the equality constraint illustrated in figure 6.2 to be used. As discussed in section 3.4, when managing individuals with disorders of lipid and lipoprotein metabolism, we are concerned with the shift from one steady state to the next that occurs when treatment is altered, so that for any individual visit to the lipid clinic a patient will have reached their new steady state and hence this constraint can be adopted.

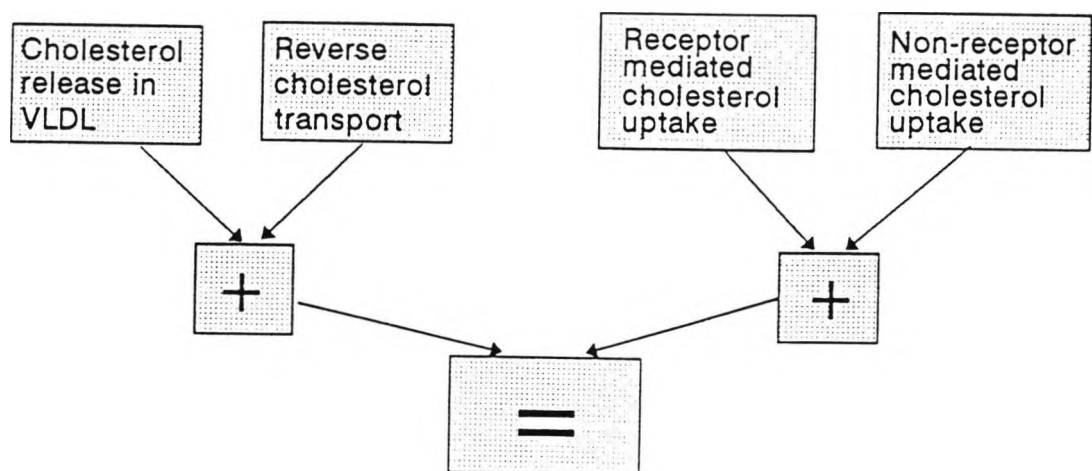


Figure 6.2- Constraint illustrating equilibrium in plasma cholesterol fluxes

However, the root nodes in figure 6.2 are not independent. For example, both the non-receptor mediated uptake of cholesterol and the cholesterol release into the plasma are dependent upon the synthesis and composition of VLDL (VLDL being the precursor to LDL, elevated concentrations of which increase non-receptor mediated uptake). Therefore, the constraint relationship, whilst useful, is an inadequate representation of these dependencies. In this situation a model of the complete VLDL and LDL metabolism is required which represents explicitly the dependencies between the root nodes in figure 6.2. These dependencies can be represented by modelling VLDL and LDL metabolism so as to predict those root nodes of the model in chapter 5 (see figure 6.3) which do not represent genetic variability, but vary as a consequence of changes in VLDL and LDL metabolism. These are VLDL triglyceride concentration, the VLDL FC:PL ratio, LDL apo B concentration, HDL free cholesterol capacity, and LDL cholesterol concentration. These variables may be thought of as being causally related to atheroma potential, as illustrated in figure 6.3. The model of VLDL and LDL metabolism must also be able to predict cholesterol release into, and receptor-mediated uptake from, the plasma so that the constraint illustrated in figure 6.2 can be used.

Representing the whole of VLDL and LDL metabolism is an enormous problem. However, in a similar way to the approach adopted for HDL metabolism, it might be possible to model relationships between the patient's state (i.e. underlying disorders and obesity) and intermediate variables measured in the plasma, e.g. the VLDL apo B / triglyceride ratio; and then between these intermediate variables and those variables in figure 6.3 which are dependent on VLDL and LDL metabolism.

This chapter describes VLDL and LDL metabolism, illustrating the different pathways involved in the delipidation and hepatic removal of these lipoproteins. It describes why lipoprotein transport via different pathways is relevant to the prediction of those root nodes illustrated in figure 6.3. The chapter discusses the significance of the composition of newly synthesised lipoproteins on their metabolic pathway and models the effects of different disorders on this composition. Insufficient data are available to construct a complete model, so that possible relationships have been

the triglyceride synthesis rate and the availability of those substrates used to package the triglyceride into lipoproteins. In VLDL this packaging consists of protein (mainly apoprotein B), free cholesterol and phospholipid.

VLDL released into the plasma has its triglyceride removed by the action of lipoprotein lipase, thus becoming smaller and more cholesterol enriched. Some of this lipolysed VLDL is removed as VLDL remnants by the liver whilst the rest, intermediate density lipoproteins (IDL), are lipolysed further forming a population of smaller lipoproteins known as low density lipoproteins (LDL), before subsequent hepatic uptake.

Lipoprotein removal is controlled by the action of apo B100/E receptors and, in the case of VLDL remnant removal, the apo E phenotype. The basic model of the VLDL "cascade" is illustrated in figure 6.4.

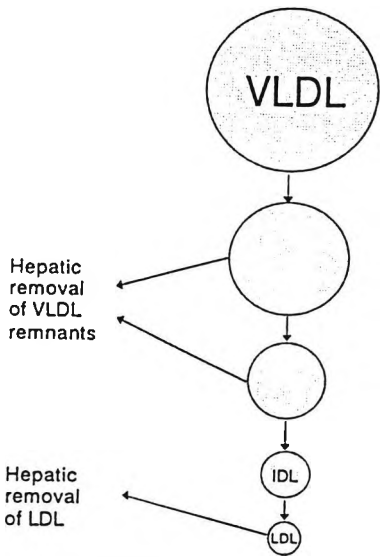


Figure 6.4 - The VLDL cascade

6.2.2 VLDL/LDL transport pathways

6.2.2.1 Apo B as a marker of VLDL/ LDL metabolism

In order to estimate the metabolic pathways of VLDL and LDL there is a need for a marker of synthesis, transport and catabolism of these lipoproteins. Apoprotein B

(apo B) serves this purpose as it remains with the lipoprotein throughout its duration in the plasma.

Apo B metabolism has been modelled in numerous studies, using compartmental models of isotope decay curves to represent its progression through the cascade. These studies are too numerous to reference here, but examples are given in the text throughout this chapter. These compartmental models often represent different physiological interpretations of VLDL and LDL apoprotein metabolism. For example, model values of apoB synthesis can mean either cascade synthesis, nascent synthesis (see section 6.2.2.3) or even apo B synthesis into different VLDL subfractions. It is necessary then, when using the data from these studies to represent the metabolism of lipoproteins, to carefully interpret their results so that :-

- a) data may be pooled, where they have the same meaning i.e. comparing like with like
- b) relationships can be generated from these pooled data
- c) consistency can be illustrated between literature data from studies where different pathways have been modelled
- d) it is possible to determine which of these pathways reported are relevant to the prediction of i) the root nodes of the model in chapter 5, and b) cholesterol input to, and receptor-mediated removal from, the plasma pool.

In the discussion of lipoprotein metabolic pathways in the whole of section 6.2 and in model construction (section 6.4), the results of studies describing apoB metabolism have been interpreted so that only data reporting the same pathways have been compared and pooled.

6.2.2.2 VLDL transport through the cascade

In normal patients the majority of VLDL released into the cascade is lipolysed becoming LDL. Abnormalities in triglyceride metabolism modify cascade throughput increasing the removal of VLDL as remnants, and hence, reducing LDL synthesis from the cascade.

Physiologically this is thought to occur in the following way. Increased triglyceride concentration in VLDL promotes cholesteryl ester transport from HDL to VLDL in exchange for triglyceride (see section 5.5.5.1). According to James et al. (1989) cholesterol rich remnants "resist further hydrolysis".. "and are thought to be catabolised via receptor mediated mechanisms. They therefore make little contribution to the production of LDL". Packard et al. (1984) also found that large triglyceride rich VLDL particles were cleared as remnants and did not appear as LDL. They comment that "within the Sf 20-100 flotation range there are at least two sub-compartments. One represents remnants of larger triglyceride-rich particles which are catabolised slowly and feeds little apo B into LDL. The other is apparently secreted directly into this flotation interval and transfers significant amounts of B protein rapidly into Sf 0-12 lipos".

Clearly, the quantity of cholesterol in lipolysed VLDL, and hence its susceptibility to removal as remnants, depends upon the triglyceride loading of newly synthesised VLDL. Triglyceride loading of VLDL might then be a marker of the metabolic pathway of VLDL. Indeed, the triglyceride loading on newly synthesised VLDL (as measured by the VLDL triglyceride/apoB synthesis ratio) has been shown to be modified in different disorders which alter lipoprotein metabolism, for example:-

Familial combined Hyperlipidaemia (FCHL) - Controversy exists as to the action of FCHL. Assuming that FCHL causes overproduction of apoB, i.e. hyperapobetalipoproteinemia, is consistent with its multiple phenotypic presentation in the same family. That is, when FCHL is the only disorder present, it results in a higher concentration of LDL, presumably because of the production of smaller VLDL

particles, which would not be removed as remnants. However, in some situations FCHL presents in conjunction with a secondary hypertriglyceridaemia such that both apoB synthesis and triglyceride synthesis are elevated and hence VLDL is of normal composition. In this situation normal or increased amounts of VLDL remnant removal may occur resulting in a lower cascade production of LDL and hence lower LDL concentrations.

Familial hypertriglyceridaemia (FHTG) increases production of triglyceride resulting in the synthesis of larger VLDL into the cascade (Kissebah et al., 1981), and hence greater removal of VLDL as remnants.

Obesity increases the synthesis of both apoB and triglyceride, resulting in overproduction of particles of normal composition (Egusa et al., 1985). This is consistent with the results of Ginsberg et al. (1985), who found no pattern of change in the VLDL triglyceride/apoB synthesis ratio during weight loss in obese patients.

Diabetes has been shown either to result in an overproduction of particles of normal composition (Kissebah et al., 1982; Abbott et al., 1990), or (Howard et al., 1987) to result in the release of larger lipoproteins due to an "increased production of VLDL triglyceride, but not VLDL apo B" increasing the removal of VLDL as remnants. This apparent contradiction may be due to the strong links between obesity and diabetes, which often preset together. As Howard et al. (1987) note "the obese state in this population, whether or not diabetes is present, causes overproduction of VLDL-apoB and VLDL triglyceride, while the imposition of NIDDM in obese subjects mainly accentuates the excessive production of VLDL triglyceride".

It is clear from this discussion that disorders perturbing the coupling of triglyceride and apoprotein B in the formation of VLDL result in different metabolic pathways for the transport and catabolism of VLDL. The synthesis ratio of triglyceride/ apoB into the VLDL cascade might then be a good surrogate measure describing VLDL transport.

The relationships between disorders, VLDL composition, and the resultant metabolic pathways are examined further in section 6.4. That section concludes that the average triglyceride/ apoB synthesis ratio may be insufficient to describe VLDL metabolism, but that the triglyceride / apo B synthesis ratio in different VLDL subfractions may prove very informative.

6.2.2.3 Direct LDL apo B synthesis

In several studies which have modelled compartmentally the complete VLDL and LDL apo B metabolism it has been necessary to postulate direct synthesis of LDL (Ginsberg et al., 1985; Kissebah et al., 1984; Kesaniemi et al., 1985; Egusa et al., 1985; Vega et al., 1988; Abbott et al., 1990; Taskinen, 1990; James et al., 1989; Vega et al., 1989). As Grundy and Vega (1990) note "When isotope kinetic studies examine the metabolism of VLDL apo B and LDL apo B simultaneously, the levels of LDL apo B are not explained by the input from VLDL apo B. This finding fostered the concept that the liver can secrete LDL particles directly into the circulation". As will be discussed in section 6.3.5, synthesis of LDL via this pathway might alter the eventual concentration and composition of LDL, and is therefore relevant to the modelling described in this chapter.

A physiological explanation for direct synthesis of LDL has been hypothesised by Shames and Havel (1991). They suggest that all lipoprotein synthesis is into a nascent pool of VLDL with a great deal of heterogeneity of particle size. Then, in a number of these nascent particles, triglyceride is thought to be catabolised rapidly resulting in an apparent direct synthesis of LDL particles, the remaining particles entering the VLDL cascade as normal, as illustrated in figure 6.5 .

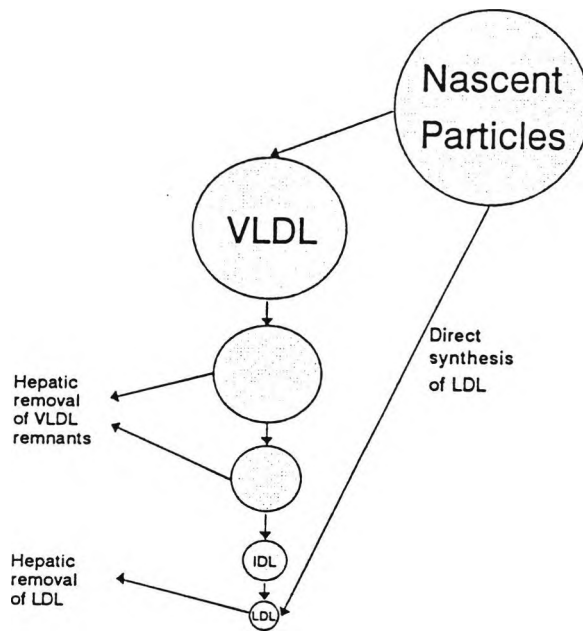


Figure 6.5 - The VLDL cascade

Several studies have illustrated direct synthesis of LDL in different disorders:-

Parhoefer et al. (1991) found that in normal patients "37% of LDL apo B was derived directly from the rapidly turning over compartment". Janus et al. (1980) on the other hand found that in normals there was no direct synthesis, i.e. all LDL was synthesised via the cascade.

Kissebah et al. (1984) found significant direct synthesis of LDL in a group of FCHL.

In FH heterozygous both Janus et al., (1980) and Fischer et al., (1980) found that significant direct LDL synthesis occurred, the latter finding found little direct synthesis in unclassified hypertriglyceridaemics.

Both Taskinen et al., (1990) and Howard et al., (1987) found significant direct synthesis of LDL in diabetic patients.

As Shames and Havel (1991) note direct LDL synthesis may be no more than a population of VLDL which is lipolysed very quickly. Since, as noted in section 6.2.2.2, increased triglyceride loading on newly synthesised VLDL delays the

particle's transport through the cascade, one would assume that disorders with very low triglyceride/ apoB synthesis ratios, e.g. FCHL, would increase the transport rate of VLDL through the cascade, the extreme case resulting in direct LDL synthesis. This is consistent with the results of Kissebah et al., (1984) who found a strong negative correlation ($r = -0.88$) between the VLDL triglyceride/ apo B synthesis ratio and the percentage of LDL synthesised directly in a group of patients with FCHL. It is also consistent with the results of Fischer et al., (1980) who found little direct synthesis of LDL in unclassified hypertriglyceridaemic patients.

Once again perturbation in the coupling of triglyceride and apo B synthesis might be responsible for modification in the metabolic pathway of VLDL. However, Taskinen et al., (1990) and Howard et al., (1987) both found significant direct synthesis of LDL in diabetics. As noted in section 6.2.2.2, diabetes is associated with either elevated or normal VLDL triglyceride/ apoB synthesis ratios, which, assuming the above hypothesis, is inconsistent with increased LDL direct synthesis during this disorder.

As described in section 6.4.2.4, the VLDL triglyceride/ apoB ratio in different VLDL subfractions (VLDL₁ and VLDL₂) might be more indicative of direct synthesis, such that small particles in each VLDL subfraction cause greater LDL direct synthesis. This hypothesis is still consistent with the results of Kissebah et al. (1984), i.e. a good relationship between the percentage LDL directly synthesised and the average VLDL triglyceride/ apoB ratio. This study was performed in a group of FCHL patients, and homogeneity within this group could result in very similar compositions of VLDL₁ and VLDL for in the different phenotypes. The hypothesis could also explain the results of Taskinen et al. (1990) and Howard et al. (1987) since, in diabetes, a normal or increased average VLDL triglyceride/apoB synthesis ratio could represent a greater amount of VLDL₁ at the smaller end of the VLDL₁ spectrum, causing increased direct LDL synthesis as found in these studies.

6.2.3 Flaw in the hypothesis of VLDL and LDL pathway regulation

As noted in section 6.2.2.1 it is necessary when pooling the data from these studies to be careful in interpreting the meaning of the transport pathways modelled. In section 6.4.2.2 data describing the apo B synthesis rate into the nascent pool have been combined from many studies and correlated with the genetic disorder or level of obesity. When pooling these data it has been assumed that total nascent apo B synthesis reported in studies describing the apo B metabolism is analogous to apo B release into the plasma from the liver. Using this assumption presumes that no apoB is catabolised directly from the nascent pool or its precursor pools. Catabolism from these pools could reduce the Apo B₁₀₀/E receptors available for VLDL remnant and LDL removal, reducing the removal of atherogenic lipoproteins.

This assumption is contradicted by the compartmental model structures of Beltz et al., (1985) and Vega et al., (1988) where lipoprotein catabolism is illustrated directly from the nascent pool. Similarly, Grundy and Vega (1990) note the problem when "equating VLDL apo B flux rates with hepatic secretion rates for VLDL apo B " in that "newly secreted VLDL may be removed rapidly from the VLDL compartment, too rapidly to be traced accurately".

To improve the accuracy with which apo B production can be estimated recent studies have used endogenous tracers of apo B. Beltz et al. (1990) have modelled apo B metabolism using both exogenous and endogenous tracers. They conclude that "exogenous labelling underestimates the true production rate of apo B-containing lipoproteins to a greater extent than does endogenous labelling". However, even when using endogenous tracers the nascent apo B pool was not fully identified, as there was still a need to hypothesise direct LDL synthesis. Endogenous tracers simply "identified apo B much earlier in the VLDL chain than exogenous labelling.", but probably did not identify the true hepatic release of apo B into the plasma .

In the correlations performed in section 6.4.2.2 nascent apo B synthesis rates have been approximated by summing apo B cascade synthesis rates and LDL apo B direct

synthesis rates, but as indicated above, this does not cater for apo B losses from the nascent pool and its precursors even when the study has used endogenous tracers.

6.3 Relevance of the modelling of lipoprotein transport pathways.

6.3.1 Introduction

As stated earlier, the aim of chapter 6 is to construct a model linking disorders of VLDL and LDL metabolism to those variables providing information on atheroma potential, illustrated in figure 6.3. So far, section 6.2 has discussed the pathways of VLDL synthesis and catabolism, and the composition of VLDL during metabolic disorders.

This section describes the most likely effects of perturbations in VLDL and LDL metabolic pathways on the variables illustrated in figure 6.3, showing the relevance of modelling these pathways to the prediction of potential for atheroma. The relationship between the VLDL and LDL metabolism and each of the dependant root nodes in figure 6.3 are described in terms of causal dependencies, with section 6.4, investigating quantitative relationships for these dependencies. It has not been possible to model all the causal relationships of section 6.3 quantitatively, as in many cases the necessary experiments have not been performed.

6.3.2 HDL_{2/3} free cholesterol levels

In section 5.5.6 and 5.6.3.1 the HDL_{2/3} free cholesterol (FC) level was used as a description of the capacity of HDL for cholesterol esterification. This FC level was described as being limited by the apoprotein AI level, with a greater amount of apo AI increasing the HDL FC capacity. In turn, apo AI release during the lipolysis of

lipoproteins for use in HDL was predominantly from larger VLDL and chylomicrons. It is logical therefore to assume a causal relationship between the composition of newly synthesised VLDL and the HDL_{2/3} FC level.

6.3.3 VLDL triglyceride concentration

The physiological reason for VLDL release into the plasma is to supply triglyceride to the tissues in the fasting state. Consequently the VLDL triglyceride concentration is modified by the composition and amount of newly synthesised VLDL, and the lipolysis of triglyceride. This relationship is modelled in section 6.4.1.

6.3.4 VLDL FC:PL ratio

The surface composition of VLDL, as indicated by its FC:PL ratio, and its modification during different disorders of VLDL and LDL metabolism is well documented, and has been described in section 5.5.5.1. The physiological mechanisms behind modification of lipoprotein surface composition in atherogenic hyperlipidaemias (i.e. those due to diabetes, type III hyperlipidaemia, and hyperbetalipoproteinaemia (Fielding, 1984)) are not known, so that it has not been possible to construct a causal model linking disorders of the VLDL and LDL metabolism to the FC:PL ratio.

6.3.5 LDL apo B concentration, LDL cholesterol concentration

The following five factors have been considered when representing the LDL apo B and cholesterol concentration:-

1) *The amount of LDL apo B produced* - Increased production of LDL apo B from either direct or cascade pathways will result in an increase in LDL apoB

concentration, depending upon the hepatic removal capacity.

2) *The metabolic pathway through which LDL is produced* - Kissebah et al. (1984) found a positive correlation between the LDL cholesterol/ apoB ratio and the percentage LDL apo B synthesised by the direct route in a group of patients with FCHL, suggesting that LDL synthesised by the direct route is cholesterol enriched.

3) *The nature of the cascade lipolysis of VLDL* - Hypertriglyceridaemics, in which a large quantity of VLDL is removed as remnants, produce LDL via the cascade which is thought to be cholesterol depleted, as indicated by the polydisperse LDL of hypertriglyceridaemia (Fischer et al., 1980). In this case, according to Fischer (1980), the most predominant band of LDL "is a considerably smaller and more dense lipoprotein than are the lipoproteins found in most individuals with mono-disperse LDL."

4) *Hepatic removal of LDL* is also likely to alter the concentration and composition of LDL. As discussed in chapter 4, it is possible that a hepatic pool of cholesterol is responsible for apo B₁₀₀/E receptor generation, so as to preserve the balance within this pool. If true, then the greater the cholesterol enrichment of LDL particles the less LDL removal will be required to fill the hepatic pool. Similarly, if a large amount of apo B₁₀₀/E receptor capacity is used in the catabolism of VLDL remnants then this might lower hepatic removal of LDL.

Both 3) and 4) are consistent with the results of several studies in which synthesis of cholesterol depleted LDL via the cascade (due to increased VLDL remnant removal) increases the FCR of LDL apo B. For example Vega et al. (1988) found that in patients with type III hyperlipidaemia, in which VLDL is cholesterol loaded and hence LDL synthesised via the cascade is cholesterol depleted, FCRs for LDL apo B were abnormally high. Also Egusa et al. (1985) found that the small LDL produced in obesity increased LDL FCR. Ginsberg et al. (1985) also notes that in obese patients the FCR of LDL apo B was increased and the LDL cholesterol/ apoB ratio decreased. Indeed, Kissebah et al. (1984) found that in a group of FCHLs a

good negative correlation existed between the LDL apoB FCR and the LDL cholesterol/ apoB ratio.

5) *Disorders of hepatic LDL removal (e.g Familial hypercholesterolaemia)* reduce the fractional catabolic rate of LDL removal. This is due to genetic abnormality in apo B₁₀₀/E receptor expression. As illustrated by Vega et al, (1989) FH results in a lower FCR of LDL apo B and hence an increased LDL cholesterol / apo B ratio. As noted in Vega et al. (1991), LDL which remains in the plasma for longer periods than normal due to disorders of receptor mediated removal is likely to become cholesterol enriched.

The overall factors regulating the LDL apo B and LDL cholesterol concentration are therefore:

- the amount and composition of LDL synthesised via the cascade
- the amount of LDL synthesised via the direct path, and
- the FCR of LDL apo B.

A possible causal structure describing these relationships is illustrated in figure 6.6.

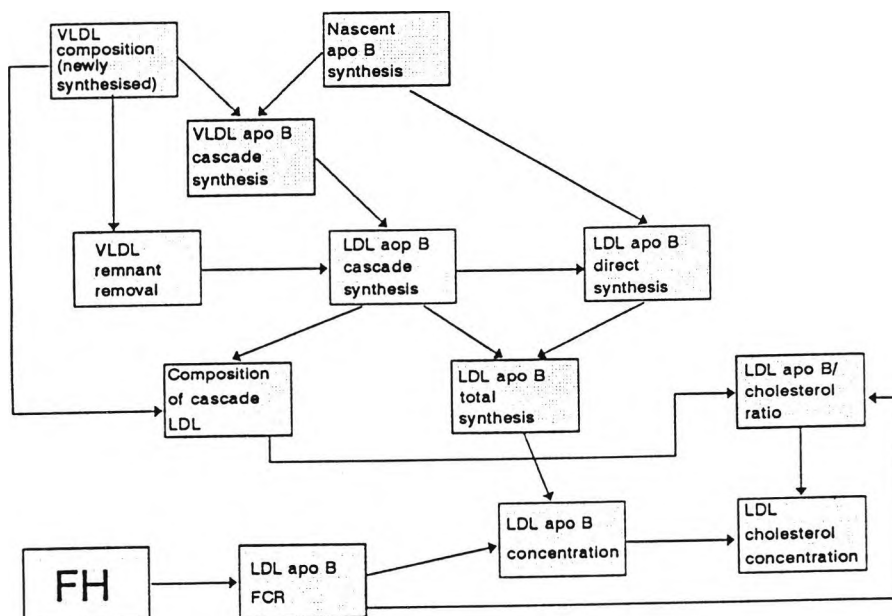


Figure 6.6 - LDL metabolism, a causal model

Many of the relationships illustrated in figure 6.6. are mathematical constraints. For example "LDL apo B total synthesis" is the sum of "LDL apo B cascade synthesis" and the "LDL apo B direct synthesis". The most critical relationship to quantify in order to automate this network is the prediction of the LDL cholesterol/ apo B ratio. This is dependant upon: the composition of LDL transported via the cascade, the direct synthesis of LDL apo B and the LDL apo B fractional catabolic rate (FCR). Compositional data for LDL transported from the cascade has not been reported so that, when examining this relationship, the LDL cholesterol /apo B ratio should be correlated instead with: the composition of newly synthesised VLDL, LDL apo B cascade synthesis, LDL apo B direct synthesis and the LDL apo B FCR. Unfortunately, no studies have been discovered which report all four of these variables so that quantification of this relationship, and hence the model, is not possible.

6.3.6 Non - receptor mediated uptake.

As described in section 5.3.1 non-receptor mediated uptake is normally dependent upon the LDL apo B concentration, elevated levels of which increases non-physiological uptake of LDL at the periphery.

In some abnormalities of VLDL metabolism LDL may not be the only source of non-receptor mediated uptake. This occurs in type III hyperlipidaemia where an apo E2/E2 phenotype blocking VLDL remnant removal co-exists with a secondary hyperlipidaemia which increases remnant formation. In this situation an elevated VLDL cholesterol/ triglyceride concentration ratio is present, the cholesterol enriched VLDL being known as β -VLDL.

β -VLDL associated with type III hyperlipidaemia are atherogenic, and as such must be removed from the plasma by non-receptor mediated pathways. No literature has been found which estimates the non-receptor mediated uptake of β -VLDL, and as such no quantitative model has been generated.

Non-receptor mediated uptake of β -VLDL does not invalidate the model constructed in chapter 5 (see section 5.7). Cholesterol deposition via this route only occurs in type III patients, the diagnosis of which is an apo E2/2 phenotype and a VLDL cholesterol/ triglyceride molar ratio greater than 0.8 (Turner et al., 1985).

6.4 Modelling VLDL and LDL metabolism

6.4.1 Modelling Triglyceride metabolism

In order to determine both VLDL triglyceride concentration and the composition of newly secreted lipoproteins it is necessary to model the triglyceride metabolism, relating variation in triglyceride synthesis, catabolism and concentration with genetic disorders and obesity.

6.4.1.1 Triglyceride synthesis

It is possible that hepatic triglyceride synthesis is regulated predominantly by the free fatty acid (FFA) levels in the plasma. Indeed Taskinen et al. (1990) in a study of 7 diabetic patients before and after insulin treatment conclude "that a limitation of substrate supply (free fatty acid and glucose) to the liver is the most likely cause of the reduction in VLDL triglyceride output". FFA levels are generally increased in situations of obesity where the large adipose store releases increased amounts of FFA into the circulation. This is heightened in poor diabetic control where there might be flushing of fatty acids from adipose stores. Whether diabetes and obesity increase triglyceride synthesis alone or in conjunction with an increase in apo B synthesis has been discussed in section 6.2.2.2.

6.4.1.2 Triglyceride Catabolism

Ginsberg et al. (1985), in a study of 6 patients before and after weight loss found little effect of obesity on triglyceride catabolism "The mean FCR for VLDL triglyceride in the study patient during either study period was not different from that which we have reported in normals". Similarly Taskinen et al. (1990) implies that diabetes and obesity have little effect on the fractional catabolic rate of triglyceride, noting that "the lack of effect of insulin on the rate of delipidation in the VLDL₁, VLDL₂, IDL and LDL cascade is consistent with the concept that muscle tissue is a more important site for triglyceride hydrolysis than adipose tissue".

Grundy and Vega (1990), discuss the lack of consensus as to how familial hypertriglyceridaemia modifies triglyceride metabolism, indicating speculation on whether it is a production or removal defect of triglyceride.

6.4.1.3 Modelling triglyceride synthesis and catabolism

One hypothesis as to how disorders effect triglyceride synthesis, concentration, and catabolism can therefore be represented as in figure 6.8. These relationships are investigated quantitatively in the following sections

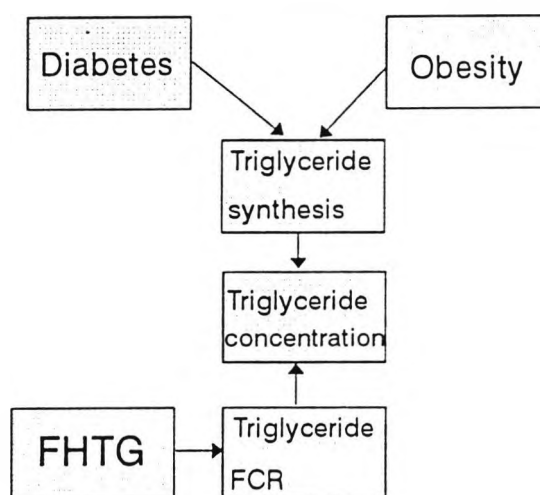


Figure 6.8 - Triglyceride synthesis, concentration and catabolism.

6.4.1.4 Model construction

Triglyceride Synthesis

The data from 10 studies (Abrams et al., 1982; Beil et al., 1982; Howard et al., 1987; Nikkila et al., 1971; Dunn et al., 1984, 1985; Angelin et al., 1990; Pietri et al., 1983; Kissebah et al., 1981, 1982;) were pooled to model triglyceride synthesis. In determining triglyceride synthesis rates, the only correlation possible was that between percentage ideal body weight (% IBW) and triglyceride transport expressed in mg/kg IBW/h (where kg IBW is ideal body weight in kilograms), ($R^2 = 25\%$). This is depicted in figure 6.9.

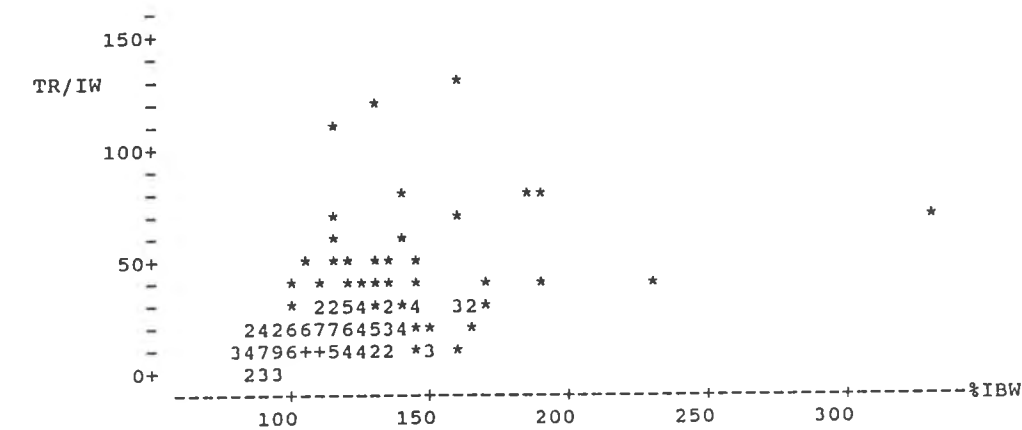


Figure 6.9 - Variation in triglyceride transport (per kg ideal body weight) with level of obesity (numbers on graph indicate the number of data points in this position)

When performing this correlation it was necessary to convert those triglyceride transport rates reported in mg/kg/d to mg/kg IBW/h. As both Grundy et al. (1979) and Dunn et al. (1984) note, triglyceride synthesis rates should be normalised to reflect the fact that increasing weight in obesity is not proportional to an increasing plasma volume, the majority of the increase in weight being due to greater amounts of adipose tissue. Representing triglyceride transport rates in units of weight could,

in obesity, result in underestimates of triglyceride transport, and hence poor relationships when correlating % IBW with triglyceride transport rates.

Including values of fasting plasma glucose in the % IBW and triglyceride transport relationship, using multiple regression analysis, did not improve this relationship. This is probably due to the complex relationship that exists between obesity and diabetes, so that it is not possible here to represent them as two independent variables controlling triglyceride synthesis. As Grundy et al. (1979) note "insulin levels are often enhanced in obesity and this has been proposed to stimulate VLDL triglyceride synthesis".

Insufficient data were available to investigate the effects of FHTG on the % IBW, triglyceride transport relationship illustrated in figure 6.8. It was not possible to explain the uncertainty in the % IBW, triglyceride transport relationship using any disorders. Therefore, to represent individual variation in this relationship, a sensitivity factor was included in the model as illustrated in figure 6.10. This node has five states and, given an individual's triglyceride transport rate and their % IBW, represents their susceptibility to increased triglyceride production. This node is given an improper *a priori* distribution, so that all states have equal likelihood initially. As further information presents, it may be possible to learn about the individual's sensitivity to triglyceride production.

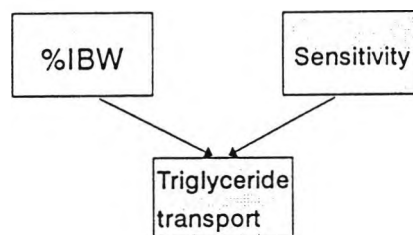


Figure 6.10 - Individual sensitivity to triglyceride transport

As discussed previously, the relationship between obesity and plasma volume is not proportional. Since VLDL triglyceride concentration is represented in the units of mg/dl it is necessary to determine the plasma volume during the obese state.

Normally, plasma volume (PV) in dl is calculated as 0.45 times weight as measured in kg. To adjust for the effects of obesity, a correction factor was used, which, when multiplied by the plasma volume calculated using weight (i.e. $PV = 0.45 * \text{weight}$), gives a more accurate estimate of the true plasma volume (as used by Greenfield et al., 1980). The relationship between the correction factor and the level of obesity (% IBW) was obtained from data reported Nikkila (1973), and is illustrated in figure 6.11. R^2 was 90% when the correction factor was correlated with the level of obesity using linear regression.

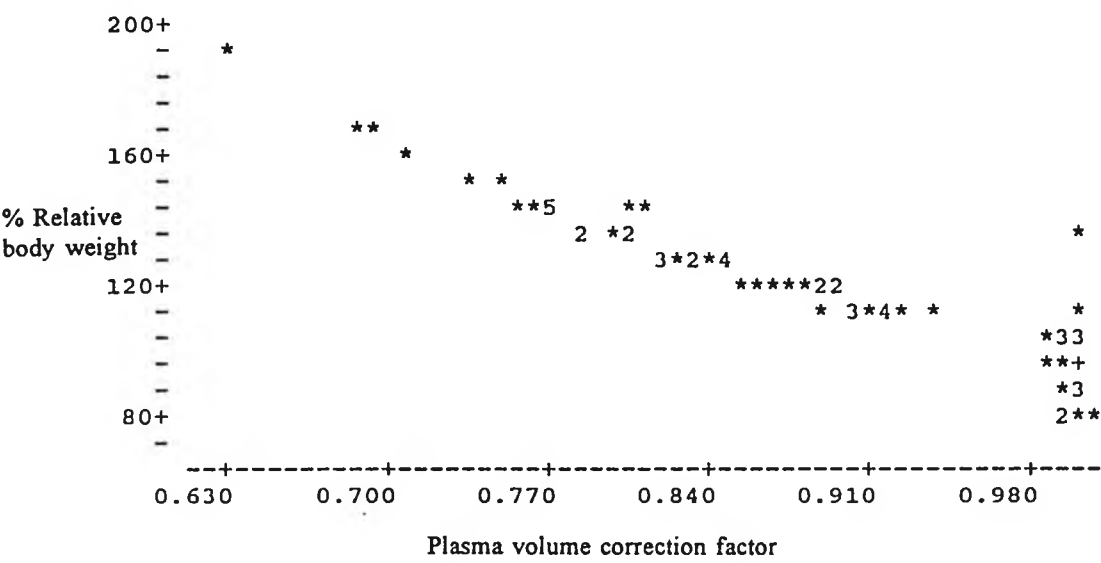


Figure 6.11 - Variation in the plasma volume correction factor with obesity
(Numbers on graph indicate number of data points in this position)

To complete the model of triglyceride synthesis, illustrated in figure 6.12, the relationship between height, sex and ideal body weight (IBW) was modelled from the data of Metropolitan Life Tables.

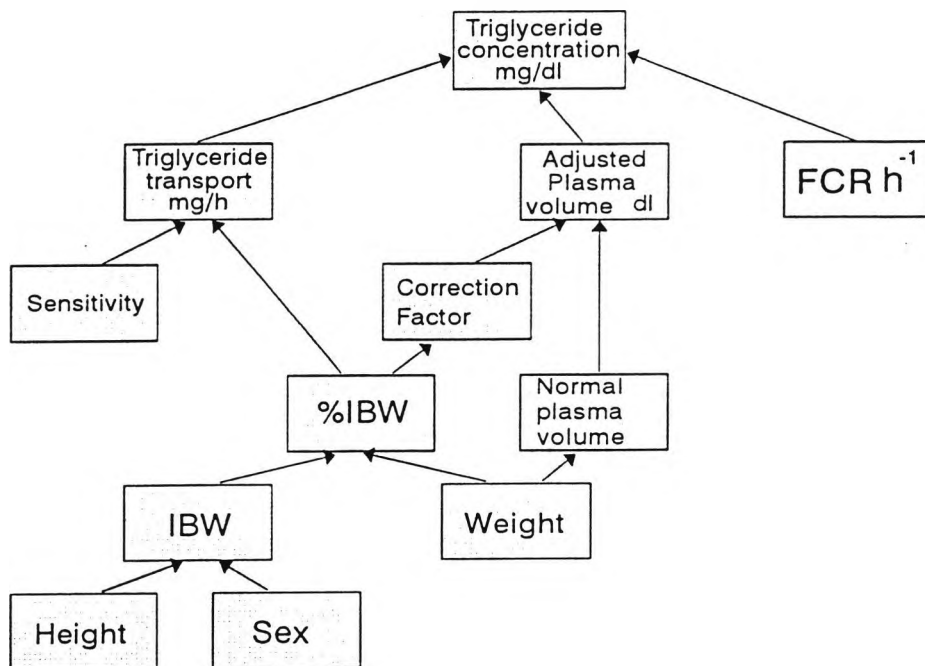


Figure 6.12 - Triglyceride synthesis, a causal model

Triglyceride fractional catabolic rate.

If triglyceride kinetics behaved according to normal saturation kinetics then as triglyceride transport increased, triglyceride concentration would increase slowly (the FCR remaining constant) up to the point at which the removal mechanism was saturated. When saturation was reached, the triglyceride concentration would increase rapidly for any subsequent increase in triglyceride transport, and the FCR would decrease. Hypothetical graphs of (a) triglyceride transport against triglyceride concentration and (b) triglyceride fractional catabolic rate against triglyceride transport rate (figure 6.13), illustrate these points.

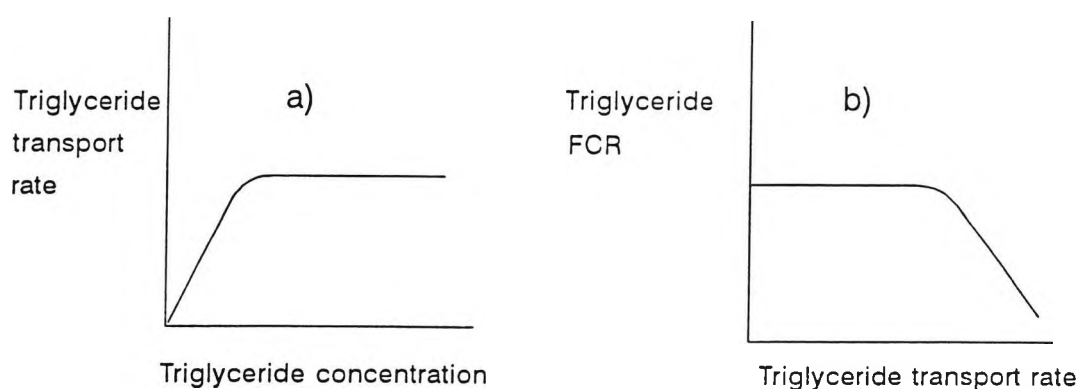


Figure 6.13 - Hypothetical graphs of triglyceride metabolism assuming saturation kinetics

However, triglyceride kinetics are not thought to obey Michaelis-Menten saturation kinetics (Grundy et al., 1982) and as these authors note, it is normal to see a steadily increasing triglyceride FCR when triglyceride transport rates are increased. A possible physiological explanation for this phenomenon is that usually a great deal of latent capacity is available for triglyceride removal. This extra capacity is necessary to cope with normal fluctuation in triglyceride synthesis during fasting and feeding, where to avoid severe chylomicronaemia in the fed state triglyceride catabolism must be increased above normal.

More accurate representations of the relationships between (a) the triglyceride transport rate and triglyceride concentration, and (b) between the triglyceride fractional catabolic rate and triglyceride transport rate might then be those illustrated in figure 6.14.

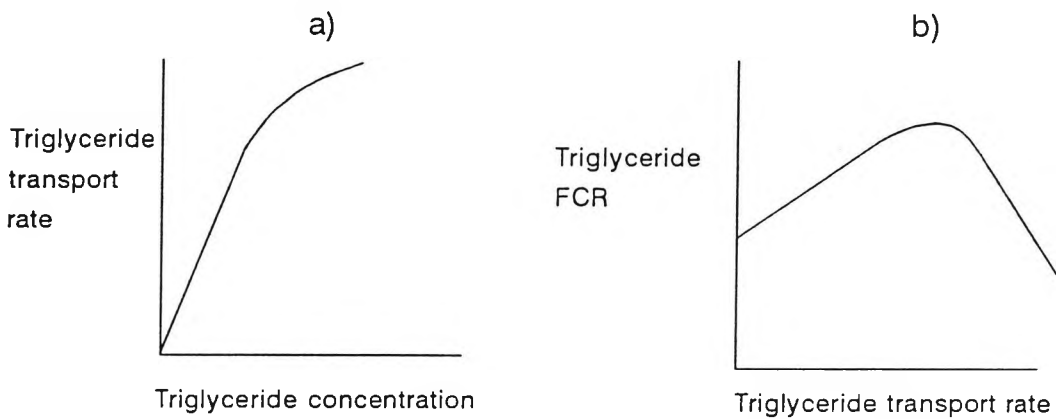


Figure 6.14 - Hypothetical graphs of triglyceride metabolism without assuming saturation kinetics

Figure 6.15 explores further the relationship between triglyceride transport and fractional catabolic rate by graphing the results of studies (Abrams et al., 1982; Beil et al., 1982; Kissebah et al., 1981; Nikkila et al., 1971; Egusa, 1985, Ginsberg et al., 1985; Kissebah et al., 1982; Howard et al., 1987) reporting both these variables. This graph was constructed as follows. For each of the studies, linear correlations were performed relating the triglyceride transport rate with triglyceride catabolic rate for different patient subgroups within the studies, i.e. obese, normals, and those with genetic disorders. Where these correlations were significant, lines were plotted on figure 6.15 illustrating this relationship, the range over which it applied, and the

standard deviation associated with that relationship. Where no such correlation was possible, a block was drawn on the graph illustrating the full range of data for that group in the study. Some studies reported only mean and standard deviation values for triglyceride transport rates and fractional catabolic rates, in which case a block was drawn on the graph illustrating the mean and standard deviation. Whilst no rigid conclusions can be drawn from figure 6.16, interestingly the studies fall broadly into the following four classifications in terms of triglyceride removal capacity.

1) Normals - In normals the FCR would appear to increase rapidly as transport rate increases. This group includes the studies of Nikkila (1971) on normal patients, Kissebah (1981) on normal patients, Kissebah (1982) on mild diabetics with no hyperlipidaemia, Egusa (1985) on normals, and to a lesser extent Egusa (1985) on obese patients.

2) Patients with moderate downregulation of their fractional catabolic rate - In this class of patient severe obesity or diabetes may disturb normal clearance mechanisms. The FCR increases with increasing TR but not as quickly as in normals, probably resulting in mild hypertriglyceridaemia. This group includes the studies of Ginsberg (1985) on obese patients after weight loss, Howard (1987) on diabetic Pima indians (Pima indians have no genetic disorders of lipid and lipoprotein metabolism), and Ginsberg (1985) on obese patients before weight loss.

3) and 4) These groups represent those patients with disorders of triglyceride removal. Interestingly none of these patients had FCRs greater than 0.3 and where it was possible to regress FCR on triglyceride transport rate the slopes of the resulting lines were negative suggesting that these individuals had reached saturation of triglyceride removal mechanisms, so that FCR decreased with increasing triglyceride transport. Group 4 is an arbitrary subdivision for those individuals with a more severe removal defect. The studies included in these groups are Abrahams (1982) on insulin dependent diabetics (IDDMs), Beil (1982) on FCHL and probable FCHL, Kissebah (1981) on FHTGs, Kissebah (1982) on mild diabetics with hypertriglyceridaemia and Kissebah (1982) on severe diabetics with

hypertriglyceridaemia.

It is clear from these groupings that disorders associated with triglyceride metabolism, that is diabetes, obesity, FHTG and perhaps FCHL, and the severity of these disorders result in lower limits for the saturation of triglyceride removal.

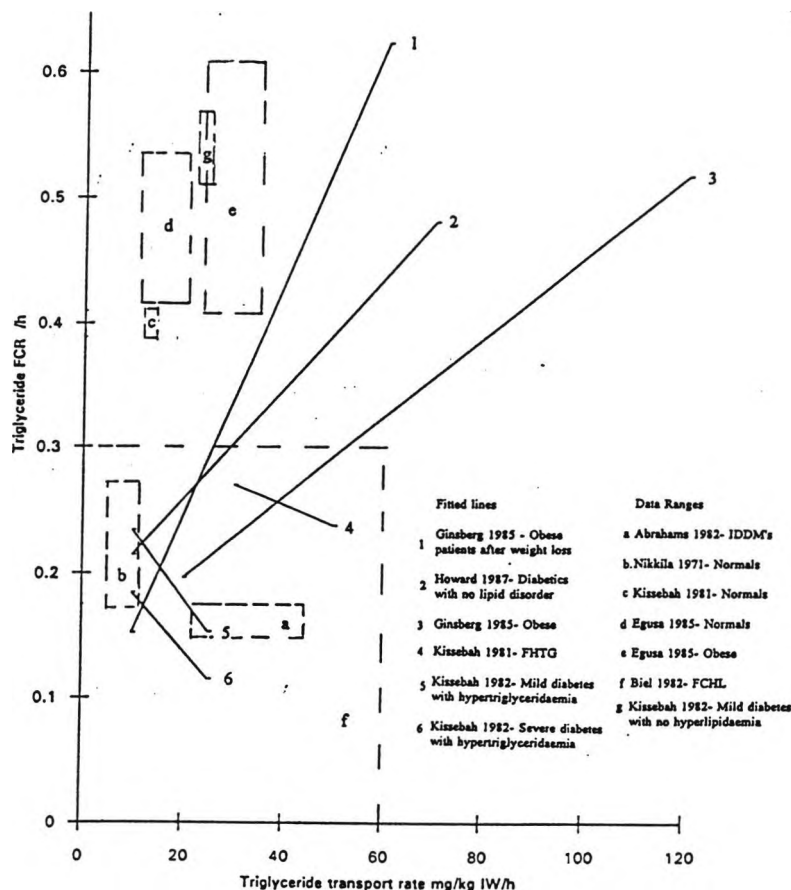


Figure 6.15 - Variation in triglyceride FCR with different triglyceride transport rate in studies representing different disorders of triglyceride metabolism

A hypothetical graph of triglyceride FCR against triglyceride transport rate, illustrating these four groups with different triglyceride removal defects is given in figure 6.16.

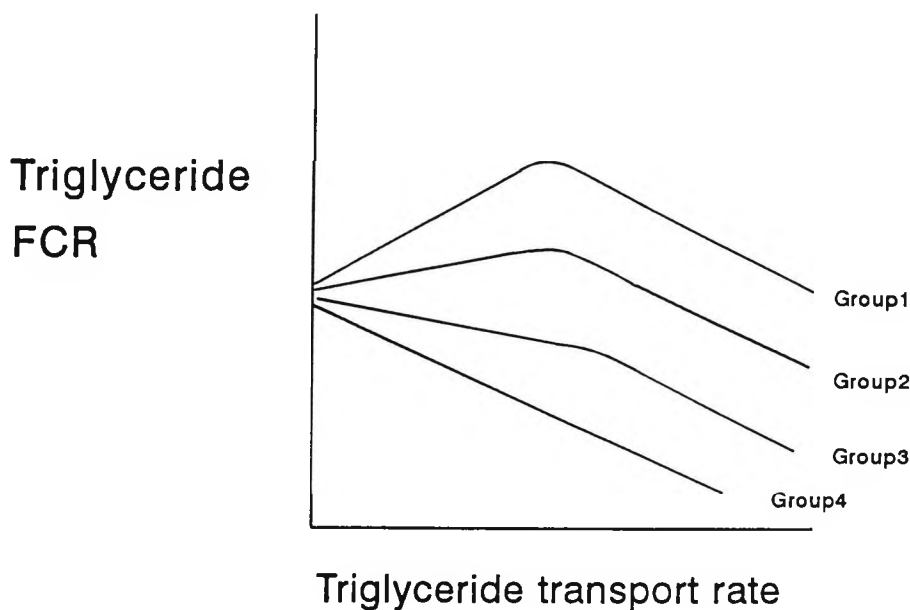


Figure 6.16 - Hypothetical graph illustrating four classifications of triglyceride removal capacity

Triglyceride removal was modelled by estimating sample values for each of the four groups from the graph of figure 6.15, and modelling them using a tabulated-normal model (see section 3.6.2). Large uncertainty estimates were encoded in this model to reflect the innacuracy in the sampling procedure. Figure 6.17 illustrates the complete model of both triglyceride transport and removal. In the same way that the triglyceride sensitivity node represents individual variation to triglyceride transport, so the removal defect node represents individual variation in the triglyceride removal capacity. The triglyceride transport rate ("trigtran 2" mg/h) is calculated from the triglyceride transport rate "Trigtran" (mg/kg IBW/h) and the ideal body weight ("IBW"). The triglyceride transport rate (mg/h) and the fractional catabolic rate (h^{-1}) are then used to calculate the VLDL triglyceride pool size (mg) which along with the adjusted plasma volume ("plas vol" dl) is then used to calculate the VLDL triglyceride concentration (mg/dl).

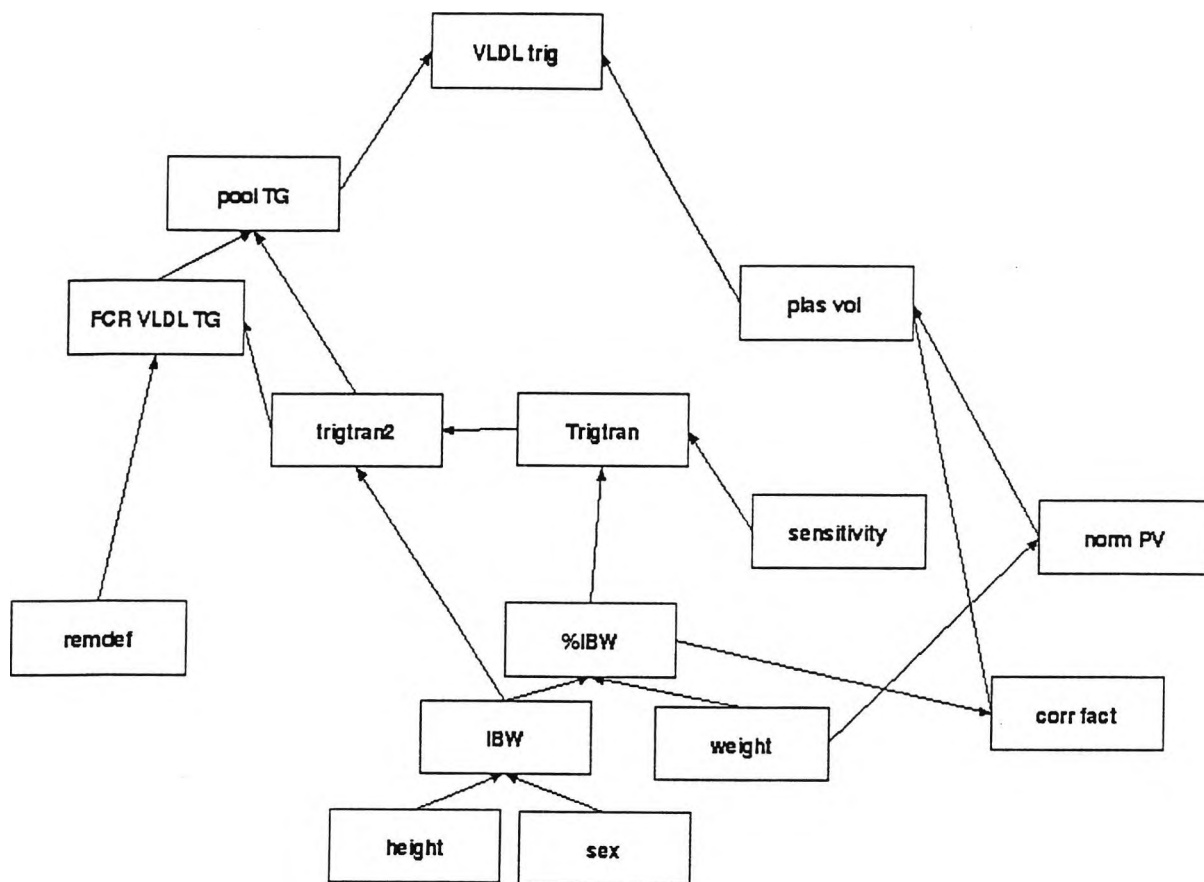


Figure 6.17 - A model of triglyceride transport and catabolism in the VLDL cascade

6.4.2 Modelling apo B synthesis and VLDL composition

6.4.2.1 Introduction

As noted in sections 6.2.2.2 and 6.2.2.3 the composition of newly synthesised VLDL, as indicated by the VLDL triglyceride/ apo B synthesis ratio, may provide information about the metabolic pathways of VLDL and LDL their concentration and composition, and the amount of direct LDL synthesis.

In order to model the VLDL triglyceride/ apo B synthesis ratio and investigate these relationships, sections 6.4.2.2 and 6.4.2.3 describe the modelling of apo B synthesis during FCHL, FH and obesity, combining this model with that constructed for triglyceride metabolism, as described in section 6.4.1.

Section 6.4.2.4 then investigates the relationship between the VLDL triglyceride/ apo B cascade synthesis ratio and VLDL removal as remnants in the cascade. It concludes that this ratio might be insufficiently detailed to explain differences in cascade removal of VLDL, and LDL direct synthesis, and that compositional ratios describing the VLDL subfractions (VLDL₁ and VLDL₂) might be more informative.

6.4.2.2 Apo B Synthesis

As described in section 6.2.2.2, both obesity and FCHL increase apo B synthesis. Obesity increases it in conjunction with elevated triglyceride synthesis such that the average size of newly synthesised VLDL is normal. FCHL results in the production of smaller VLDL, unless accompanied by a secondary disorder which increases triglyceride synthesis. For the purposes of this model development hyperbetalipoproteinaemia was assumed to be synonymous with FCHL.

Seven studies were found that reported raw patient data for percentage ideal body weight (% IBW), genetic disorder (in some studies the patients were Pima indians and therefore had no genetic disorder of their lipid metabolism), cascade synthesis of apoB, and nascent synthesis of apo B. These were Kesamiani et al., (1985); Egusa et al., (1985); Turner et al., (1985); Vega et al., (1988); Teng et al., (1986); Vega et al., (1986); and Chait (1980). Three of these studies did not report nascent synthesis. In the others all four of these variables were reported, although it was sometimes necessary to calculate nascent synthesis as illustrated in section 6.2.3. Some of these studies reported synthesis rates in mg/kg/d. Where this occurred, either actual values of weight or average values (based upon sex, height, and % IBW) were used to calculate synthesis rates in mg/d.

A graph of cascade apo B synthesis against %IBW for the pooled data is illustrated in figure 6.18. A linear regression fit of this graph giving an R² value of 14.7%

due to FH. This would present as a reduced amount of cascade synthesis of apo B without a reduction in nascent synthesis. Cascade synthesis of apoB is reduced during FH compared with normals, so that on multiple regression

$$\text{casc} = 306 + 8.88 \%IBW + 1129 \text{ FCHL} - 489 \text{ FH} \tag{6.3}$$

with an R² value of 43.7% (The P values on a t test for this equation gave the following results: P(constant)= 0.2, P(%IBW)= 0, P(FCHL)= 0, P(FH)= 0.021).

Equations 6.2 and 6.3 enable the nascent and cascade synthesis to be modelled as a function of %IBW and genetic disorder (FH, FCHL) as illustrated in figure 6.20. LDL direct synthesis can then be modelled as the difference between nascent and cascade synthesis. However, it is possible to improve the modelling of LDL direct synthesis by including the relationship between FH and LDL direct synthesis shown in figure 6.19. This figure illustrates the mean and standard deviation of "the fraction of nascent synthesis transported directly to LDL" in normals and in FH, which can then be used to constrain direct LDL synthesis as illustrated in figure 6.20. The mean and standard deviation are: mean = 0.163, SD=0.16 in normals, and mean = 0.365, SD = 0.189 in FH.

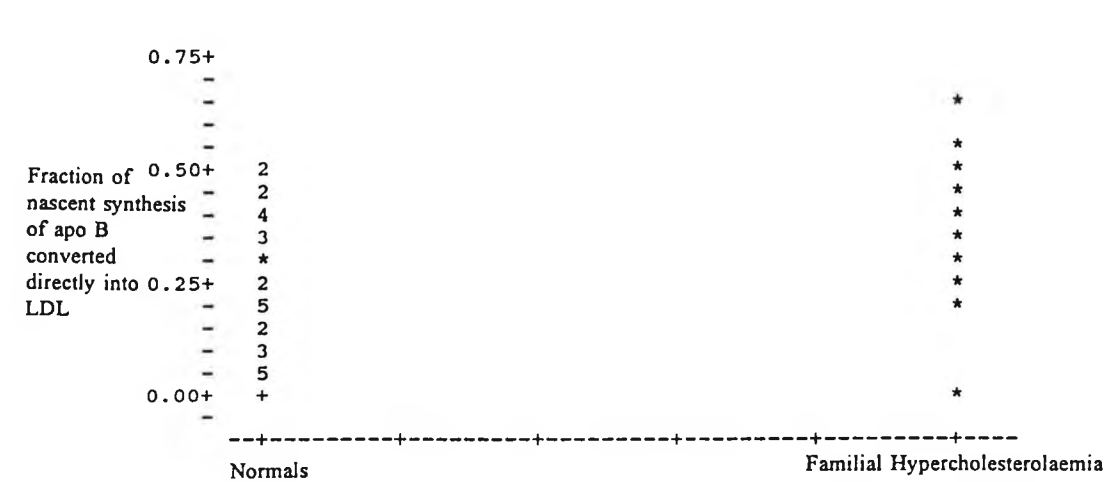


Figure 6.19 - Variation in LDL direct synthesis with familial hypercholesterolaemia

In FCHL whilst the absolute amount of direct LDL synthesis was increased, the LDL direct synthesis as a percentage of total nascent was normal. FCHL appears then to increase apoB transport by both the direct and cascade pathways.

No further explanation could be found for the uncertainty in either of the models of nascent or cascade synthesis, i.e. equations 6.2 and 6.3, so that the final model of apo B synthesis is that illustrated in figure 6.20.

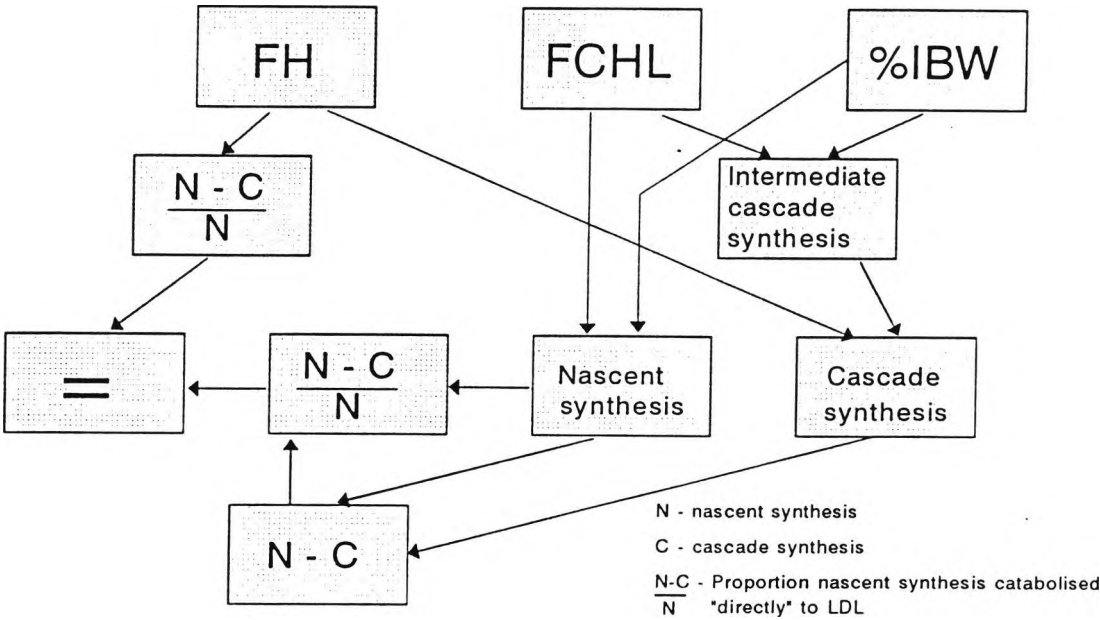


Figure 6.19 - Apoprotein B synthesis, a causal model

6.4.2.3 Combining apo B synthesis with triglyceride synthesis

The complete model combining apo B and triglyceride synthesis is illustrated in figure 6.21.

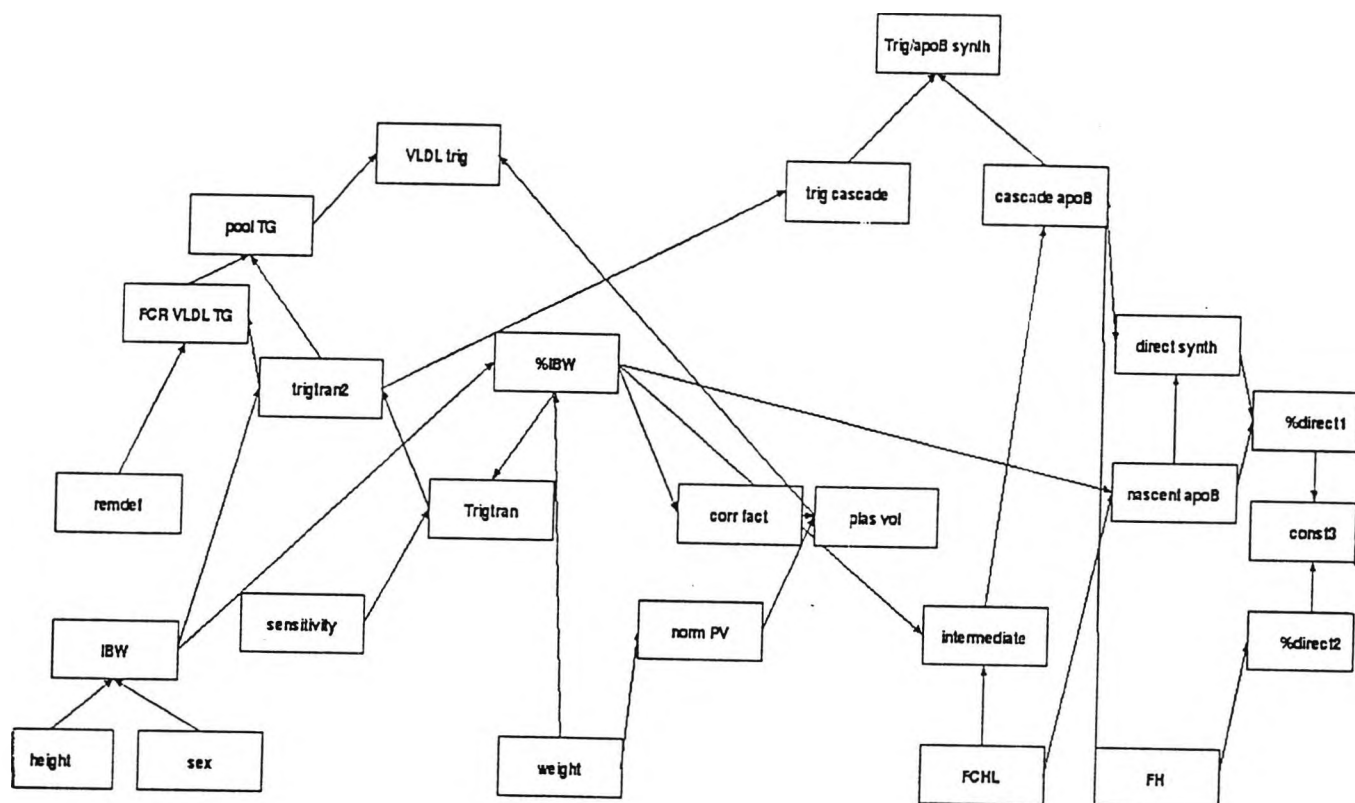


Figure 6.21 - Model of VLDL triglyceride metabolism and VLDL apoB release into the cascade

6.4.2.4 The effects of VLDL triglyceride : apo B coupling (i.e. the VLDL TG: apo B synthesis ratio) on the metabolic pathways of VLDL and LDL

As described in sections 6.2.2.2 and 6.2.2.3 lack of coupling between the VLDL triglyceride and apo B synthesis can result in disturbances in the metabolic pathways of both VLDL and LDL and hence alter the composition and concentration of many of the factors affecting the potential for atheroma. This section investigates the relationship between the VLDL triglyceride/ apoB ratio and the removal of VLDL either via the cascade or through transport to LDL. No model is created as insufficient data are available. The section comments on how the composition of newly synthesised sub-fractions of VLDL i.e. VLDL₁ and VLDL₂ might be more indicative of both the VLDL and LDL metabolic pathways than the average

composition ratio (i.e. VLDL triglyceride/ apo B ratio), and how future studies should examine these issues.

Relationship VLDL trig/ apo B synthesis ratio and LDL apo B synthesis via the cascade.

Six studies (Kissebah et al., 1981; Kissebah et al., 1982; Kesamiemi et al., 1985; Egusa et al., 1985; Taskinen et al. 1990; Howard et al., 1987) provided the necessary information to investigate relationships between the composition of VLDL and the amounts of cascade VLDL lipolysed to LDL rather than being removed as VLDL remnants. A graph of the LDL apo B cascade synthesis against the VLDL apoB cascade synthesis, created from the data in these studies, is illustrated in figure 6.22.

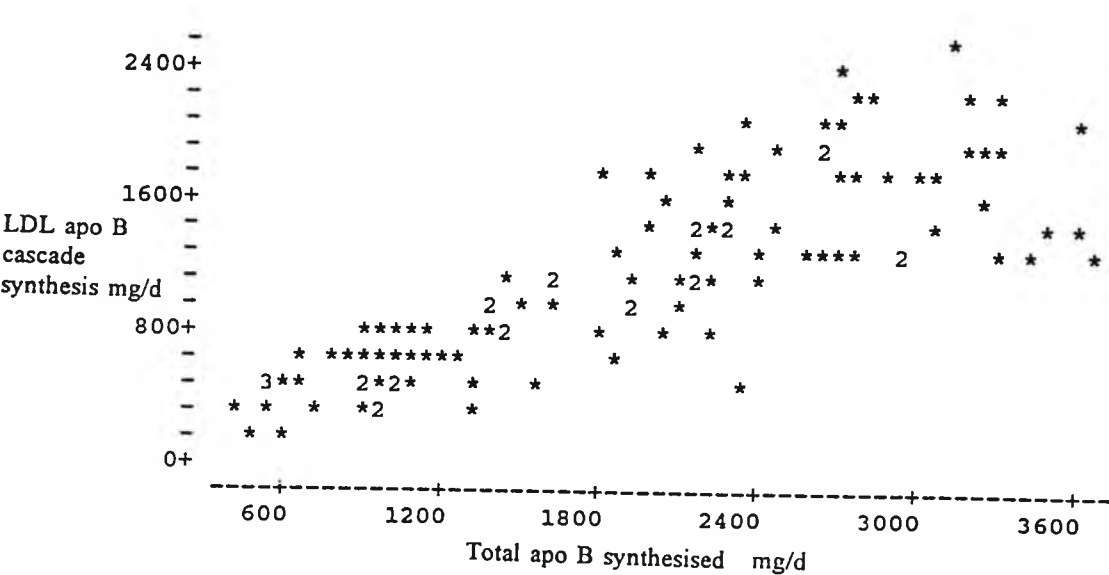


Figure 6.22 - Variation in LDL apo B cascade synthesis with VLDL apo B cascade synthesis (numbers on graph indicate number of data points in this position)

When the relationship illustrated in figure 6.22 was fitted using linear regression, a value of $R^2 = 67\%$ was obtained. If a fixed quantity of apo B was catabolised during the cascade then one would expect this relationship to be linear with very little

variation, i.e. a high value of R^2 .

The uncertainty in the relationship described by figure 6.22 might be due to either random variation or bias between the studies. However, as described in section 6.2.2.2, during disorders of triglyceride metabolism and apo B metabolism (which modify the coupling and hence size of VLDL), variation in VLDL remnant catabolism is thought to occur such that triglyceride-rich VLDL are more likely to be catabolized as VLDL remnants. Part of the uncertainty in the relationship illustrated in figure 6.22 might therefore be due to disturbances in the triglyceride/apoB coupling of VLDL particles. The relationships between LDL apo B cascade synthesis, the total apo B synthesis and (a) VLDL triglyceride concentration, (b) VLDL triglyceride: apoprotein B concentration ratio, and (c) VLDL triglyceride: apoprotein B synthesis ratio, were therefore investigated using multiple regression. Unfortunately none of these variables describing VLDL composition provided any extra information describing the LDL apo B cascade synthesis.

Two studies from the same group (Kissebah et al., 1981; Kissebah et al., 1982) describe the LDL apo B synthesis from the cascade and the total VLDL apo B cascade synthesis in numerous disorders. Graphs of these effects have been generated using linear regression and re-plotted in figure 6.23.

The correlation coefficients of these lines are : Normolipidaemic mild diabetes, $r=0.913$; hyperlipidaemic mild diabetes, $r=0.979$; hyperlipidaemic moderately severe diabetes, $r=0.882$; normals, $r=0.995$; FHTG, $r=0.723$; and FCHL, $r=0.969$. Thus, a good fit is obtained in each case.

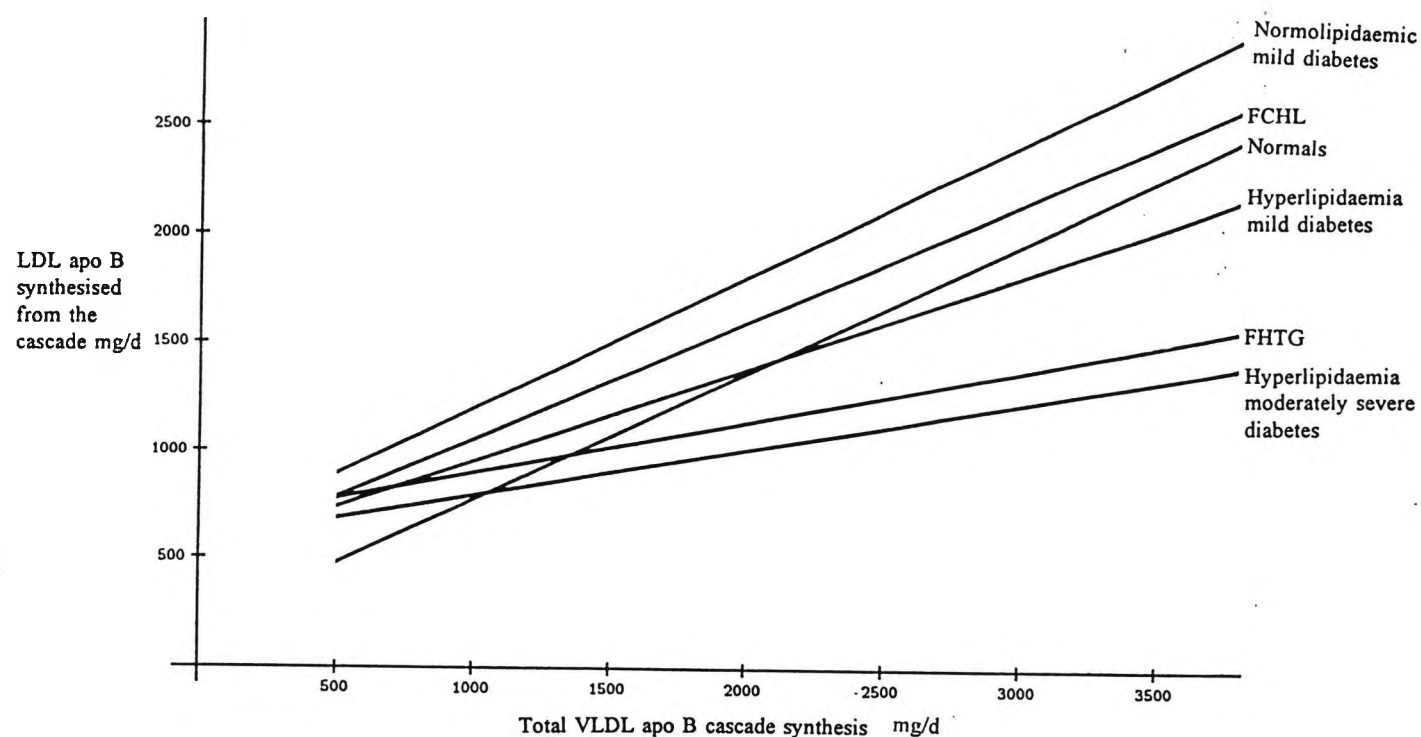


Figure 6.23 - Variation in LDL apo B cascade synthesis with VLDL apo B cascade synthesis in studies describing patients with disorders of VLDL metabolism

By visually inspecting this graph it is clear (apart from normolipidaemic mild diabetes) that those disorders in which apo B is overproduced, i.e. FCHL, have a decreased catabolism of VLDL remnants and hence an increased LDL cascade synthesis of apo B; whereas in those patients with an overproduction of triglyceride, i.e. FHTG, the inverse is true. This is consistent with the hypothesis that larger triglyceride rich VLDL are more susceptible to catabolism as remnants. However, neither the investigation above nor in Kissebah's (1981, 1982) own investigation of these two separate studies did the VLDL triglyceride/ apo B synthesis ratio provide any useful information in determining the LDL apo B cascade synthesis. In fact in the studies from Kissebah (1981, 1982) only one disorder, FHTG, modified the average VLDL triglyceride: apo B synthesis ratio.

An apparent contradiction exists such that the disorders illustrated in figure 6.23 modify the metabolic pathway of VLDL in a manner consistent with their perturbation of apo B and triglyceride synthesis and modification of the VLDL

triglyceride: apo B synthesis ratio, but that in reality this ratio is not modified.

An hypothesis explaining this contradiction is implicit in the work of Hugh et al., (1991). They suggest that both the VLDL₁ and VLDL₂ sub-fractions contain sub-populations with different metabolic fates. They classify these sub-fractions as bound and unbound (depending on their binding to heparin-sepharose), noting that the difference between these sub-populations is the apoE/apoC ratio (greater in the bound fraction) and the subfraction size, that is "as the particles become smaller (within each VLDL₁, VLDL₂ subfraction) the proportion of bound particles increased" (brackets added). They note that "In both VLDL₁ and VLDL₂ the unbound fraction had higher triglyceride / apo B ratios than found in the bound fraction".. These authors illustrate that the metabolic pathway of apo B is dependent upon the amount of each of the bound and unbound sub-populations that are present in each of the VLDL sub-fractions VLDL₁ and VLDL₂. Direct synthesis of LDL coming from the bound fraction alone.

Direct synthesis of LDL represents a group of nascent synthesised lipoproteins which rapidly pass through the cascade, these coming predominantly from the smaller VLDL within each of the VLDL₁ and VLDL₂ sub-fractions i.e. the "bound" particles. Whilst increasing the bound fraction increases the speed of VLDL cascade throughput, increasing the number of larger lipoproteins in each subfraction, i.e. the "unbound" particles, would decrease the speed of throughput, making the VLDL more susceptible to cholesterol loading, and hence removal as remnants. As Nestel et al. note (1983) "the precursor of IDL was predominantly within the bound rather than the unbound fraction of VLDL". Therefore, it might be possible to correlate the sub-fraction size distribution, i.e. the VLDL triglyceride/ apo B synthesis ratio in both VLDL₁ and VLDL₂, with both VLDL remnant removal and the amount of LDL direct synthesis from each sub-fraction.

The speed of lipoprotein throughput in the cascade is likely to depend upon the triglyceride removal from lipoproteins. Different lipases act on different size lipoproteins, i.e. lipoprotein lipase catabolises the triglyceride of large lipoproteins

(VLDL₁) and hepatic lipase catabolises the triglyceride of smaller lipoproteins (VLDL₂). It is possible then that direct synthesis of LDL from each of these sub-fractions is independent, related instead to the different lipase activities.

Unfortunately, insufficient data are available to correlate VLDL sub-fraction sizes with either the VLDL remnant removal or LDL synthesis via the direct pathway.

6.5 Summary

This chapter endeavoured to extend the modelling of chapter 5, enabling the effects of lipoprotein disorders and obesity to be represented in terms of CHD risk. In doing so this chapter has described the transport and catabolism of VLDL and LDL apoprotein B and VLDL triglyceride, modelling part of this system. Further it has suggested how disorders of triglyceride and apoprotein B synthesis and catabolism effect the composition of VLDL, and how compositional change in VLDL may alter its metabolic fate, determining whether or not it becomes an atherogenic lipoprotein.

In this study, no association has been shown between the average VLDL composition and its eventual metabolic fate. This chapter has presented possible hypotheses for this finding suggesting that different VLDL subfractions might be metabolised in different ways. It is possible then that the composition of different VLDL subfractions might indicate the metabolic fate of VLDL more accurately, although, further studies are required to confirm this hypothesis.

Chapter 7 - Model Validation

7.1 Introduction

In chapters 5 and 6 models were constructed representing the potential for atheroma (i.e. the difference between forward and reverse cholesterol transport) and the modification of lipoprotein metabolism during different disorders. This chapter examines the validity of these models in qualitative and quantitative terms.

Section 7.2 provides evidence for the validity of the model constructed in chapter 5. Section 7.2.1 illustrates that model predicted "potential for atheroma", at the *a priori* population level, is consistent with slowly progressing atheroma in a population on a western style diet. The range of "potential for atheroma" is then checked with that estimated from studies of whole body cholesterol metabolism. This was found to be of the same order of magnitude, illustrating consistency between the modelling of chapter 5 and studies of whole body cholesterol metabolism. In general, when examining this model in qualitative terms, model inference is shown to be consistent with assumptions made in chapter 5, regardless of the quantitative values assigned to individual nodes.

Section 7.2.2 illustrates the validity of the model constructed in Chapter 5 in quantitative terms. In this section values of variables taken from Castro et al. (1985) are assigned to model nodes. These data were independent from those used in model formulation and were not available for all model nodes. Validation proceeded by examining the behaviour of other model nodes for which data were reported in the literature source. Some quantitative consistency was shown between reported and model predicted variables for this study

Section 7.3 examines the validity of the model constructed in chapter 6. In that chapter it was not possible to construct a complete model of VLDL and LDL

metabolism linking disorders to variables in the model of "potential for atheroma" described in chapter 5. Instead, a tentative model describing VLDL apo B cascade synthesis and triglyceride cascade synthesis was constructed. The ratio between these two synthesis rates being thought to determine the size and hence transport pathway of lipoproteins entering the VLDL cascade.

Validation of this model is in qualitative terms only, illustrating that this model obeys the assumptions made during its construction.

7.2 Testing the validity of the model of "potential for atheroma"

7.2.1 Model validation in qualitative terms

7.2.1.1 Validity of *a priori* state

Data used in model construction have been taken from studies examining a western population. This population as a whole are at elevated risk of CHD (Lewis et al., 1989), in part due to the high saturated fat content of their diet. This means that slow atheromic progression, occurring over a period of decades, is normal.

If slow atheromic progression is the norm in a western population then the potential for cholesterol deposition at the peripheral tissues must be slightly weighted toward transport to the tissues, yet be close to zero. The small negative atheromic potential predicted by the model in the initial state, and illustrated in figure 7.1, is consistent with this hypothesis. When the model is initialised to the population distributions, the atheroma potential has a mean of -0.5 nmol/ ml/ h (-ve indicating transport toward the tissues). This value is extremely small when we consider total cholesterol turnover which has a range of $35 - 105 \text{ nmol/ ml/ h}$ (1- 3 g/d) (see chapter 4) .

An important result can be inferred from this validation. Since the atheromic potential approximates to zero this validates the assumption of representing transport to the peripheral tissues as being analogous to the non-receptor mediated uptake of cholesterol; that is, ignoring both receptor mediated uptake and *de novo* cholesterol synthesis at the peripheral tissues. As noted in section 5.2 of chapter 5, if this assumption was invalid then the model predicted estimate of reverse cholesterol transport would have been greater than the predicted non-receptor mediated uptake of cholesterol, and the population value of atheromic potential would have had a positive mean.

7.2.1.2 Checking range of atheroma potential with studies of whole body cholesterol metabolism.

In chapter 4 it was suggested that the difference in turnover between the two and three pool models of isotope study might indicate cholesterol deposition in the peripheral tissues and hence atherogenic potential. It was also noted that this might be approximated by the difference between the two pool isotope cholesterol turnover and cholesterol turnover inferred from sterol balance studies such that

$$\begin{array}{lcl} \text{Peripheral tissue} & = & \text{2 pool isotope} \quad - \quad \text{Sterol balance} \quad (7.1) \\ \text{cholesterol loss} & & \text{cholesterol turnover} \quad \quad \quad \text{cholesterol turnover} \end{array}$$

In this section a range of peripheral tissue cholesterol loss is determined from studies reporting both 2 pool cholesterol turnover and sterol balance cholesterol turnover in the same patients. This range is compared with the range of "potential atheroma" represented in the model constructed in chapter 5 and illustrated in figure 7.1.

As noted in chapter 4, any consistency illustrated by this comparison provides valuable evidence for model validation, since the studies of whole body cholesterol metabolism and those used in chapter 5 to construct the model of potential atheroma involve different methodology and are performed over different lengths of time.

Three studies (Nestel et al., 1973; Grundy et al., 1969; Samuel et al., 1978) were found which reported cholesterol turnover in the same patients using the 2 pool compartmental modelling and sterol balance methods. Together, these studies reported a total of 59 patients.

Using equation 7.1 the range of peripheral tissue cholesterol loss was estimated and is illustrated in figure 7.2. This range is in g/d where -ve illustrates transport from the tissues and +ve transport to the tissues.

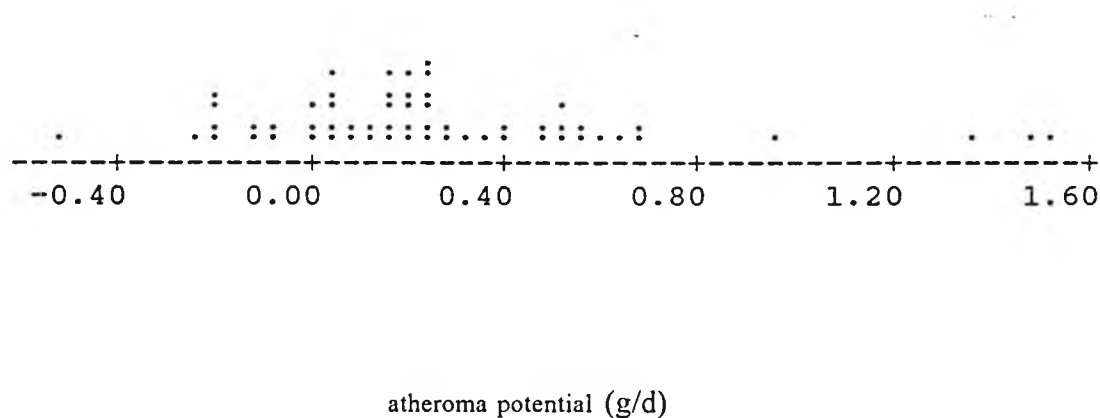


figure 7.2 - Range of "potential for atheroma" inferred from studies of whole body cholesterol metabolism.

So as to compare this range with the range of potential for atheroma predicted by the model constructed in chapter 5, a number of extreme physiological cases were investigated.

1) The postprandial state

The postprandial state represents an extreme physiological state since reverse cholesterol transport is elevated, caused by an increased VLDL triglyceride concentration and VLDL and LDL which are not saturated with cholesterol ester;

whilst forward cholesterol transport is normal, i.e. a normal LDL cholesterol concentration. The model, when instantiated to the postprandial state, would therefore appear as in figure 7.3.

Figure 7.3 illustrates increased reverse cholesterol transport during the post prandial state. The distribution for "potential for atheroma" having a mean of 20 nmol/ml/h and an upper limit of 100 nmol/ml/h (i.e. mean 0.58 g/d, upper limit 2.92 g/d).

Whilst the upper limit is outside the range of that deduced in figure 7.2, the mean transport in the post prandial state is of a similar order of magnitude as the lower limit on the graph of figure 7.2. That is, a maximal value for negative potential atheroma.

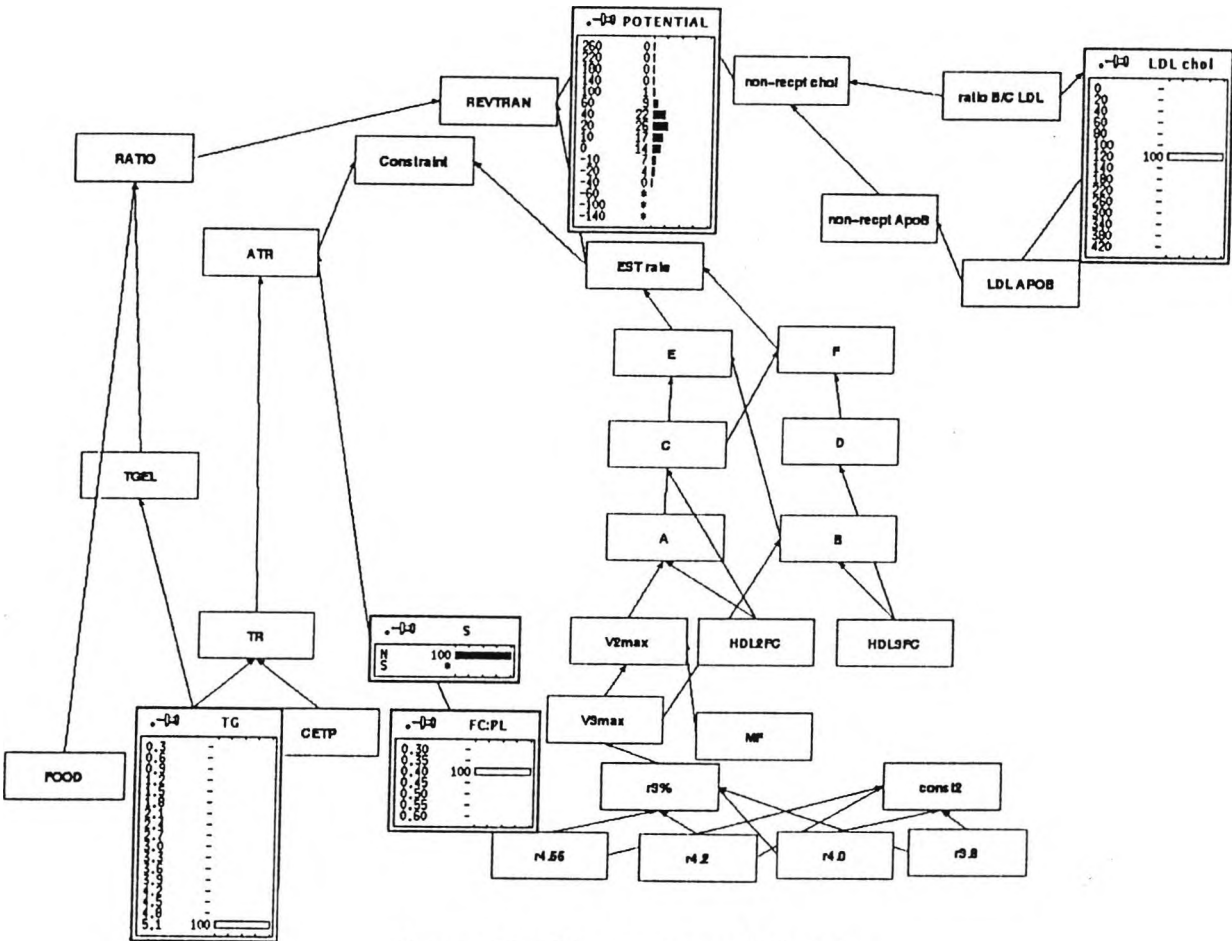


figure 7.3 - The post-prandial state

2) High LDL cholesterol and saturated receiving particles.

Individuals with a high potential for atheroma are likely to have elevated LDL cholesterol levels and abnormal composition of VLDL and LDL preventing reverse cholesterol transport. When both these conditions occur, the resulting potential for atheroma is likely to be maximal, and can therefore be compared with the upper limit of the potential for atheroma distribution, estimated from studies of whole body cholesterol metabolism and illustrated in figure 7.2.

To perform this comparison the model was instantiated with an LDL cholesterol level of 300 mg/dl, and a free cholesterol phospholipid ratio of 0.5. The resulting model is illustrated in figure 7.4.

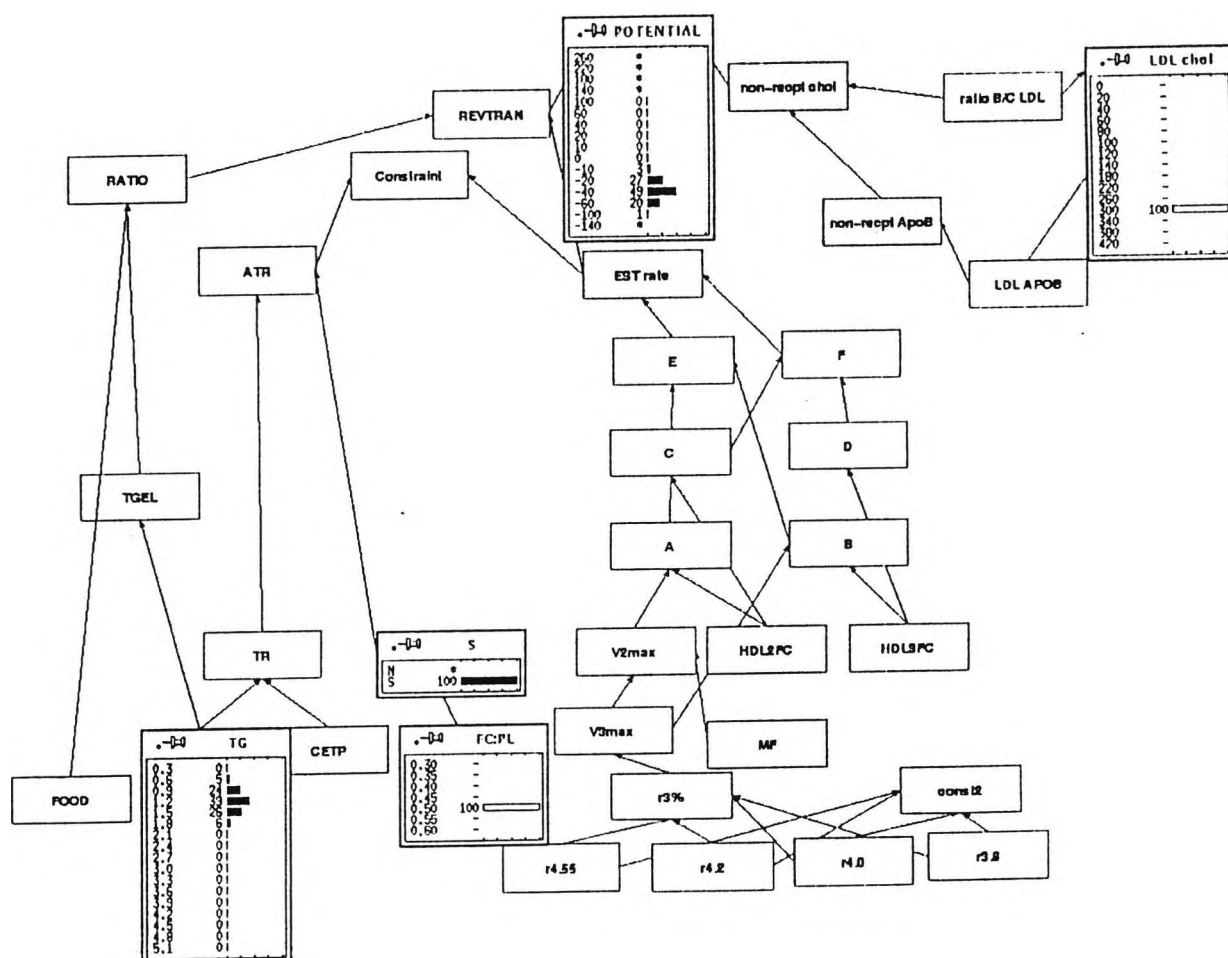


figure 7.4 - An extreme case of increased "potential for atheroma"

Figure 7.4 illustrates a distribution indicating increased potential for atheroma in an extreme case. This distribution having a mean of 40 nmol/ml/h and an upper limit of 100 nmol/ml/h (i.e. mean 1.16 g/d, upper limit 2.92 g/d).

The mean transport in this state is of a similar order of magnitude as the upper limit on the graph of figure 7.2. That is, a maximal value for potential atheroma.

In both of these examples model predicted extremes of atherogenic state have been shown to be comparable with a range of possible values for cholesterol deposition and regression as determined from studies of whole body cholesterol metabolism. The upper limits of the distributions on the potential for atheroma in each of these examples are not comparable with this range being approximately 5g/d. These are large numbers and are unlikely to be present in any individuals as they are greater than the total cholesterol turnover. In terms of the model, however, these large distributions simply represent a great deal of uncertainty in the model as a whole. As will be discussed in chapter 8, many of the issues and relationships highlighted in this thesis are yet to be resolved. Indeed one of the advantages of this modelling representation is that enables identification of these issues. We should not be surprised then that a great deal of uncertainty exists in model predictions. If it were possible to improve the model, reducing its uncertainty, then the range over which potential for atheroma is represented could be narrowed, and the resolution of the states on this node increased.

7.2.1.3 Qualitative validation of model subsections

Testing the model qualitatively is performed by first instantiating evidence in the model and observing the results. Comment is then provided describing the reasonableness of model inference and its consistency with the assumptions used in model construction.

In the model of potential for atheroma the cholesterol esterification rate ("EST rate" see figure 7.4) and the cholesterol ester transfer rate from HDL to larger lipoproteins ("ATR" see figure 7.4) are constrained to be equal so as to preserve equilibrium in cholesterol transport. This means that evidence obtained about one of these two variables (and its parents) will infer the most likely values of the other (and its parents). These inferences can be validated qualitatively, for example:

- 1) Information on the esterification rate should provide information on the VLDL triglyceride concentration and the CETP level.
- 2) Similarly the VLDL triglyceride concentration should provide information as to the most likely esterification rate.
- 3) Information on the VLDL triglyceride concentration and the esterification rate should determine the most likely composition of VLDL.
- 4) Modifying the GGE profile should provide information on the most likely esterification rate and hence the most likely VLDL triglyceride concentration.

The following qualitative testing illustrates these points:-

The *a priori* state of VLDL triglyceride, CETP activity, VLDL FC:PL ratio, the cholesterol esterification rate and the HDL₃ gradient gel electrophoresis are illustrated in figure 7.5.

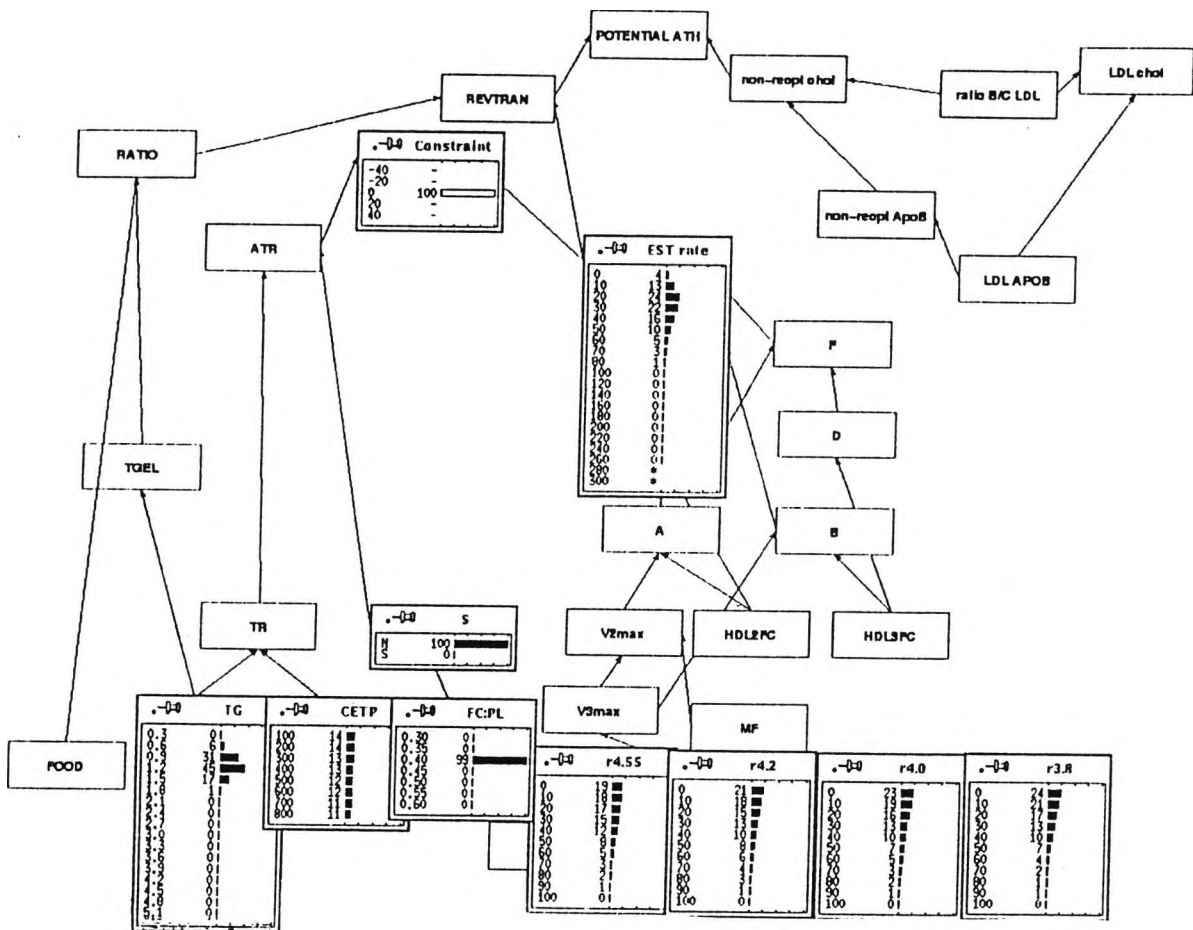


figure 7.5 - "Potential for atheroma" *a priori* state

As illustrated in figure 7.6, when the VLDL triglyceride concentration is elevated so the model predicts an increased cholesterol esterification rate i.e. an increase in the total throughput of cholesterol through the HDL cycle.

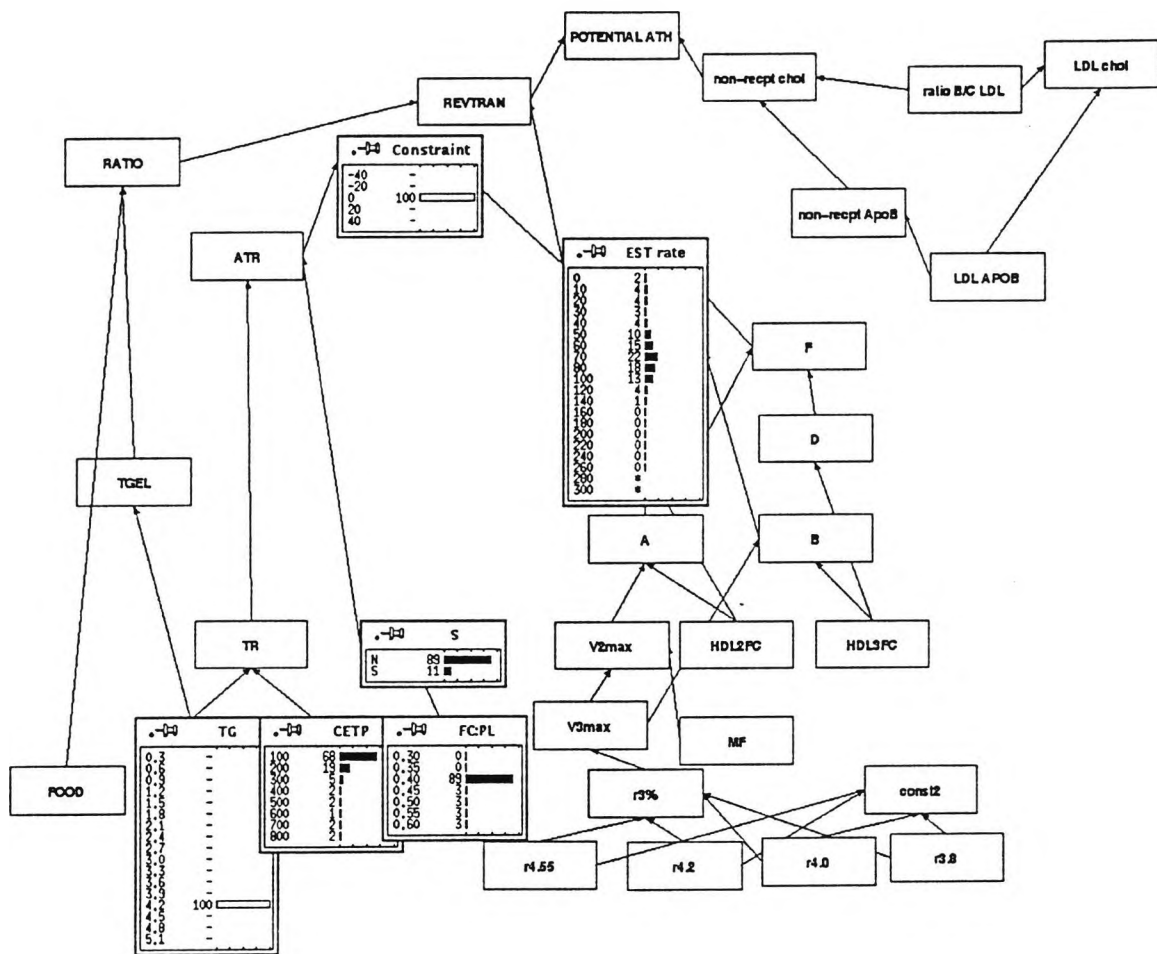


figure 7.6 - "Potential for atheroma" increased VLDL triglyceride as evidence

Similarly if the only information available was that the esterification rate was elevated then figure 7.7 illustrates the most likely pattern of VLDL triglyceride concentration and CETP activity, indicating increased cholesterol throughput in the HDL cycle.

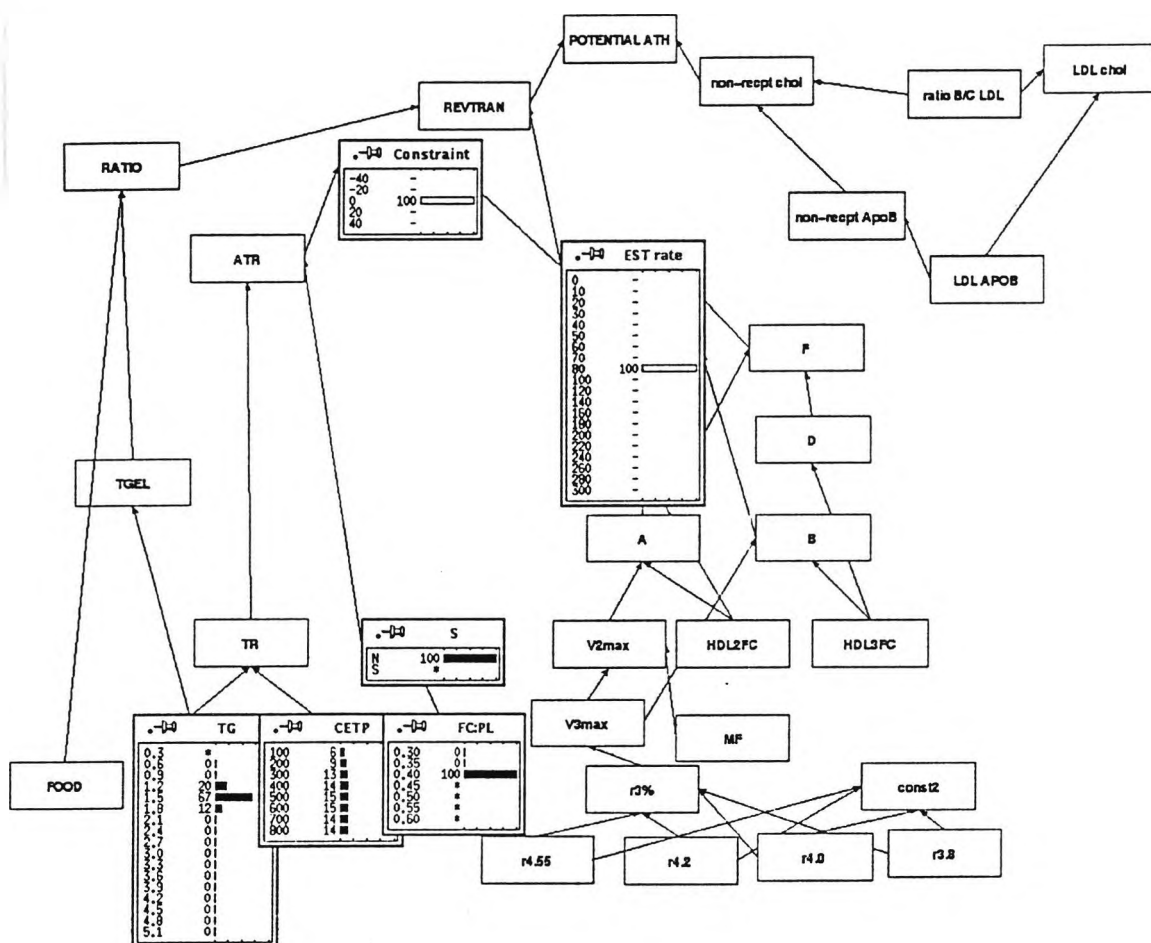


figure 7.7 - "Potential for atheroma" increased cholesterol esterification as evidence.

If it is known that the esterification rate is low (e.g 20 nmol/ml/h) and that the VLDL triglyceride concentration is high (e.g. 4.5 mmol/l) then the model infers a saturated VLDL, i.e. a high VLDL FC:PL ratio (see figure 7.8). The explanation for this is consistent with assumptions made in model construction. That is, whereas a high VLDL triglyceride concentration would drive cholesterol ester transport from HDL to VLDL/LDL, the decreased esterification illustrates that the throughput has not in fact been elevated. The only possible explanation for this is saturation of VLDL/LDL which would prevent cholesterol ester uptake from HDL.

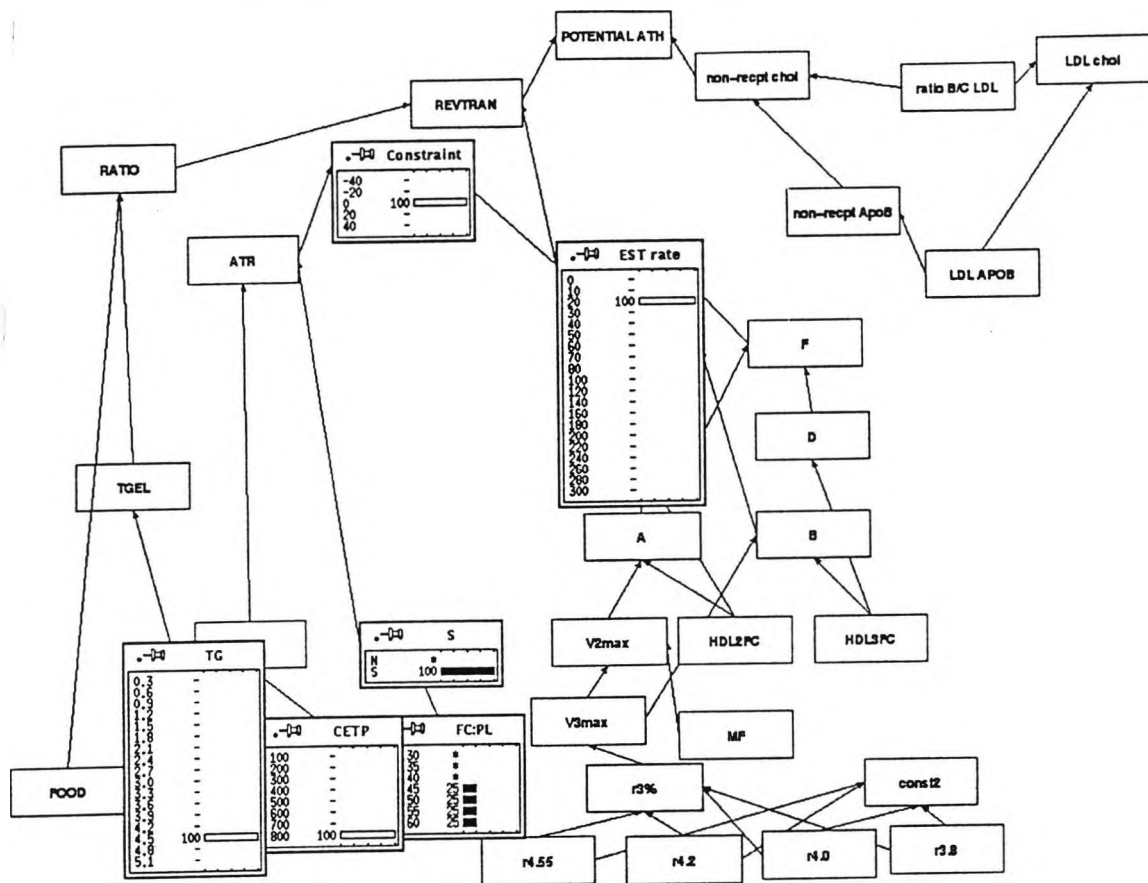


figure 7.9 - "Potential for atheroma", inferred saturation of VLDL when CETP levels are high.

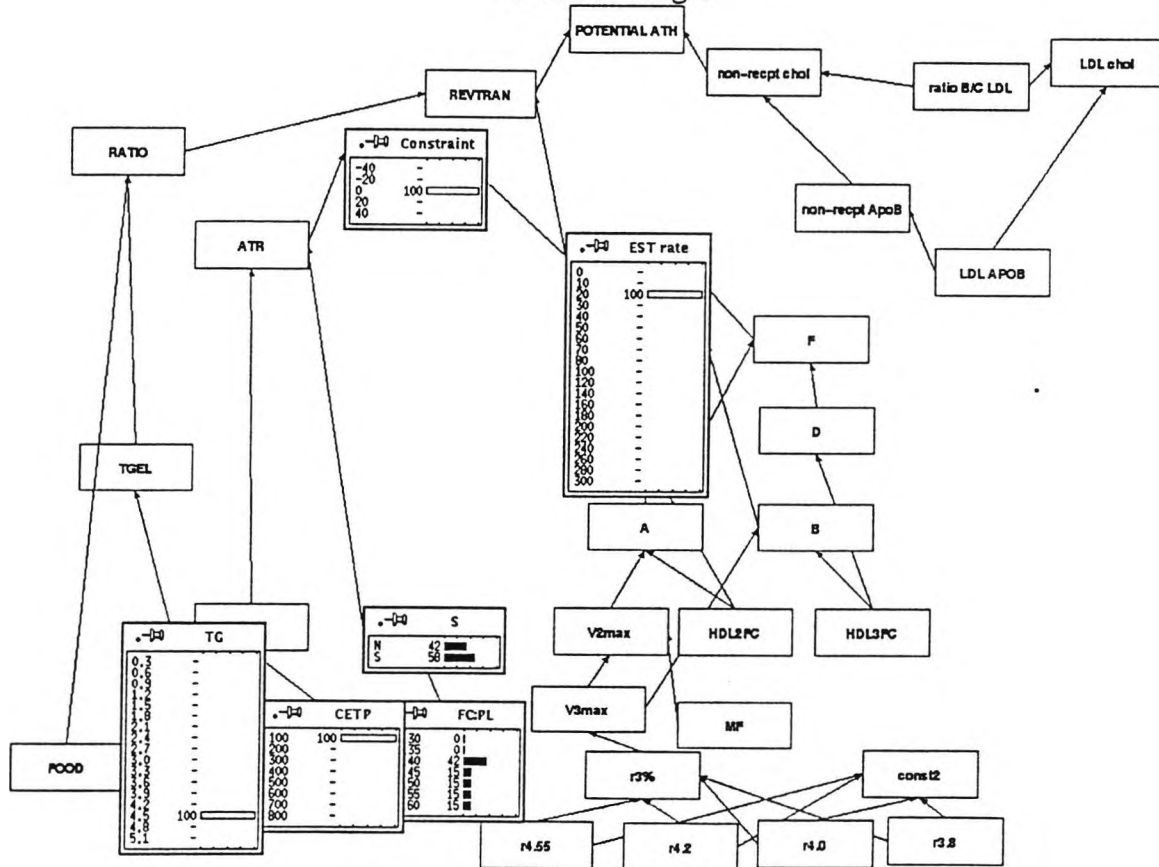


figure 7.10 - "potential for atheroma", inferred saturation of VLDL when CETP levels are low

Finally, in this section, the relationship between model predicted variables and different HDL₃ gradient gel electrophoresis (GGE) profiles is examined. The assumptions behind this section of the model are that a modification in esterification rate can be explained by either: a) changes in the HDL₃ GGE profile, such that profiles with a greater amount of smaller HDL₃ particles indicate an increased esterification rate, or b) changes in the HDL apoprotein AI concentration, increases in which are assumed to increase the potential loading of HDL with free cholesterol and hence its capacity for cholesterol esterification.

As discussed in section 5.6.3.2, the relationship between the HDL₃ GGE profile and the cholesterol esterification rate is extremely uncertain. What is of interest here then is the way in which the two possible explanations for an increased esterification rate combine. That is, changes in the HDL₃ profile and changes in the HDL free cholesterol (FC) concentration.

If the cholesterol esterification rate was known to be 70 nmol/ml/h (which, as illustrated in figure 7.15, is a typical high esterification rate for high VLDL triglyceride concentrations and unsaturated receiving particles), then the model can be used to indicate possible causes of this. With HDL FC at very low concentrations the only explanation for the elevated esterification rate would be an abnormal GGE profile as illustrated in figure 7.11.

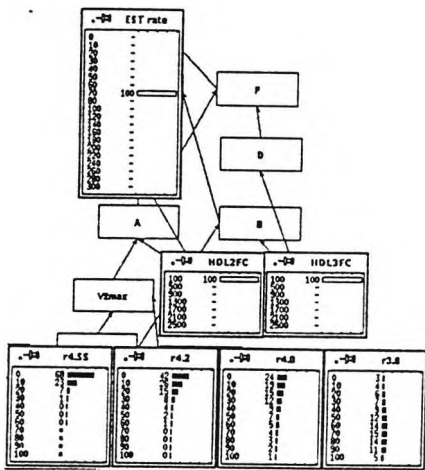


figure 7.11 - An abnormal HDL₃ profile on high cholesterol esterification rate and low HDL free cholesterol.

However, as HDL FC levels are increased they begin to explain the change in the esterification rate so that the HDL₃ GGE profile gradually normalises (see figure 7.12).

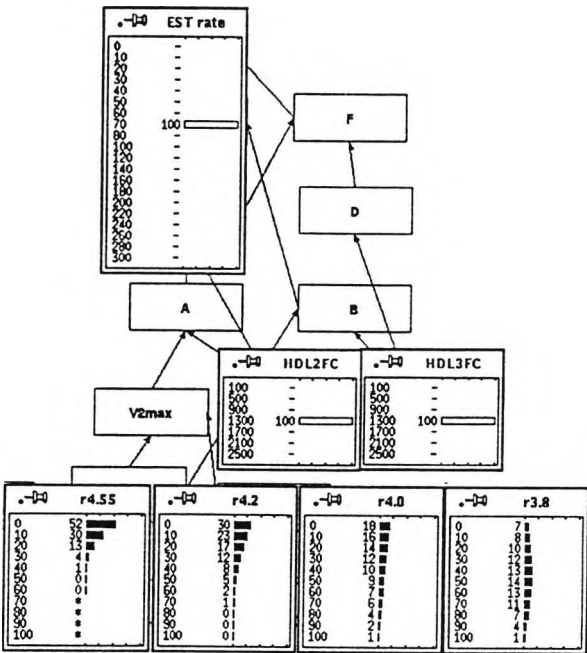


figure 7.12 - A normalising HDL₃ profile on increasing HDL free cholesterol.

Indeed at high levels of HDL FC the most likely HDL₃ GGE profile is virtually normalised (see figure 7.13).

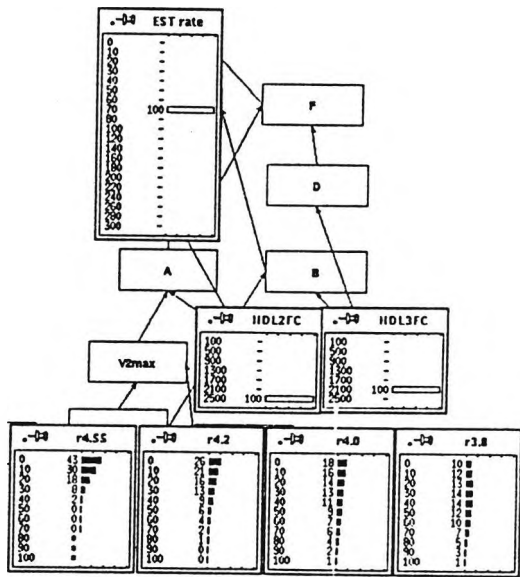


figure 7.13 - A normal HDL₃ profile on elevated HDL free cholesterol

The actual HDL₃ GGE distributions illustrated in figures 7.11 - 7.13 are somewhat irrelevant as the relationship between the GGE profile and the esterification rate (via V3 max) is extremely uncertain (see section 5.6.3.2). What is more interesting here is the qualitative behaviour of the model, providing alternative explanations for high esterification rates.

As noted above, and discussed extensively in section 5.5.6, it is possible that an elevated cholesterol esterification rate may present in both an atherogenic state (as indicated by a HDL₃ GGE profile with a preponderance of small particles) and in a normal state. The above testing illustrates that, when the cholesterol esterification rate is high, an increase in the HDL FC concentration provides an explanation for this such that an abnormal GGE profile becomes less likely.

In this respect the model obeys assumptions made in chapter 5 which were based upon the work of Hopkins and Barter. These authors found that by adding apo AI to a cholesterol esterification process, that modification of the HDL₃ GGE profile to an atherogenic profile was prevented. Apo AI is the main HDL lipoprotein and as such the carrier for HDL FC. Increasing HDL Apo AI may well be analogous to increasing HDL FC.

The above validation does, however, indicate an apparent limitation in the model. Currently, an increase in the model's cholesterol esterification rate (EST rate) will cause an increase in reverse cholesterol transport (REVTRAN). Since increases in the esterification rate can be caused by either atherogenic states (i.e. preponderance of small HDL₃), or non-atherogenic states (i.e. elevated HDL FC) then model propagation can result in contradictory situations. For example, figure 7.15 illustrates a situation where a high VLDL triglyceride concentration is thought to drive HDL cholesterol throughput resulting in an elevated reverse cholesterol transport when compared with the *a priori* state (see figure 7.14). However, this example also illustrates an abnormal HDL₃ GGE profile with a preponderance of small HDL₃, i.e. an atherogenic profile, hence the contradiction, an atherogenic HDL₃ profile whilst at the same time an increased reverse cholesterol transport.

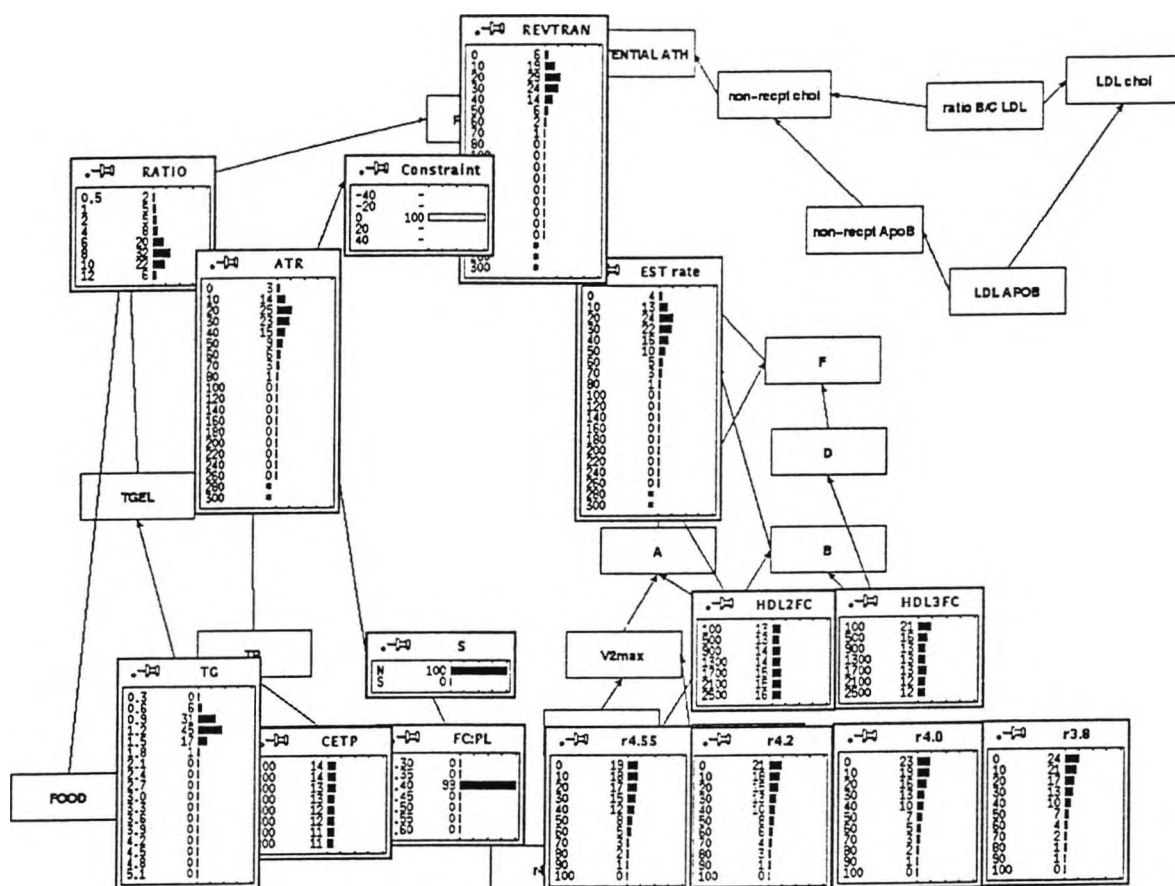


figure 7.14 - Reverse cholesterol transport, *a priori* state

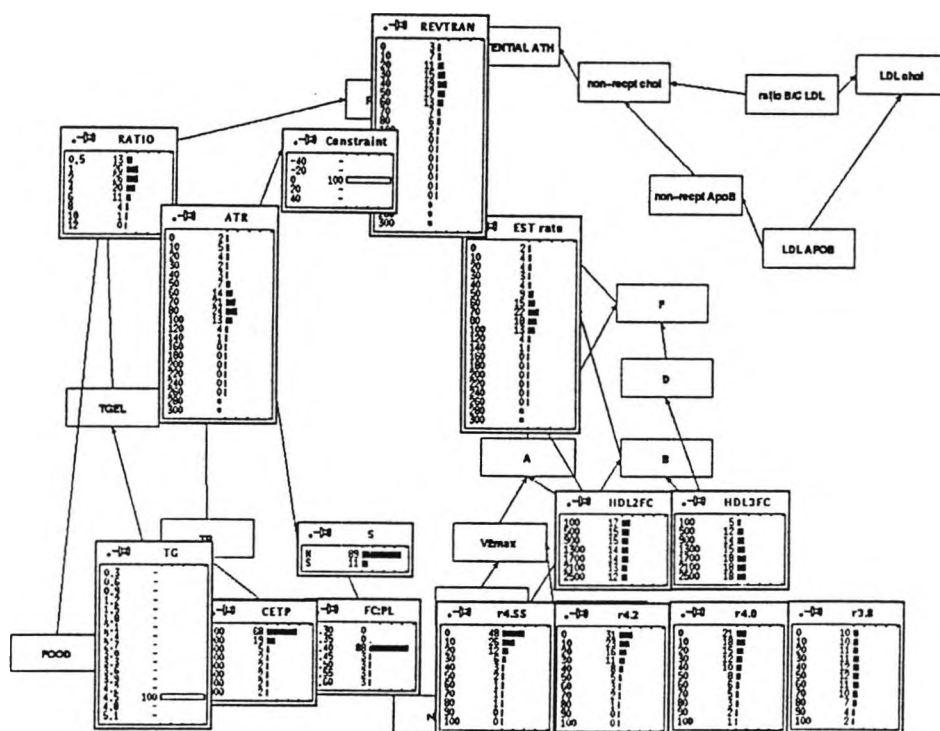


figure 7.15 - Illustrating the contradiction between increased reverse cholesterol transport and an atherogenic GGE profile.

This example illustrates a model limitation which is yet to be resolved. In physiological terms the solution lies in the source of free cholesterol for elevated esterification. In the normal case an increase in apo AI should encourage free cholesterol uptake from the tissues. In the abnormal case, it is likely that free cholesterol for esterification is that from cholesterol enriched VLDL rather than from the tissues. This could be modelled by linking the GGE profile to the RATIO node (ratio of free cholesterol from tissues: free cholesterol from lipoproteins used in cholesterol esterification) such that this ratio is decreased when the GGE profile becomes atherogenic. Unfortunately no data could be found to formulate this relationship.

7.2.2 Model validation in quantitative terms.

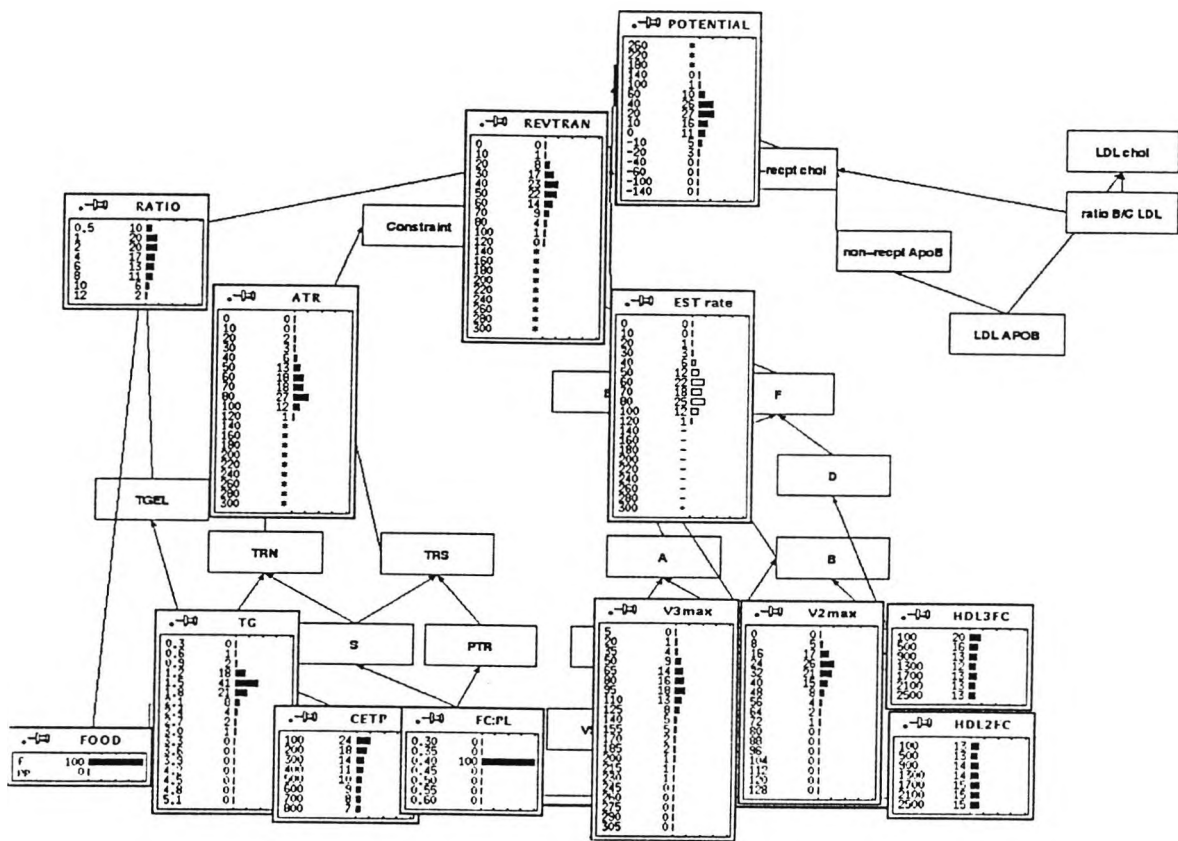
Castro et al. (1985) report several of the variables included in the model of potential for atheroma constructed in chapter 5. This information could not be used to formulate any of the local parent-->child relationships in the model as the study did not report variables which were directly causally related. It did, however, report variables which were indirectly related, for example the VLDL triglyceride concentration (TG) and the cholesterol esterification rate (EST). These data can be used to provide some preliminary quantitative validation of the model, and illustrate the mechanism by which further validation could be performed given more data.

By fixing the model esterification rate to that reported in the study, and all other variables to the *a priori* population values, the model can be propagated and we can compare the model predicted VLDL triglyceride concentration with that reported in the paper.

Since Castro et al. report results for groups of patients instead of individuals, i.e. means and standard deviations, it was necessary to instantiate likelihood distributions rather than single values on the network. A computer program was therefore written to convert data reported as mean and standard deviation values into discrete

probability weightings for each of the states on the node. This distribution converted the *a priori* distribution on that node to the mean and standard deviation reported in the study.

For the patients investigated in this study Castro et al (1985) report an esterification rate of $68.65 \pm 19.17 \mu\text{mol/ml/h}$. When this evidence was instantiated and propagated throughout the model it resulted in a network as illustrated in figure 7.16.



Several deductions can be made from figure 7.16 when comparing node distributions to the *a priori* distributions of the population illustrated in figure 7.14.

1) The reverse cholesterol transport "REVTRAN" predicted by the model has increased to a mean level of approximately 40-45 $\mu\text{mol/ml/h}$, compared with a value reported in Castro et al. (1985) of $43.52 \pm 19.69 \mu\text{mol/ml/h}$.

2) The RATIO node (i.e. the proportions of free cholesterol for esterification coming from peripheral tissues or large lipoproteins) has decreased from an *a priori* mean of 8.2 to one much closer to that reported in Castro et al. (1985) for this population, i.e. 4.5 ± 1.5 .

3) The VLDL triglyceride concentration predicted by the model has increased to a mean of approximately 1.5 mmol/l from an initial mean of about 1.1 mmol/l. Total triglyceride concentration for the patients reported in Castro et al. (1985) is $1.89 \pm 0.53 \text{ mmol/l}$ which is consistent with a VLDL triglyceride concentration in the region of 1.5 mmol/l.

7.3 Testing the validity of the model of VLDL apoprotein B and triglyceride cascade synthesis

As noted in the introduction to this chapter, and discussed in detail in chapter 6, it was not possible to construct a complete model of VLDL and LDL metabolism. Model validation has therefore been limited to examining its behaviour in qualitative terms.

The model of VLDL triglyceride metabolism and VLDL cascade synthesis was constructed using several assumptions about the effects of obesity and disorders. In this section the model is tested to see if its behaviour is consistent with these assumptions. More specifically, whilst obesity was assumed to increase both VLDL

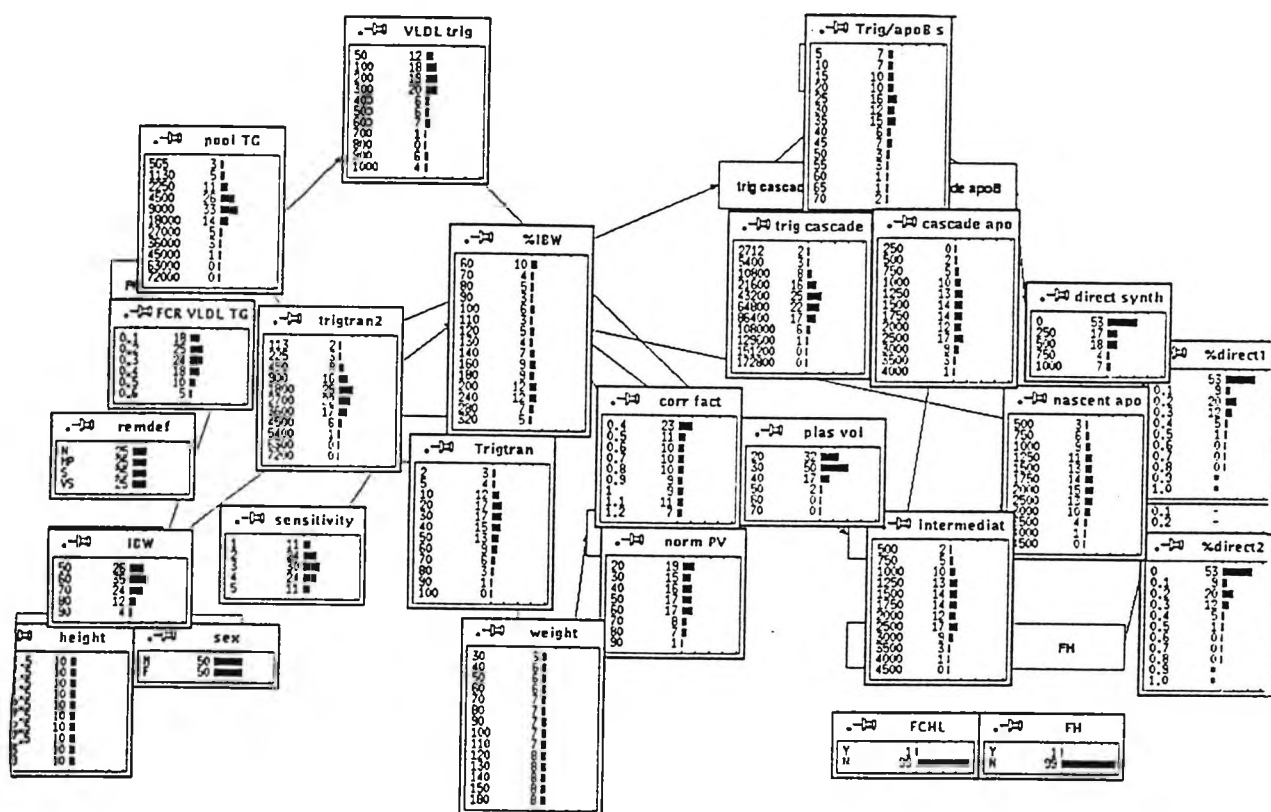


figure 7.18 - A model of VLDL triglyceride metabolism and VLDL synthesis into the cascade

To examine the behaviour of increasing obesity in the model, figures 7.19 and 7.20 illustrate model propagation when obesity is instantiated to its extremes. As illustrated in these figures, an increasing obesity elevates both triglyceride synthesis (trig cascade) and apo B synthesis (cascade apo). This results in less modification of the VLDL triglyceride/ apo B cascade synthesis ratio, particularly when compared with disorders of either triglyceride or apoB synthesis alone (see figures 7.21- 7.24).

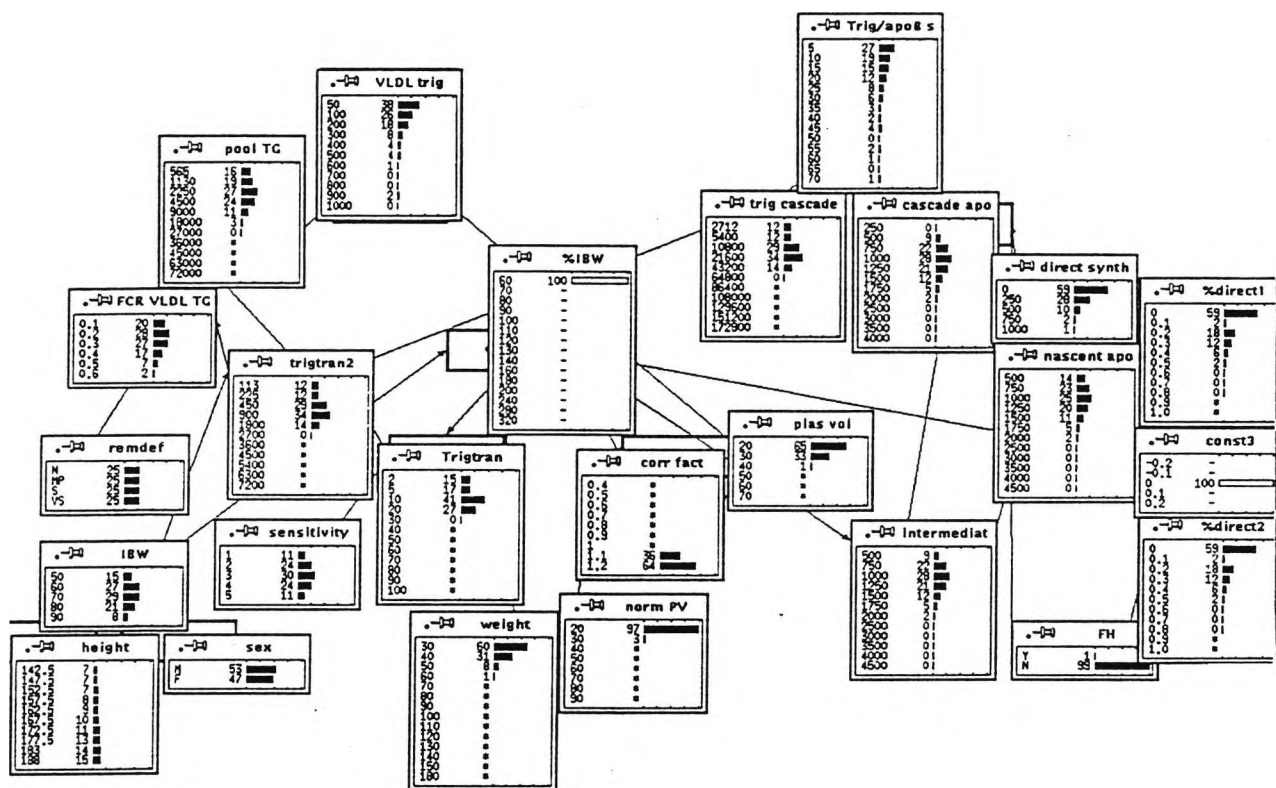


figure 7.19 - VLDL cascade synthesis when ideal weight is lower than normal

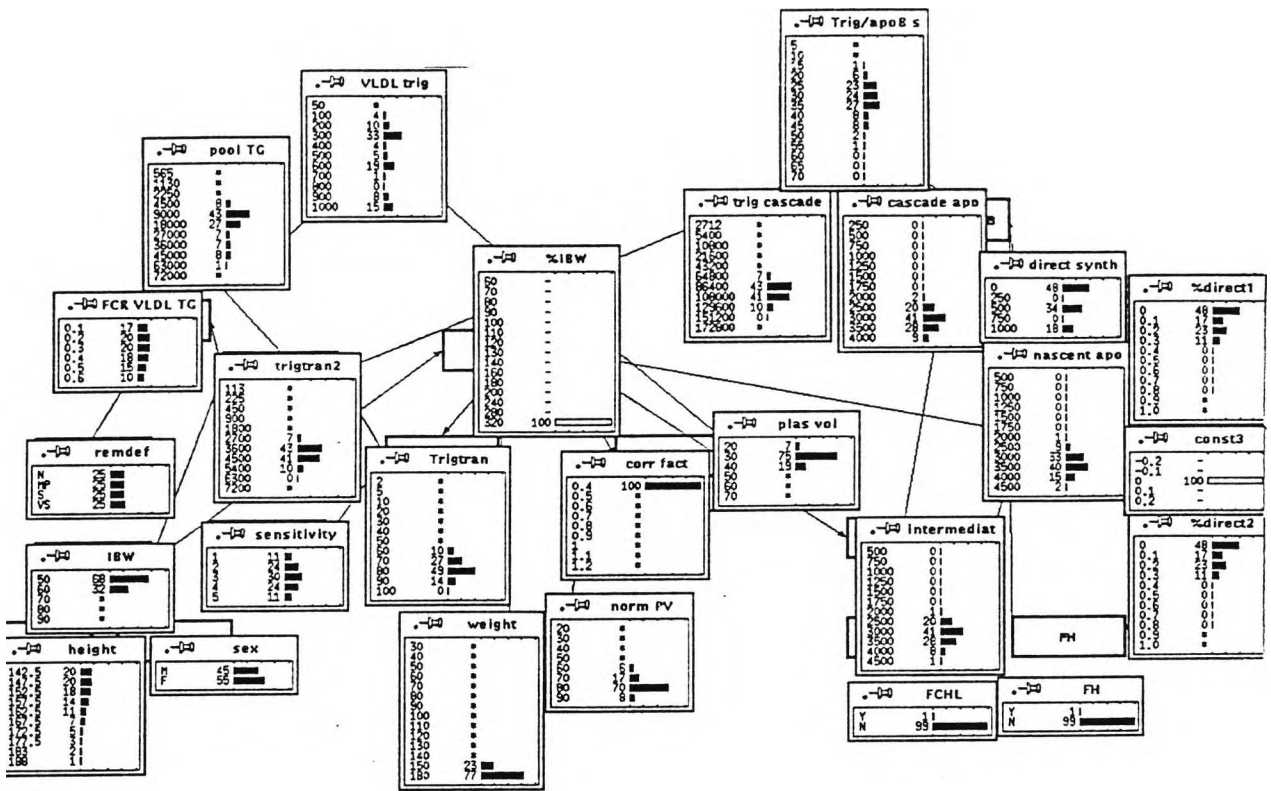


figure 7.20 - VLDL cascade synthesis during obesity

Figures 7.21 and 7.22 illustrate the effect of individual variation in triglyceride production (sensitivity) when obesity is not present. As illustrated in these figures individual variation in triglyceride production modifies the composition of VLDL synthesised via the cascade, in a way consistent with initial assumptions.

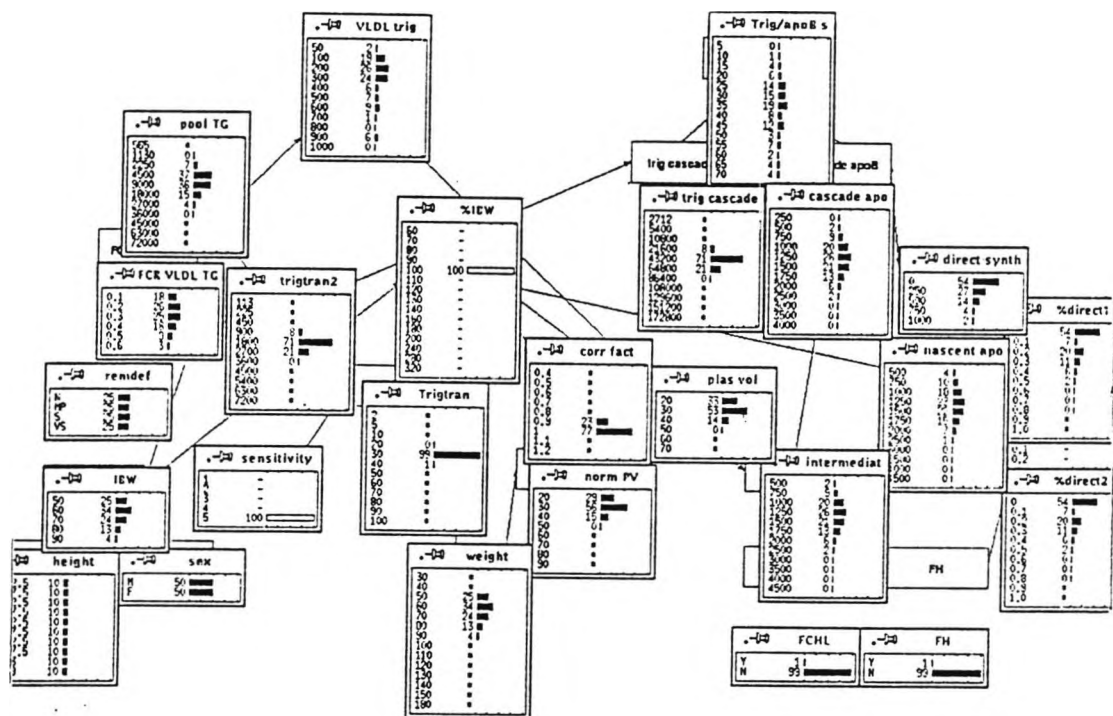


figure 7.21 - VLDL cascade synthesis in individuals who oversynthesise triglycerides

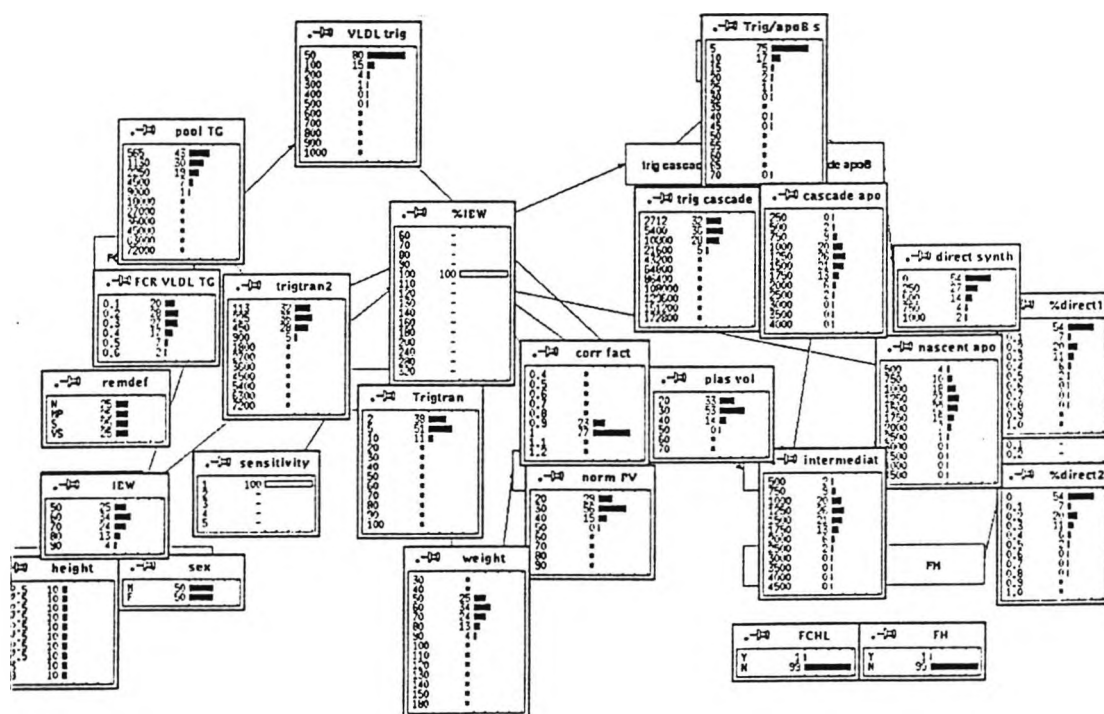


figure 7.22 - VLDL cascade synthesis in individuals who undersynthesise triglycerides

Figures 7.23 and 7.24 illustrate the effect of familial combined hyperlipidaemia, which during model construction was assumed to increase apo B synthesis. The multiple phenotypes of FCHL are explained in this thesis by the presentation of concomitant disorders of triglyceride metabolism which normalise VLDL composition resulting in an overproduction of normal VLDL. As illustrated in these figures the presence of FCHL modifies the apo B synthesis rate, modifying the composition of VLDL released into the cascade, once again this is consistent with initial assumptions.

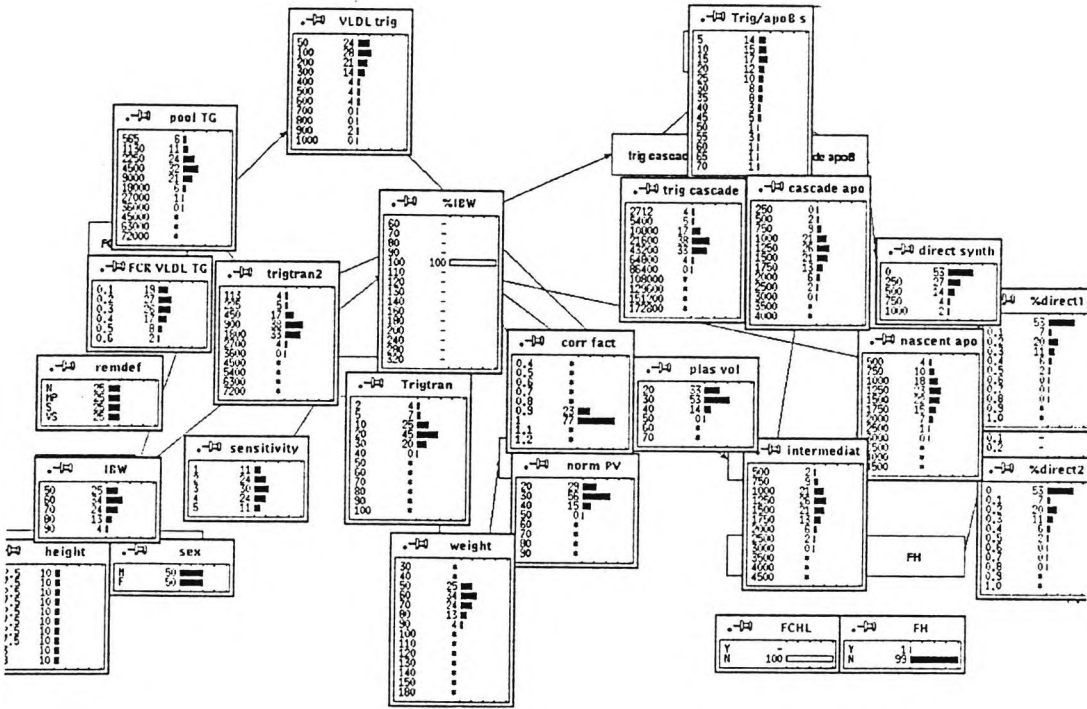


figure 7.23 - VLDL cascade synthesis without FCHL

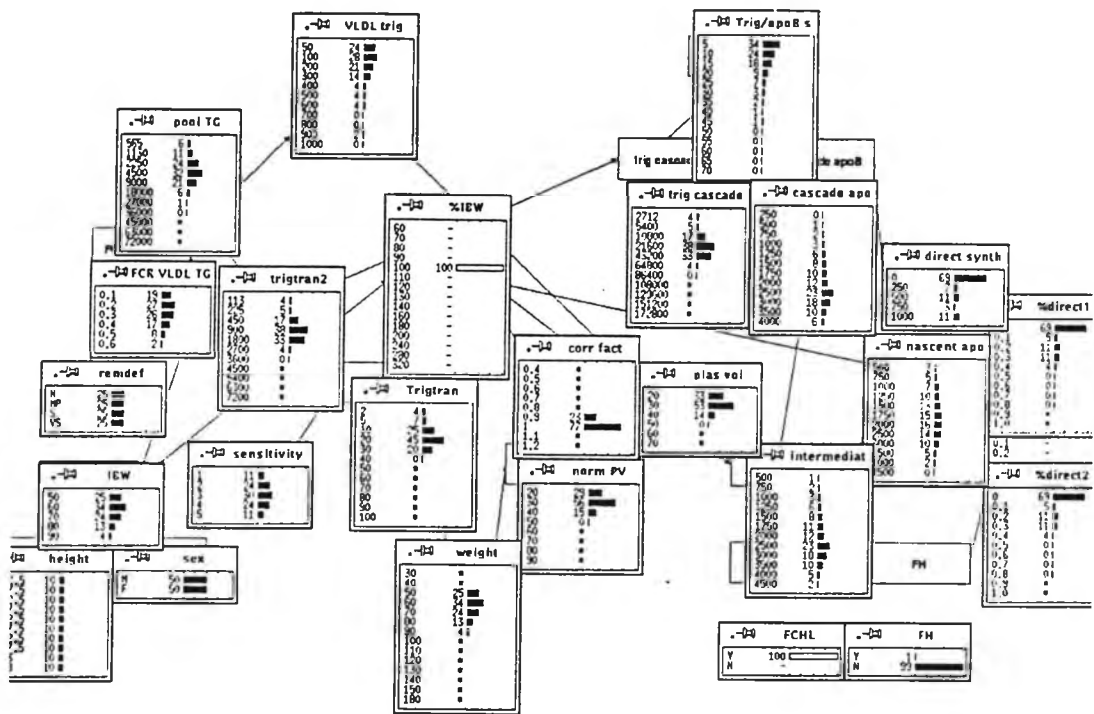


figure 7.24 VLDL cascade synthesis with FCHL

Chapter 8 - Discussion and Commentary

8.1 Introduction

In decision support systems (DSS), data, information and/ or knowledge are represented, and inferences made from this representation which direct the user toward the most appropriate decision. In the medical domain two sources of data, information and/ or knowledge exist. These are: a) the qualitative knowledge of the clinician, i.e. their opinions as to the efficacy of treatment and the most plausible physiology and pathophysiology; and b) quantitative literature data describing physiology and pathology.

To the researcher building DSS both these information sources are valuable. Whilst literature data quantitatively describe physiological mechanisms, it can often be the case that insufficient data exist to describe the complete physiological domain of interest, preventing the construction of decision support systems based on data alone. Conversely, clinical expertise can and has (Lewis et al., 1989; Thompson et al., 1990; The Working Group, 1991; The Study Group EAS, 1987; The Expert Panel, 1988; Shepherd et al., 1987;) been used to define patient management protocols, these being based on consensus between experts. However, these protocols often fail to address the complex trade-offs between benefit and risk involved in managing an individual (as illustrated by the letter to the Lancet - Olbright, 1991- quoted in section 2.6 of this thesis), and rather than highlighting areas of contention where further research is required, often force a consensus where none truly exists. The problem presents, how can we use both limited quantitative data and qualitative expert opinion in the patient management process.

Currently, reconciliation between quantitative data and qualitative knowledge has been performed in the published literature review. Here an expert interprets experimental studies in their specialist area and suggests hypotheses describing physiological mechanisms and their clinical implications. However, the processes of

validating and modifying these hypotheses when new data present, directing future research so as to examine contentious areas of these hypotheses, and including the implications of these hypotheses into a quality clinical decision making process are, on the whole, unstructured. Indeed Eddy et al. (1992) have commented on the poor linkage between experimental data and their eventual use as part of the clinical decision making process.

"The synthesis of evidence is a crucial link in the chain of events that connects an idea to evidence, to decision, to actual changes in people's lives. A break in the chain at the point where evidence is interpreted to draw conclusions can undo decades of hard work, miss opportunities for benefit, and even cause harm. Other links are fairly strong. Entire disciplines such as statistics and epidemiology have been developed to design individual experiments and perform face value interpretations. Billions of dollars are spent each year conducting experiments. Hundreds of billions are spent delivering interventions, and hundreds of millions of lives are affected by their outcomes. In contrast, relatively little attention has been paid to the synthesis of evidence and application of results to real decisions."

The objectives of this thesis, as described in chapter 1, were therefore "to illustrate how expert opinion and literature data can be incorporated into a model describing lipid and lipoprotein metabolism; and to show how (given sufficient data) such a model can be used to predict an individuals' health outcome, as part of a strategy for clinical decision support."

Both clinical and experimental advantages were thought to occur because of these objectives:-

- Predicting the health outcome of an individual, enables clinical decisions regarding treatment to be directed toward improving this health outcome i.e the definition of a

"quality" treatment decision given by Lohr (1990).

- In combining expert opinion and literature data in a more traceable way, a more formal checking of consistency between these two information sources can be performed, and areas identified where current opinion cannot be quantifiably validated, and hence further experimental research is required.

The remainder of this chapter is divided into three sections. Section 8.2 summarises this thesis, linking the chapters which describe: the problem, the most appropriate techniques for its solution and a theoretical framework for including these techniques as part of the decision making process (chapters 1-3); with an implementation of models of lipid and lipoprotein metabolism illustrating the use of these techniques (chapters 4-7).

Section 8.3 describes the contributions of this thesis both in the short and long term. In the long term this work is beneficial in that the models constructed in chapters 5 and 6 form part of a strategy consistent with a quality treatment decision making process. The more immediate contributions of this thesis are to show that, when modelling using the techniques described, data, information and knowledge from different sources can be incorporated into the same model, and consistency can be illustrated between different sources, the resultant model being a summary of current knowledge. This model-based summary can then aid in the interpretation of the results of future experiments, and highlight beneficial areas for future research into lipid and lipoprotein metabolism.

In section 8.3 examples are given of consistency between different information sources describing hyperlipidaemia e.g experimental and epidemiological data, and of beneficial areas for future research into lipid and lipoprotein metabolism.

8.2 Thesis summary and commentary

So as to construct the most appropriate models to achieve the objectives outlined in section 8.1, the first step was to investigate the decision making process involved in the management of hyperlipidaemia. This was performed in chapter 2. In brief, it was found that the goal of treating hyperlipidaemia was to minimise the risk of coronary heart disease, and that currently several tools exist which are useful when screening the population (identifying individuals at elevated risk relative to the population) and during first line management. These tools are: formulae for the calculation of CHD risk, management guidelines, and computer- based automation of these guidelines. However, these tools are of limited use when considering the management of an individual at elevated risk of CHD; as they do not enable a comparison between treatment options for the individual, including the complex trade-offs defining the benefit to an individual of treatment during more complex second line management. It was concluded then that a more detailed physiological representation of CHD risk was required which did not include inappropriate fixed baseline measurements of risk.

Representing individual risk of CHD at a more detailed level suggested the most appropriate level of modelling in later chapters (5 & 6). In these chapters model construction was directed toward representation of CHD risk in terms of cholesterol fluxes to and from peripheral tissues, and based upon the incorporation of qualitative and quantitative knowledge.

Chapter 3 described why a physiological model-based approach to knowledge representation is most appropriate when supporting the complex treatment decisions of second line management, and how both qualitative and quantitative knowledge can be included in such a physiological model.

More specifically, the chapter commented on other studies where a physiological model based approach to knowledge representation was required to support patient management decisions. This occurs in situations where no clear guidelines exist, diagnosis and the effects of treatment are uncertain and treatment decisions involve

trade-offs between goals. In these studies knowledge representation using physiological models enables consideration of the complete patient context during decision support i.e. a representation of patient state and the likely effects of treatment on that state. Treatment selection is then a trade off between the most likely effects of treatment offset against costs and patient preferences.

Chapter 3 then described methods for representing knowledge in context of the whole patient state rather than as context independent rules, i.e. intensional approaches. In particular it focused on the Causal Probabilistic Network technique. This technique not only provides a model-based context sensitive (intensional) approach to knowledge representation, but also allows the representation of uncertainty in model relationships and provides a framework for the incorporation of qualitative and quantitative knowledge into model construction. More specifically, qualitative knowledge is incorporated into modelling using expert opinion on causal pathways existing in the physiology, and conditional independence and irrelevance assumptions within these pathways; whilst quantitative knowledge is incorporated via the use of literature data to quantify local causal relationships in the network. This method of being able to use literature data to describe local causal relationships, i.e. to estimate conditional probability tables in the model, was particularly useful in the field of lipid and lipoprotein disorders. In this field data describing the whole metabolism are extremely limited. Instead, data are available describing small localised areas. Annotating the qualitative structure with quantitative relationships enables consistency checking between a) different quantitative studies and b) qualitative assumptions and quantitative studies. This was illustrated in the validation chapter (chapter 7), and is discussed further in section 8.3.

Whilst the CPN methodology provides a framework for the incorporation of qualitative and quantitative knowledge, it is necessary to have techniques which enable the dispersed quantitative knowledge, describing each local relationship, to be drawn together. This is important since many studies describing lipid and lipoprotein metabolism are performed by different groups and under different conditions, such that study biases exist. It is necessary therefore to have techniques for : a) adjusting the

data from different studies to make them comparable and b) generating relationships between variables for these adjusted data. Chapter 3 described techniques for meta-statistical analysis referring to the work of Eddy et al. (Eddy et al., 1989, 1990, 1992). These authors have used CPN techniques (called influence diagrams in their work) to tackle the problems of meta-statistical analysis, such that study biases are explicitly represented in the model. When using these techniques in later chapters, rather than combining reported average effects, raw data from different studies are combined. In doing so it has been possible to visually inspect for heterogeneity within the data, highlighting situations where lipid metabolism can behave in a number of different ways. In this way consistency has been shown between different studies performed by different groups but examining the same local relationship, and where this consistency does not exist the thesis has highlighted areas of contention and tried to provide reasonable hypothesis to explain the contention (see section 8.3)).

Chapter 3 concluded by describing a framework for decision support in the management of hyperlipidaemia within which all of these techniques fit. This framework illustrates that, given sufficient knowledge describing the lipid and lipoprotein metabolism and patient preferences, it possible to use the models constructed from this knowledge in a way so as to provide quality decision support. This chapter also described the tools used in constructing the CPN models of lipid metabolism constructed in chapters 5 and 6 which form part of the framework.

Chapters 4 - 6 dealt with the modelling of lipid and lipoprotein metabolism, representing the health outcome as an individual specific measure of CHD risk due to modifications in the lipid and lipoprotein metabolism. This measure, described as the "potential for atheroma" in chapter 5, is the difference between cholesterol transport to and from the peripheral tissues.

Chapter 4 established the necessary level of abstraction for modelling the lipid metabolism so as to distinguish between individuals in terms of their risk of CHD (potential for atheroma). It examined studies of whole body cholesterol metabolism and concluded that a more detailed level of modelling was required to describe how

modifications in lipid and lipoprotein metabolism effect cholesterol deposition at the periphery, this level of abstraction being that modelled in chapters 5 and 6. Some information on the range of cholesterol deposition as inferred from these studies and has been compared (in chapter 7) with the range of atheroma potential modelled in chapter 5. This consistency check provides further evidence for model validation and is discussed in further in section 8.3.

In Chapter 5 the potential for atheroma was modelled, this being represented as the difference between forward and reverse cholesterol transport. The model was constructed as a CPN from expert opinion on physiological pathways, and pooled literature data describing the quantitative nature of relationships on these pathways. In this chapter many controversial issues of reverse cholesterol transport were addressed. In doing so, one model has been constructed which is consistent with expert opinion, experimental data and epidemiological data.

Chapter 6 endeavoured to extend the model of chapter 5 so that the effects of lipoprotein disorders and obesity could be represented in terms of CHD risk modification. It was not possible to complete this link, the limit of the modelling in chapter 6 being to illustrate how disorders of apo B and triglyceride metabolism would effect VLDL triglyceride concentration and the apo B / triglyceride ratio of VLDL entering the cascade. The latter of these variables was thought to indicate the metabolic fate of VLDL and hence have a large effect on atherogenicity.

Chapter 7 illustrates the validity of the models constructed in chapters 5 and 6 in qualitative and quantitative terms. This chapter shows how the model of "potential for atheroma", constructed in chapter 5, is consistent with a population on a western style diet, studies of whole body cholesterol metabolism, the assumptions used in model construction, and the results of a study not used in model construction. Validation in qualitative terms was performed on the model of apoprotein B and triglyceride production constructed in chapter 6, illustrating that the model obeys the assumptions made during its construction.

8.3 Contributions of this Thesis

8.3.1 Introduction

This thesis has described, and illustrated the use of, techniques for combining quantitative data and qualitative knowledge into models of lipid and lipoprotein metabolism. These models predict modifications in the health outcome associated with disorders of lipid and lipoprotein metabolism, i.e. the potential for atheroma, and as such could be included in a DSS for the management of hyperlipidaemia. Constructing such a system, using the framework outlined in chapter 3 (section 3.5), would enable the clinician to predict the results of an individual patients' treatment in terms of its effects on their risk of CHD (atheroma potential), and hence aid them in selecting the most beneficial treatment when this change in risk is offset against likely side-effects and patient preferences.

The lack of quantitative information available describing lipid and lipoprotein metabolism means that, currently, it has not been possible to construct a DSS system using these techniques. However, other, more immediate contributions of this work exist, particularly in the fields of knowledge representation and lipid and lipoprotein metabolism. These are:-

- a) The modelling approach allows consistency checks between numerous sources of data, information and knowledge e.g. data describing detailed metabolic experiments, epidemiological data, and clinical expert opinion.
- b) The modelling approach enables identification of areas where future research is necessary and most beneficial, either because that area is contentious or because few data exist.
- c) Having models which summarise current knowledge allows us to interpret the results of new studies, comparing them with the model's behaviour.

The following section expands on these conclusions giving examples of consistency between different information sources and areas where future research investigating lipid and lipoprotein research is beneficial.

8.3.2 Illustration of the consistency of different sources of data, information and knowledge describing lipid and lipoprotein metabolism

The modelling approach taken in this thesis has enabled consistency checks to be performed between quantitative data, and qualitative information and knowledge, describing both detailed physiology, and more general epidemiology. The following text (sections 8.3.2.1- 8.3.2.4) illustrates, with examples, how consistency has been shown between:-

- detailed knowledge describing metabolic pathways and the behaviour of the model as a whole.
- studies reported by different groups examining the same metabolic relationship
- experimental, and epidemiological data
- detailed experimental data and studies of whole body cholesterol metabolism

8.3.2.1 Consistency between detailed knowledge describing the metabolic pathways and the behaviour of the model as a whole.

The model of "atheroma potential" constructed in chapter 5 was built from quantitative data and expert opinion describing the metabolic pathways involved in cholesterol transport. Although the local relationships in the model were constructed at a detailed level from quantitative data, the model's behaviour as a whole is consistent with qualitative assumptions about the nature of the lipid and lipoprotein metabolism. For example, in the model of potential for atheroma it was possible to examine how instantiated evidence in part of the model indicated the most likely values of variables in other parts of the model, and compare this with the assumptions

as to how the whole system should work.

In this way the model predicted results were consistent with (see section 7.2.1)

- co-existing high cholesterol esterification rates and high VLDL triglyceride concentrations.
- VLDL cholesterol saturation as an explanation for evidence of high triglyceride concentrations co-existing with low esterification rates.
- high esterification rates being atherogenic or antiatherogenic depending upon the HDL free cholesterol concentration and the HDL₃ GGE profile, these two factors providing alternate explanations for apparently high esterification rates.

Similarly, in the model of VLDL triglyceride and apo B metabolism described in chapter 6, the qualitative behaviour of the model was shown to be consistent with the assumptions describing the action of obesity and disorders of apo B and triglyceride synthesis (see section 7.3).

Further consistency has been illustrated between the behaviour of the model of 'potential for atheroma' and current knowledge of atheroma in a population on a western style diet. The model, when instantiated at the population (*a priori*) state, predicted a small negative atheromic potential (-ve indicating cholesterol transport toward the tissues) (see section 7.2.1.1). Western populations are at elevated risk of CHD relative to other populations, meaning that slow progression of atheroma, occurring over a period of decades, is normal. Thus the model predicts results consistent with knowledge describing western populations as a whole.

In all these cases representing detailed knowledge in a model was consistent with qualitative expert opinion at a higher level of abstraction.

8.3.2.2 Illustrating consistency between different groups studying the same local relationship

The techniques described and used in this thesis allow for consistency checking between data describing the same local relationship in the metabolism. Data are often reported in the literature by different groups using different protocols or techniques, and hence have different biases. By pooling data from different studies to generate model relationships, it was possible to try and explain uncertainties in generated relationships in terms of heterogeneity within the study groups. Where this uncertainty could not be explained, then using the CPN technique to model the relationship enabled representation of the remaining uncertainty in the model.

For example, in chapter 5 (section 5.3.2), data from nine studies, reported by six different research teams, were used to construct a relationship between the uptake of apo B by the non-receptor mediated pathway and the concentration of LDL apo B. By pooling these data it was possible to show consistency within the study results even though there was a wide range of heterogeneity of primary lipid disorder present within the patients in the study groups.

8.3.2.3 Illustrating consistency between experimental and epidemiological data.

The model of 'atheroma potential' (see chapter 5) was constructed using both experimental data and expert opinion describing lipid and lipoprotein metabolism. In this thesis this model has been shown to be consistent with epidemiological studies correlating different lipoprotein patterns with the risk of CHD. For example:-

1) In the model, an extremely small subfraction of HDL₃ indicated a high cholesterol esterification rate, where much of the free cholesterol for this esterification was from the larger lipoproteins rather than the peripheral tissues, i.e. an atherogenic presentation. Epidemiologically, Cheung et al. (1991) have illustrated a correlation between patients presenting with an abundance of small HDL₃ and a high risk of

CHD.

2) In the model a high FC:PL ratio indicated saturation of VLDL/LDL with cholesterol, this saturation inhibiting cholesterol transport along the reverse pathway and resulting in increased atherogenic potential. Epidemiologically Kukis et al. (1982) have illustrated that an increase in the FC:PL ratio of large lipoproteins is associated with an increased risk of CHD.

3) In the model, just as an elevated cholesterol esterification rate combined with an abnormal phenotypic pattern of small HDL₃ indicated greater free cholesterol use from larger lipoproteins, so an increased esterification rate combined with a normal HDL pattern indicated greater free cholesterol uptake from the tissues. According to Hopkins et al., (1989) the lack of very small HDL₃ in this situation is caused by a greater concentration of apo AI in HDL, which normalises the size distribution of HDL. This increase in HDL Apo AI increases the HDL capacity for both free and esterified cholesterol resulting in a high HDL cholesterol concentration as a whole.

Numerous epidemiological studies have found a relationship between high concentrations of HDL cholesterol and decreased risk from CHD. This finding, consistent with the metabolic description given above, is now common clinical knowledge, and it is normal for a total cholesterol measure to be subfractionated into LDL and HDL cholesterol concentrations.

4) Contention exists concerning the value of triglyceride concentrations as indicators of CHD risk. As noted by expert guidelines (The Expert Panel, 1988), in epidemiological studies hypertriglyceridaemia "is not an independent risk factor (for CHD), i.e. the association usually disappears when adjusted statistically for plasma total cholesterol levels and HDL-cholesterol levels" (brackets added).

Once again this is consistent with the model of 'atheroma potential' described in chapter 5. In this model high triglyceride concentrations drive HDL cholesterol throughput, increasing the cholesterol esterification rate. However as illustrated in points 1 and 3, a high esterification rate can either cause atheroma or be protective against it depending upon the nature of HDL. HDL consisting of smaller HDL₃ lipoproteins being indicative of atheroma whilst larger cholesterol enriched HDL are protective. The value of triglyceride concentrations as indicators of atheroma would therefore depend upon the HDL composition which, as noted in point 3, determines the HDL cholesterol concentration.

5) Brinton et al. (1991) have commented on the association between lipase activities and the concentration of HDL cholesterol, noting that an increase in hepatic lipase (HTLP) activity is associated with a decreasing HDL cholesterol level, whilst an increase in lipoprotein lipase activity is associated with increasing HDL cholesterol concentrations.

This is consistent with the modelling of chapter 5. HTLP lipolyses smaller lipoproteins, whilst lipoprotein lipase lipolyses triglycerides from very large VLDL and chylomicrons. As noted in section 5.5.1 and 5.5.5.1 it is the larger VLDL and chylomicrons which are likely to release apo AI and other HDL surface constituents during triglyceride lipolysis, meaning that: lipoprotein lipase is more likely to be involved in the production of normal, apo AI rich, HDL; and HTPL is more likely to be involved in the production of very small, atherogenic, HDL₃.

6) As noted in section 5.5.3, two types of HDL exist which are thought to be more or less protective against atheroma (Rader et al., 1991; Zech et al., 1983). These are:- Lp AI and Lp AI/AII, the former of which contains apo AI alone and is thought to be protective against atheroma, whilst the latter contains both apo AI and apo AII and is thought to be indicative of atheroma.

In point 3 it was suggested that increasing apo AI increases HDL free cholesterol

concentrations, which are protective against atheroma. Here it should be noted that increasing apo AI concentrations are also more likely to increase the Lp AI fraction of HDL, which is consistent with greater protection against atheroma.

In all of these cases (1-6 above) the qualitative behaviour of the model constructed from detailed knowledge and data describing individual metabolic relationships can be shown to be consistent with epidemiological studies relating patterns of lipid and lipoprotein presentations with risk of CHD due to atheroma. As in section 8.4.2.1 the representation of detailed knowledge in the model was consistent with knowledge at a higher level of abstraction.

8.3.2.4) Illustrating consistency between the modelling of chapter 5 and the studies of whole body cholesterol metabolism described in chapter 4.

In chapter 7 a range of peripheral tissue cholesterol loss was determined from studies reporting both 2 pool cholesterol turnover and sterol balance cholesterol turnover in the same patients. This range was compared with the range of "potential atheroma" predicted by the model of chapter 5 when the model was instantiated to extremes of physiological state. Some consistency was illustrated between the range of peripheral tissue cholesterol loss and the mean predictions of the model, these values being of the same order of magnitude.

8.3.3 Using the model as a summary of current knowledge to interpret future work

Since the model of 'atheroma potential' described in chapter 5 is consistent with knowledge/data from many different levels of abstraction, it therefore represents a summary of the domain against which the results of new experimental studies can be compared. These new studies, if compatible with this summary, can then be used to re-formulate subsections of the model, this process being relatively simple since each

of the model relationships are constructed locally as subsections of the whole model. Chapter 7 (section 7.2.2) illustrated how the results of studies not used in model construction can be checked against the model.

Newer CPN techniques are also being developed (Spiegelhalter and Lauritzen, 1989) which allow conditional probability tables to automatically adjust as new data present, automating the maintenance of CPN models.

8.3.4 Illustrating that the modelling approach can be used to direct further research.

In constructing the models described in chapters 5 and 6 many areas for further research have been identified. These fall into two groups:-

- areas where controversy exists in expert opinion on lipid and lipoprotein metabolism and,
- areas where insufficient data exist to substantiate model formulation.

Identification of these areas is extremely important as limited resources can then be directed toward the most profitable areas for research. New studies performed in these areas can then be compared with, and included in, the model which summarises previous knowledge.

This section highlights areas of contention in lipid and lipoprotein metabolism and areas where insufficient data exists. These problem areas having come to the fore during, and because of, the modelling process.

8.3.4.1) Areas of contention within lipid and lipoprotein metabolism highlighted during the modelling process.

What effect has the composition of newly synthesised VLDL on its eventual metabolic pathway, and how do disorders of lipoprotein metabolism effect this composition?

As discussed in section 6.2.2.2 triglyceride enriched VLDL are more prone to cholesterol uptake from HDL in an equi-molar exchange of cholesteryl esters for triglyceride. The VLDL remnants of initially triglyceride rich VLDL are therefore likely to become more cholesteryl enriched than normal and, as noted in section 6.2.2.2, are therefore more susceptible to hepatic uptake. It follows then that larger triglyceride rich VLDL would result in fewer LDL being produced from the cascade, and indeed in clinical practice it is often the case that elevated triglyceride concentrations are associated with below normal concentrations of LDL. As in the case of LDL direct synthesis illustrated below, the composition of newly synthesised VLDL may well determine its metabolic fate.

In order to model the VLDL triglyceride/ apo B synthesis ratio and investigate these relationships, sections 6.4.2.2 and 6.4.2.3 describe the modelling of apo B synthesis during FCHL, FH and obesity, combining this model with that constructed for triglyceride metabolism, as described in section 6.4.1. Chapter 6 concludes that the VLDL triglyceride / apo B cascade synthesis ratio might be insufficiently detailed to explain differences in cascade removal of VLDL, and LDL direct synthesis, and that compositional ratios describing the VLDL subfractions (VLDL₁ and VLDL₂) might be more informative. Synthesis of LDL coming from either of these subfractions independently (as different lipases act on each subfraction), dependent upon the triglyceride / apo B ratio in each subfraction.

Direct synthesis of LDL.

As described in section 6.2.2.3 many studies modelling apo B metabolism have found it necessary to postulate direct hepatic synthesis of LDL. Direct production of LDL can alter the eventual concentration and composition of LDL and as such is relevant when representing potential for atheroma.

One explanation for direct hepatic synthesis of LDL is presented in section 6.2.2.3. According to Shames and Havel (1991) direct synthesis is no more than heterogeneity within the VLDL pool, such that part of the VLDL subfraction is lipolysed rapidly appearing as directly synthesised LDL. If this hypothesis is true then it is possible that the amount of LDL directly synthesised depends upon the composition of newly synthesised VLDL. As indicated in the previous point, some evidence was found to suggest that the composition of the newly synthesised VLDL subfractions VLDL₁ and VLDL₂ was more indicative of the amount of LDL direct synthesis than the composition of newly synthesised whole VLDL.

The physiological mechanisms behind modification of the surface composition of VLDL during atherogenic hyperlipidaemias.

As noted in section 8.4.2.3 increased VLDL free cholesterol: phospholipid (FC:PL) ratios have been associated with increased risk of CHD. However, the experimental literature has not, to any great extent, dealt with the physiological reasons behind perturbation of the surface composition of VLDL. Disorders such as FCHL are thought to perturb the overall composition of VLDL resulting in smaller VLDL with a greater proportion of surface to core components. However, both free cholesterol and phospholipid exist on the surface of the lipoprotein, so that modification in whole lipoprotein size does not explain perturbation in the FC:PL ratio.

One possible hypothesis explaining modification in the FC:PL ratio concerns the action of hepatic triglyceride lipase (HTLP). HTLP is involved in the lipolysis of triglyceride from smaller lipoproteins. Therefore, in cases such as FCHL, HTLP is likely to lipolyse a greater amount of VLDL triglyceride. Brinton (1991) has commented that HTLP consumes phospholipid during triglyceride lipolysis such that the triglyceride lipolysis of small surface rich VLDL by HTLP is likely to result in a high FC:PL ratio in these particles. This hypothesis is consistent with an increased activity of HTLP being associated with an increased risk of CHD as described

previously (see section 8.4.2.3, point 5) .

Is peripheral tissue cholesterol synthesis/ receptor mediated uptake of cholesterol at the peripheral tissues significant in the progression/regression of atheroma?

In representing cholesterol fluxes to and from peripheral tissues in chapter 5 both receptor mediated uptake of cholesterol at the periphery and peripheral cholesterol de novo synthesis were ignored (see section 5.2). In doing so it was noted that if either of these assumptions were incorrect then the model would predict reverse cholesterol transport rates high than model predicted forward cholesterol transport even in the models population state. As illustrated in chapter 7 the population *a priori* state of the model illustrates an extremely small cholesterol transport toward the tissues consistent with a western population, hence justifying these assumptions.

It might be assumed that these two physiologically controlled mechanisms cancel each other out such that increased peripheral tissue cholesterol synthesis might increase reverse cholesterol transport, whilst also decreasing receptor mediated cholesterol uptake at the periphery. Decreasing receptor mediated uptake would be likely to increase LDL apo B and cholesterol concentrations and hence increase non-receptor mediated uptake at the periphery. The resultant increase in reverse and forward cholesterol transport cancelling.

Logically, this cancelling is to be expected. Both receptor-mediated cholesterol uptake and peripheral tissue cholesterol synthesis are physiologically regulated and, unlike non-receptor pathways of cholesterol deposition, are unlikely to be involved in an increased potential for atheroma.

8.3.4.2 Identification of areas of lipid and lipoprotein metabolism where little information exists

What proportion of free cholesterol for esterification is from the tissues and what proportion is from larger lipoproteins?

The esterification rate alone provides very little information about the removal of cholesterol from the periphery. As noted in section 5.5.6 increased esterification rates can indicate either increased or decreased atherogenicity, depending upon the amount of apo AI released on triglyceride lipolysis of large lipoproteins, and the nature of the HDL₃ subfraction. Whilst 'relatively' large amounts of work have been performed estimating cholesterol esterification rates, little has been done examining the source of this free cholesterol in different states of atherogenicity i.e. for different compositions (cholesterol loadings) and concentrations of VLDL, for different HDL₃ subfraction profiles, and for different apo AI (or HDL free cholesterol) concentrations.

What is the relationship between the saturation of VLDL with cholesteryl esters and the cholesteryl ester transport from HDL to VLDL?

Currently, only the work of Fielding et al. (1984a) and Castro (1985) have provided any quantification as to how VLDL saturation (FC:PL ratio) effects cholesteryl ester transport. Even in these studies no information is given on how variations in CETP activity and VLDL triglyceride concentration combine with the saturation to determine the eventual transport. Since VLDL saturation has been shown to be indicative of CHD risk further examination of these relationships would be beneficial.

What is the relationship between the GGE profile of HDL₃ and the cholesterol esterification rate.

As noted in section 5.6.3.2 it is now possible to enumerate GGE profiles automatically by integrating gaussian curves fitted to peaks in the profiles. In an attempt to represent how modification in HDL₃ GGE profile effected the maximum esterification rate ($V_{3_{max}}$) GGE profiles, taken from Barter et al. (1985), were visually inspected, the area under each calculated by counting squares on graph paper, and relationships constructed from this data. Further studies are required if these relationships are to be anything more than approximations.

Fortunately, (as illustrated by Barter et al., 1984, 1985, 1987), when performing these studies it is possible to isolate the effects of HDL₃ profile and the HDL₃ FC level on the cholesterol esterification rate, by using Intralipid as a substrate. Intralipid is apoprotein free and hence does not release apo AI on triglyceride lipolysis which normalises HDL composition.

Non-receptor mediated uptake of β -VLDL.

Whilst excellent information exists on the non-receptor mediated uptake of LDL (see section 5.3), to the author's knowledge, no studies exist which quantify non-receptor mediated uptake in type III hyperlipidaemia, where atherogenic VLDL remnants occur. Although threshold levels of VLDL cholesterol/ triglyceride ratio (cholesterol / triglyceride molar ratio greater than 0.8 (Turner et al., 1985)) have been reported for the diagnosis of type III, no examination of the relationship between non-receptor mediated uptake and the concentration of atherogenic VLDL remnants has been performed.

The saturation of triglyceride lipolysis

As described by Grundy et al. (1982), triglyceride kinetics are not thought to obey Michaelis-Menten saturation kinetics. These authors suggest that triglyceride FCR increases for increasing triglyceride transport rates until saturation of the removal mechanism occurs, at which point the FCR would decrease with increasing triglyceride transport. The implication of this hypothesis is that a great deal of latent capacity exists in the triglyceride removal mechanism, possibly to cope with the normal fluctuations in triglyceride transport during everyday fasting and feeding.

In this thesis, the quantitative nature of triglyceride removal kinetics was examined by plotting raw data, means and standard deviations, and data ranges for the triglyceride FCR as it varies with increased triglyceride transport in different disorders. These data were taken from numerous studies.

Intuitively it was possible to split the population into four groups depending upon disorder and consequently the nature of their triglyceride catabolism. As illustrated in figure 6.15 and discussed in 6.4.1.3, in individuals with disorders associated with triglyceride metabolism, that is diabetes, obesity, FHTG and perhaps FCHL lower limits of capacity for triglyceride removal were present, these limits varying with the severity of the disorder. However, further data are required if more detailed relationships are to be obtained.

The relationship between the amount and composition of newly synthesised VLDL and the apo AI released from VLDL triglyceride lipolyses for use by HDL.

As noted in section 8.4.2.3 of this chapter, apo AI is released during the lipolysis of larger lipoproteins and directly effects the amount, composition and hence atherogenicity of HDL. However, to the author's knowledge, no data exist relating the

amount, composition and lipolysis of VLDL to the amount of apo AI available for HDL formation. Future studies will clarify these issues and enable the model to be completed.

8.4 Summary

This chapter has commented on this thesis describing: a) the major conclusions of each chapter, and b) the contributions of this thesis, in particular highlighting consistency between different information sources and areas of contention within lipid and lipoprotein metabolism. The next chapter concludes this thesis describing whether original goals have been met, the general contributions of this thesis and future work required as a result of this thesis.

Chapter 9 - Conclusions

9.1 Introduction

This chapter summarises briefly the conclusions of this thesis in terms of: a) the original objectives, and whether these were met, b) the contributions of this thesis to the modelling and understanding of lipid and lipoprotein metabolism and, c) future research required in these fields.

9.2 Thesis objectives - Were these met?

As described in section 1.2, the objectives of this thesis were to illustrate how expert opinion and literature data could be incorporated into a model describing lipid and lipoprotein metabolism; and to show how (given sufficient data) such a model could be used to predict an individual's health outcome, as part of a strategy for clinical decision support.

Both clinical and experimental advantages were thought to occur because of these objectives:-

- Predicting the health outcome of an individual, enables clinical decisions regarding treatment to be directed toward improving this health outcome, i.e. the definition of a "quality" treatment decision (Lohr, 1990).
- In combining expert opinion and literature data in a more traceable way, a more formal checking of consistency between these two information sources can be performed, and areas identified where current opinion cannot be quantifiably validated, and hence further experimental research is required.

In order to construct models which may be useful in the clinical setting it was first necessary to identify the health outcome(s) of interest when managing individuals with hyperlipidaemia. These individuals are identified as being at risk of CHD relative to the population, part of this risk being due to their susceptibility to atheroma which occurs in some abnormalities of lipid and lipoprotein metabolism. A reasonable health outcome for this population was therefore their "potential for atheroma"; this being defined as the difference between cholesterol flux to and from the peripheral tissues.

Having identified the "potential for atheroma" as the most appropriate health outcome for the population attending a lipid clinic, models were constructed describing lipid and lipoprotein metabolism, enabling the prediction of atheroma potential from variables describing the metabolism. In this way it was possible to define a strategy for decision support based upon the most likely reduction in atheroma potential for a given treatment option, offset against costs and patient preferences. Once sufficient literature data become available it would be possible to complete these models, and examine their use in the clinical setting.

Using the techniques described in this thesis, it was possible to include expert opinion on the most likely physiological pathways describing lipid and lipoprotein metabolism with experimental data, so as to construct models of lipid and lipoprotein metabolism. These models have been shown to be beneficial experimentally in that: a) they illustrate consistency (or otherwise) between epidemiological and physiological data, b) they highlight areas of contention within the field of lipid and lipoprotein metabolism, and c) they identifying areas where insufficient data exist to support hypotheses describing physiological mechanisms.

9.3 Contributions to knowledge

This thesis has described, and illustrated the use of, techniques for combining quantitative data and qualitative knowledge in models of lipid and lipoprotein

metabolism. In the long term this work is beneficial in that the models constructed in chapters 5 and 6 form part of a strategy consistent with a quality treatment decision making process. The more immediate contributions of this thesis are to show that, when modelling using the techniques described, data, information and knowledge from different sources can be incorporated into the same model, and consistency can be illustrated between :-

- detailed knowledge describing metabolic pathways and the behaviour of the model as a whole (see section 8.4.2.1).
- studies reported by different groups examining the same metabolic relationship (see section 8.4.2.2)
- experimental, and epidemiological data (see section 8.4.2.3)
- detailed experimental data and studies of whole body cholesterol metabolism (see section 8.4.2.4)

The resultant model is therefore a summary of current data, information and knowledge from numerous sources which has been checked for consistency. Having a model based summary of current knowledge is extremely useful since, unlike the published literature review, it can be used to interpret of the results of future experiments, as illustrated in Chapter 7, comparing their results with current understanding.

The research has also demonstrated the applicability of advanced modelling methods and techniques, as exemplified by Causal Probabilistic Networks, in addressing the complexities of metabolic dynamics as have been examined in the context of lipid and lipoprotein metabolism.

9.4 **Future research**

If, in future, these models are to be used in a clinical setting then further experiments are required so that the models may be improved. However, since the models provide

a summary of current understanding of lipid and lipoprotein metabolism they can identify areas of contention and areas where few data exist to support hypotheses describing physiological pathways. These models can therefore be seen as useful tools for directing future research into lipid and lipoprotein metabolism, and indeed many important questions requiring further research were raised during the modelling process and are discussed in Chapter 8.

Currently, in many medical fields, decision support is based upon expert guidelines where expert consensus is forced so that management protocols can be defined. By forcing consensus where none exists areas of contention cannot be identified and experimentation cannot be directed toward these areas. In contrast, because the models described in this thesis form part of a decision making strategy, and highlight areas of contention in lipid and lipoprotein metabolism, they enable identification of experimental questions which have direct bearing on clinical decisions.

In summary, model-based approaches clearly offer potential benefits which relate to both experimental and clinical problems and as such offer substantial opportunity for fruitful research.

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