**Contemporary Diabetes**
*Series Editor:* Aristidis Veves

Alicia J. Jenkins
Peter P. Toth
Timothy J. Lyons *Editors*

# Lipoproteins in Diabetes Mellitus

Humana Press

# Contemporary Diabetes

**Series Editor**: Aristidis Veves, MD, DSc

For further volumes:
http://www.springer.com/series/7679

Alicia J. Jenkins • Peter P. Toth
Timothy J. Lyons
Editors

# Lipoproteins in Diabetes Mellitus

Humana Press

*Editors*
Alicia J. Jenkins, MD, FRACP
Health Sciences Center
Section of Endocrinology and Diabetes
University of Oklahoma
Oklahoma City, OK, USA

Peter P. Toth, MD, PhD
College of Medicine
University of Illinois
Peoria, IL, USA

NHMRC Clinical Trials Centre
University of Sydney
Sydney, NSW, Australia

Timothy J. Lyons, MD, FRCP
Health Sciences Center
Section of Endocrinology and Diabetes
Harold Hamm Diabetes Center
University of Oklahoma
Oklahoma City, OK, USA

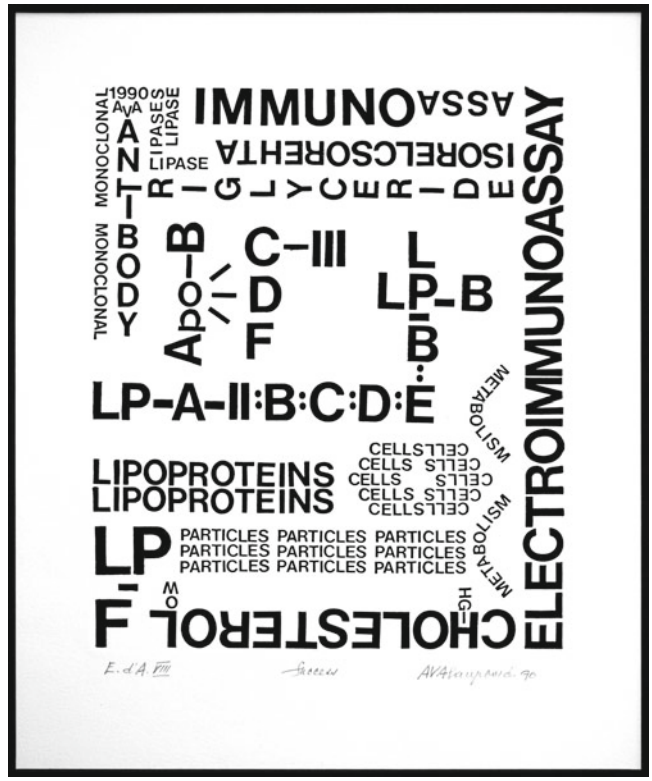
ISBN 978-1-4614-7553-8 ISBN 978-1-4614-7554-5 (eBook)
DOI 10.1007/978-1-4614-7554-5
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013944894



Printed on acid-free paper

Humana Press is a brand of Springer
Springer is part of Springer Science+Business Media (www.springer.com)




*"Success" by Alexandra Alaupovic (nee Vrbanic—born December 21, 1921, Podravska Slatina, Croatia—died January 2, 2013, Oklahoma City, OK, USA).*
*The artist created this work in recognition of the lipoprotein-related research by her husband Petar Alaupovic. Alexandra Alaupovic trained at the Academy of Visual Arts in Zagreb, University of Illinois and the University of Oklahoma, and was a Professor of Sculpture at the Oklahoma City University. Her work is exhibited in many public and private collections around the world. She is survived by her husband, daughter Betsy and grandsons Robert and Homer Clark.*

# Preface

Diabetes mellitus (DM) is becoming increasingly epidemic globally. The World Health Organization (WHO) estimates that the prevalence of DM varies between 8 and 10 % in all regions of the globe. Millions of new cases are diagnosed every year, and a substantial percentage of people with DM are undiagnosed either because they are not screened for the condition or because of inadequate access to healthcare. The epidemics of obesity, increased mechanization and reduced physical activity, cigarette smoking, and the fact that people are living longer have all contributed to the rise in Type 2 DM incidence. The incidence and prevalence of Type 1 DM is also increasing, perhaps also related to changes in the environment. Obesity, sedentary lifestyle, and cigarette smoking potentiate insulin resistance, which also promote atherosclerosis and the vascular complications of DM, as well as of Type 2 DM itself. Longer lifespan is associated with increased weight, lower levels of physical activity, and progressive loss of pancreatic islet cell mass. The US Centers for Disease Control estimates that 26.5 % of Americans 65 years of age or older have DM. According to the American Heart Association, in 2008, 18 million Americans had diagnosed DM, with another 7.1 million having undiagnosed DM; it is estimated that the prevalence of pre-diabetes in the US is 81.5 million. These staggering numbers are not unique to the United States. The worldwide rate of rise in DM is just as alarming. It is estimated that by the year 2030, 340 million people around the world will have DM and the figure is likely to be higher.

The risk for DM is strongly influenced by genetic and environmental factors. Risk for new onset DM is strongly influenced by race and ethnicity. Insulin resistance (IR) is the hallmark of pre-diabetes and Type 2 DM and is characterized by impaired transduction of insulin signaling pathways. Insulin resistance, which also occurs in Type 1 DM, results in hyperglycemia and is also associated with visceral organ steatosis, endothelial dysfunction, hypertension, increased systemic inflammatory and oxidative tone, a prothrombotic state, intracellular accumulation of toxic lipid intermediates (diacylglycerol, ceramide), as well as atherogenic dyslipidemia, among other changes. These metabolic disturbances greatly augment risk for the development of microvascular and macrovascular disease. The epidemic of DM is expected to result in one of the steepest rises in human morbidity and mortality ever observed outside of wartime. DM is the leading cause of proliferative retinopathy and adult onset blindness in working age adults, peripheral vascular disease and lower extremity amputation, end-stage renal disease and

need for dialysis and renal transplantation, peripheral and autonomic neuropathy, and it magnifies the risk of myocardial infarction, stroke, and sudden death at least two- to four-fold. In addition to the human cost of this disease, there is an enormous economic burden associated with the clinical management and treatment of complications associated with DM.

*Lipoprotein in Diabetes Mellitus* is meant to be an authoritative and comprehensive reference on the many changes wrought by IR and DM on lipid and lipoprotein metabolism. Reducing the burden of atherogenic lipoproteins in serum is unequivocally associated with reductions in risk for cardiovascular events and may also ameliorate microvascular damage. The book begins by summarizing the various techniques to measure lipoproteins and their subclasses. In addition to delineating the molecular basis for how IR and DM alter lipid and lipoprotein handling in the gut, adipose tissue, liver, blood, and blood vessel wall, this volume explores how IR induces dyslipidemia, the glycation and oxidation of lipoproteins, and how alterations in immunity and cell surface receptor expression can impact lipoprotein metabolism. The mechanistic basis for why IR and DM increase risk for atherosclerosis as well as diabetic retinopathy and nephropathy are explored in detail. The design of clinical trials and the impact of lifestyle modification and of specific approved and investigational drug classes on diabetic dyslipidemia and risk for diabetes-related complications comprise the latter third of this volume. We thank our international panel of contributors for their clinical and basic scientific expertise and many insights. It is our sincerest hope that the clinicians who care for patients with IR and DM and the basic science researchers who explore mechanisms of vascular damage and protection will find this treatment of the issues covered herein timely and relevant and that it will significantly impact patient care in a positive and lasting way.

Oklahoma City, OK, USA — Alicia J. Jenkins, MD, FRACP
Peoria, IL, USA — Peter P. Toth, MD, PhD
Oklahoma City, OK, USA — Timothy J. Lyons, MD, FRCP

# Contents

# Contributors

**Jean Claude Ansquer** ClinSciences, Dijon, France

**Michael Aviram** The Lipid Research Laboratory, Technion Faculty of Medicine, Rappaport Family Institute for Research in the Medical Sciences and Rambam Medical Center, Haifa, Israel

**Harold Bays** Louisville Metabolic and Atherosclerosis Research Center, Louisville, KY, USA

**Alex Bobik** Vascular Biology and Atherosclerosis, BakerIDI Heart and Diabetes Insitute, Melbourne, VIC, Australia

**Eliot A. Brinton** Utah Foundation for Biomedical Research, Salt Lake City, UT, USA

Utah Lipid Center, Salt Lake City, UT, USA

**Alberico L. Catapano** Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy

Center for the Study of Atherosclerosis, E. Bassini Hospital, Cinisello Balsamo, and IRCCS Multimedica, Milan, Italy

**Alan Chait** Division of Metabolism, Endocrinology and Nutrition, University of Washington School of Medicine, Seattle, WA, USA

**Peter Clifton** Sansom Institute University of South Australia, Adelaide, SA, Australia

**Michael Cobble** CMO Atherotech Diagnostics Lab, Birmingham, AL, USA

Canyons Medical Center, Sandy, UT, USA

**Thomas D. Dayspring** Foundation for Health Improvement and Technology, Richmond, VA, USA

**Christelle Foucher** ClinSciences, Dijon, France

**Val Gebski** NHMRC Clinical Trials Centre, University of Sydney, Sydney, NSW, Australia

**Per-Henrik Groop** Folkhälsan Research Center, Folkhälsan Institute of Genetics, Biomedicum Helsinki, Finland

Department of Medicine, Division of Nephrology, Helsinki University Central Hospital, Helsinki, Finland

Baker IDI Heart & Diabetes Institute, Melbourne, VIC, Australia

**Sandra J. Hamilton** Combined Universities Centre for Rural Health, University of Western Australia, Geraldton, WA, Australia

**Tony Hayek** The Lipid Research Laboratory, Technion Faculty of Medicine, Rappaport Family Institute for Research in the Medical Sciences and Rambam Medical Center, Haifa, Israel

**Andrzej S. Januszewski** Department of Medicine, The University of Melbourne, St. Vincent's Hospital, Melbourne, VIC, Australia

NHMRC Clinical Trials Centre, University of Sydney, Sydney, NSW, Australia

**Alicia J. Jenkins** Section of Endocrinology and Diabetes, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

NHMRC Clinical Trials Centre, University of Sydney, Sydney, NSW, Australia

**Steven R. Jones** Department of Medicine, Cardiology Division, The Johns Hopkins University, Baltimore, MD, USA

**Parag H. Joshi** Johns Hopkins University, Baltimore, MD, USA

**Marielle Kaplan** The Laboratory of Clinical Biochemistry, Rappaport Family Institute for Research in the Medical Sciences, Rambam Medical Center, Haifa, Israel

**Anthony Keech** NHMRC Clinical Trials Centre, University of Sydney, Sydney, NSW, Australia

**Richard L. Klein** Division of Endocrinology, Diabetes, and Medical Genetics, Medical University of South Carolina, Charleston, SC, USA

**Gerhard M. Kostner** Medical University of Graz, Graz, Austria

**Karam M. Kostner** University of Queensland, Mater Hospital, Brisbane, Australia

**Barry Lewis** University of Sydney, Sydney, NSW, Australia

**Peter J. Little** Department of Pharmacy, RMIT University, Bundoora, VIC, Australia

Monash University, Melbourne, VIC, Australia

**Maria F. Lopes-Virella** Department of Medicine, Medical University of South Carolina, Charleston, SC, USA

Ralph A. Johnson VA Medical Center, Charleston, SC, USA

**Timothy J. Lyons** Section of Endocrinology & Diabetes, Harold Hamm Oklahoma Diabetes Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

**Ville-Petteri Mäkinen** Folkhälsan Research Center, Folkhälsan Institute of Genetics, Biomedicum Helsinki, Finland

Department of Medicine, Division of Nephrology, Helsinki University Central Hospital, Helsinki, Finland

**Seth S. Martin** Johns Hopkins University, Baltimore, MD, USA

**Patrick D. Mize** Atherotech Diagnostics Lab, Birmingham, AL, USA

**Giuseppe D. Norata** Department of Pharmacological and Biomolecular Sciences, University of Milan and Center for the Study of Atherosclerosis, E. Bassini Hospital, Cinisello Balsamo, Italy

**David O'Neal** University of Melbourne, Department of Medicine, St. Vincent's Hospital, Fitzroy, Melbourne, VIC, Australia

**Narin Osman** RMIT University, Melbourne, VIC, Australia

**Daphne Owens** Diabetes Institute of Ireland, Beacon Hospital, Sandyford, Dublin, Ireland

Trinity College Dublin, Dublin, Ireland

**Angela Pirillo** Center for the Study of Atherosclerosis, E. Bassini Hospital, Cinisello Balsamo, and IRCCS Multimedica, Milan, Italy

**Vasudevan A. Raghavan** Cardiometabolic, Lipid Clinic & Medical Weight Management Services, Division of Endocrinology, Scott and White Center of Diagnostic Medicine, Temple, TX, USA

Texas A&M Health Sciences Center and College of Medicine, Temple, TX, USA

**Arthur Z. Schwartzbard** Leon H. Charney Division of Cardiology, Department of Medicine, Center for Prevention of Cardiovascular Disease, NYU School of Medicine, New York, NY, USA

University Langone School of Medicine, New York, NY, USA

**Kamalpreet Singh** Texas A&M Health Sciences Center and College of Medicine, Temple, TX, USA

**David R. Sullivan** Department of Clinical Biochemistry, Royal Prince Alfred Hospital, Camperdown, Sydney, NSW, Australia

**Nina Tolonen** Folkhälsan Research Center, Folkhälsan Institute of Genetics, Biomedicum Helsinki, Helsinki, Finland

Department of Medicine, Division of Nephrology, Helsinki University Central Hospital, Helsinki, Finland

**Gerald H. Tomkin** Diabetes Institute of Ireland, Beacon Hospital, Sandyford, Dublin, Ireland

Trinity College Dublin, Dublin, Ireland

**Peter P. Toth** University of Illinois School of Medicine, Peoria, IL, USA

**Henry A. Tran** Greenberg Division of Cardiology, Weill Cornell Medical College, New York, NY, USA

New York University Langone School of Medicine, New York City, NY, USA

**James A. Underberg** Division of General Internal Medicine, Center for Prevention of Cardiovascular, NYU School of Medicine, New York, NY, USA

**Gabriel Virella** Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC, USA

**Gerald F. Watts** Cardiometabolic Research Centre, School of Medicine and Pharmacology, Royal Perth Hospital Unit, University of Western Australia, Perth, WA, Australia

**Mingyuan Wu** Section of Endocrinology & Diabetes, Harold Hamm Oklahoma Diabetes Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

# Laboratory Assessment of Lipoproteins in Diabetes

1

David R. Sullivan and Barry Lewis

## Abbreviations

| | |
|---|---|
| Apo B | Apolipoprotein B |
| CETP | Cholesteryl ester transfer protein |
| CVD | Cardiovascular disease |
| HDL-C | High-density lipoprotein cholesterol |
| LDL-C | Low-density lipoprotein cholesterol |
| NHDL-C | Non-high-density lipoprotein cholesterol |
| TC | Total cholesterol |
| TG | Triglycerides |
| TRL | Triglyceride-rich lipoproteins |

## Introduction

### Lipids, Lipoproteins and Other Analytes in Diabetes

Type 1 and Type 2 diabetes mellitus are often regarded as abnormalities of insulin and glucose metabolism, but it is more appropriate to recognise that they disrupt the pathophysiology of macronutrient metabolism as a whole. Accordingly, it is essential to recognize the effects of diabetes on another major class of macronutrients, namely, lipids. The fundamental differences in the pathophysiology and treatment of Type 1 and Type 2 diabetes are manifest in the changes in lipoprotein metabolism that accompany these two common forms of diabetes. Consequently, the role of altered lipoprotein metabolism in the atherosclerotic process that underlies macrovascular complications may differ. Fully treated Type 1 diabetes often causes minimal disturbance to the lipoprotein profile, in fact the level of HDL-C may be slightly increased in insulin-treated patients [1, 2]. Nevertheless, glycation of the protein component of lipoproteins [3], as well as other modifications such as oxidation and immune complex formation (discussed in other chapters), may render lipoproteins dysfunctional in Type 1 diabetes. Consequently, the atherogenicity of the diabetic state in Type 1 diabetes, combined with the early age of onset, results in an increased lifelong risk of CVD that demands efforts to maintain lipoproteins at target levels or better [4]. This may be difficult to achieve in the face of complications of Type 1 diabetes such as renal impairment or the need for immune-suppressive therapy subsequent to renal, pancreas or islet cell transplantation. Hypercholesterolemia may occur in Type 1 diabetes in association with severe chronic hyperglycemia. Furthermore, insulin is required for the action of lipoprotein lipase, so early use of insulin therapy may be necessary in the massive hypertriglyceridemia associated with both forms of diabetes [5].

D.R. Sullivan (✉)
Department of Clinical Biochemistry,
Royal Prince Alfred Hospital, Missenden Rd,
Camperdown, Sydney, NSW 2050, Australia
e-mail: David.sullivan@sswahs.nsw.gov.au

B. Lewis
University of Sydney, Sydney, NSW, Australia

A.J. Jenkins et al. (eds.), *Lipoproteins in Diabetes Mellitus*, Contemporary Diabetes,
DOI 10.1007/978-1-4614-7554-5_1, 

**Table 1.1** Potential confounding factors that may affect lipoprotein assessment in Type 2 diabetes

| | |
|---|---|
| Intercurrent illness (with acute phase inflammation) | Increased VLDL (TG), reduced HDL-C, LDL-C |
| Hemoconcentration (dehydration, upright posture, prolonged tourniquet, squeezing to obtain fingerprick sample) | Proportionate increase in most analytes, including lipoproteins |
| Medications, menstrual cycle | Variable |
| Winter/summer | LDL-C lower in summer |
| Stress | Small unexplained increase is LDL-C reported |
| Food or caloric intake | Increased chylomicrons (TG) sufficient to undermine standardisation for purposes of classification and LDL-C calculation |
| Hemolysis or analytical delay | Predominantly affects other analytes (e.g. glucose and potassium levels) rather than lipids |
| Lipaemia | May require dilution<br>May interfere with turbidimetric analysis of Apolipoproteins |
| Presence and type of anticoagulant | Direct HDL-C now less likely to be affected |

On the other hand, Type 2 diabetes is associated with a well-characterized disturbance of the lipoprotein profile which features mild to moderate increase in triglyceride-rich lipoproteins (TRL), reduced HDL-C, smaller LDL size and modification of LDL particle composition, hence increased cardiovascular risk. Type 2 diabetes is by far the commoner variety and is becoming increasingly prevalent in the setting of increased dietary energy intakes and reduced activity levels both in affluent and developing societies; it will be the major focus of attention of this chapter.

Lipid abnormalities manifest as disturbances of the levels of the lipoproteins that transport lipids in the bloodstream. These disturbances contribute to the macrovascular complications of diabetes by influencing the processes that underlie atherosclerosis and thrombosis. Less frequently, they lead to massive increases in TG that greatly increase the risk of acute pancreatitis with associated loss of beta cell function. Recent evidence also suggests that disturbances in lipoprotein metabolism may contribute to some forms of microvascular complications of diabetes such as renal impairment, which is discussed in other chapters; however the relevant mechanisms are yet to be fully elucidated [6].

The laboratory assessment of lipoprotein status in diabetes relies on minimization of the effect of potential confounding factors which are summarised in Table 1.1. Sample collection procedures are designed to reduce preanalytical sources of error [7]. Sustained attention to standardisation and quality assurance has established a high level of reliability for routine lipid measurements which is maintained by a well-established system of internal and external quality assurance programmes [8, 9]. This process has been extended to include Apolipoproteins, most importantly Apo B [10] and Apo (a) [11].

One of the most clinically relevant sources of variability is the presence of intercurrent illness because the associated inflammatory response modifies the lipid and lipoprotein profile. It is important to note that the lipoprotein response to intercurrent illness shares some of the features of that associated with Type 2 diabetes, as will be described later. The magnitude of modifications associated with an inflammatory response is usually proportional to the severity of the underlying illness [12], but proportionately smaller responses should also be anticipated in association with minor intercurrent illnesses [13].

## Routine Lipoprotein Assessment

Clinical evaluation of lipoprotein metabolism in diabetes usually involves the measurement of total cholesterol, HDL-C and TG following a 12-h fast. LDL-C is derived from the fasting results by application of the Friedewald equation

[14] [LDL-C in mg/dl = TC−HDL-C−(TG/5), LDL-C in mmol/l = TC−HDL-C−(TG/2.2)], but this calculation becomes less reliable as TG levels increase beyond approximately 4 mmol/l (350 mg/dl). Non-fasting samples have been shown to be a more sensitive marker for the detection of individuals with increased risk of CVD [15], but the unstandardized nature of non-fasting samples [16] makes them unsuitable for the characterization or serial monitoring of lipid status in diabetes. Indeed, even fasting levels of TG and other lipids show considerable within-individual variability [17]. This has implications for the serial measurement of LDL-C and the fasting TG from which it was calculated. A change in a serial measurement can only be attributed to clinical factors if it is greater than would be expected due to other sources of variability [18]. The considerable biological variability of fasting TG will increase the proportion by which a serial measurement of fasting TG (and hence LDL-C) must differ in order to indicate a clinically significant alteration.

Increased levels of TRL may also cause variable interference with automated "direct" HDL-C measurements due to TRL cholesterol content. This may have resulted in a positive bias in the past. Method comparison studies prior to 2000 suggested good agreement between "separation" HDL-C methods and the reference method [19], even in the presence of Intralipid [20] or TRL [21]. Where positive bias occurred, it was attributed to incomplete precipitation with the comparator method [22–24] or the presence of Apolipoprotein E-containing HDL [25], but the sources of TG used in these studies had a relatively low cholesterol content. "Direct" HDL methods initially involved the use of α-cyclodextrin, and positive interference from TRL was described in some [26], but not all [23] studies. Since methods involving α-cyclodextrin have been superseded, several recent studies of "direct" HDL methods have reported positive biases which were attributed to TRL [27] or the presence of diabetes [28]. This is an important issue because any overestimation of HDL-C leads to a risk of under-diagnosis of the metabolic syndrome and insulin resistance, as well as under-calculation of LDL-C and NHDL-C. These combined effects would result in a substantial underestimation of absolute risk of CVD, leading to loss of opportunity to effectively identify and treat patients on the basis of their metabolic risk factors. It is possible that TRL may also interfere with "direct" LDL-C assays that utilize a similar strategy based on selective effect of detergents [29].

The accuracy of standard lipid measurements is extremely important because this quantitative information is applied directly to patient management. The atherogenic effect of LDL-C and other Apo B-containing lipoproteins and the probable anti-atherosclerotic effects of HDL-C represent independent risk factors for CVD. Whereas LDL-C (or TC) originally provided thresholds for initiation of treatment and targets for management, management decisions are now seen in a wider context that encompasses the overall (absolute) CVD risk of the patient. This incorporates the classic modifiable and non-modifiable risk factors to varying extents. The predominance of age is one of several inevitable limitations to the performance of the absolute risk calculation algorithms. Diabetes is no longer regarded as "coronary risk equivalent", but rather the presence or absence of diabetes is treated as a categorical variable, usually without adjustment for severity. Clinical uncertainty associated with intermediate levels of CVD risk has led to efforts to "reclassify" patients in this category by a variety of methods. Consequently, some algorithms allow adjustment for factors such as ethnicity, duration of diabetes, HbA1C level and the presence or absence of microalbuminuria (e.g. www.yourheartforecast.co.nz/). Whilst the additional CVD risk posed by the presence of diabetes often justifies active management of the lipid profile, clinicians need to remember that the presence of massive hypertriglyceridemia poses the more immediate risk of acute pancreatitis.

## LDL Composition and Particle Number

Clinical decision-making based purely on quantitative assessment of LDL-C and HDL-C is no longer appropriate, particularly in the presence of

elevated TG, which is often the case in Type 2 diabetes. Increased levels of TRL promote the action of cholesteryl ester transfer protein (CETP), which leads to a reduction in HDL-C levels and a depletion in the amount of cholesterol carried per LDL particle. The extent of this process may depend on the severity of postprandial lipemia [30] such that these changes in HDL-C and LDL composition [31] are not completely reflected by the accompanying fasting TG level. Furthermore, the relationship between LDL-C and CVD risk becomes confounded [32] because the formation of "small dense LDL" results in an LDL-C concentration that is low relative to the number of LDL particles. This is illustrated by the superiority of other risk markers [33] such as NHDL-C (calculated as the difference between TC and HDL-C levels) which reflects the full range of potentially atherogenic lipoproteins. This superiority is thought to reflect the greater atherogenicity of the "small dense LDL" and hence the pre-eminence of particle number as the main determinant of the pro-atherogenic effect on non-HDL lipoproteins [34]. This conclusion is based on quantitative ultracentrifuge studies which are usually too tedious to perform for clinical purposes. Electrophoresis based on sizing gel techniques has attempted to circumvent this problem, leading to designation of so-called "pattern A" and "pattern B" profiles or estimations of LDL diameter. These methods are non-quantitative with respect to the number of atherogenic lipoprotein particles, so their clinical value may be marginal.

A more promising approach is based on the measurement of serum Apo B level [35]. All particles that contain Apo B (including chylomicrons, which contain Apo B48, and VLDL, LDL IDL and Lp(a), which contain Apo B 100) are capable of transporting lipid to peripheral sites and, as such, might be considered potentially atherogenic. All such particles contain a single molecule of Apo B, so Apo B provides a direct measurement of the number of particles. Human Apo B derived from the intestine is the product of post-translational modification (m-RNA editing) that yields a product that consists of the N-terminal fragment that represents 48 % of the complete Apo B protein. These two products are designated Apo B 48 and Apo B 100, respectively. Polyclonal antibodies, or monoclonals targeting the first half of the molecule, can be used to quantify both forms. Apo B levels do not change very much after a meal because the transport of dietary fat is largely accommodated by an increase in TG content, rather than an increase in total Apo B. This also reflects the fact that the number of Apo B 100 particles is large relative to the number of Apo B 48 particles. Hence Apo B measurement need not depend on fasting [36] or the ability to differentiate the Apo B 100 isoform.

The degree to which large TG-rich Apo B-containing particles can directly damage the artery wall is debateable, but all apo B 100-contaiining particles (with the exception of Lp(a)) are potential precursors of LDL. As a result, Apo B 100, which is largely reflected by total Apo B levels, quantitatively represents the pro-atherogenic potential of the lipoprotein profile. Furthermore, the predominance of LDL particles means that Apo B largely reflects LDL particle number. Evidence suggests that Apo B measurement is superior to LDL-C or NHDL-C for CVD risk assessment [37]. When combined with LDL-C measurement, the LDL-C:Apo B ratio can reflect the degree to which cholesterol depletion of LDL has led to the formation of "small, dense LDL".

Nuclear magnetic resonance (NMR) spectroscopy is a non-destructive analytical technique applied to plasma or serum that may be used to reflect the physical composition of lipoprotein particles, particularly their size and number. Consequently, NMR spectroscopy has been used to provide a more detailed picture of lipoprotein size distributions, including HDL species. The technique is unable to distinguish between LDL and Lp(a). Nevertheless studies suggest that NMR spectroscopy may provide additional benefit in terms of the clinical assessment of lipoprotein-associated CVD risk [38].

Tables 1.2, 1.3, and 1.4 are provided as a means of extending the benefits of Apo B measurement to include diagnosis.

**Table 1.2** An algorithm for the prediction of the likely class of lipoproteins responsible for dyslipidemia in approximate order of prevalence in Type 2 diabetes (adapted from de Graaf et al.) [23]

| Apolipoprotein B level | TG > 1.5 mg/dl (Y/N) | TG:ApoB ≥10 (Y/N) | TC (mg/dl): ApoB ≥6.2 (Y/N) | Lipoprotein |
|---|---|---|---|---|
| Apo B < 1.2 g/l | N | N | N | Normal |
| Apo B < 1.2 g/l | Y | N | N | VLDL[a] |
| Apo B ≥ 1.2 g/l | Y | N/A | N/A | LDL and VLDL[a, b] |
| Apo B ≥ 1.2 g/l | N | N/A | N/A | LDL[b] |
| Apo B = 0.75–1.2 g/l | Y | Y | N | Chylomicron and VLDL[a] |
| Apo B < 1.2 g/l | Y | N | Y | IDL or "remnants" |
| Apo B < 0.75 g/l | Y | Y | N | Chylomicrons alone[a] |

[a]LDL particle size (diameter) may be reduced, as in "small, dense LDL"
[b]LDL particle number may be increased, as in increased Apo B level

## Etiological Assessment

The clinical implications of dyslipidemia depend on the type of lipoprotein responsible for the alteration in lipid levels and the etiological reason for its accumulation. The atherogenic effect of various lipoproteins may differ depending on the etiological context in which they arise, and it should not be assumed that the lipoprotein profile in Type 1 or Type 2 diabetes is solely and necessarily based on that condition alone. Other secondary causes may modify the lipoprotein abnormality, whilst intercurrent primary lipoprotein disorders may influence or even dictate the lipoprotein profile. Tables 1.2, 1.3, and 1.4 provide a framework for diagnostic considerations that may modify clinical management. The first step in this process is the consideration of which lipoprotein class is responsible for any dyslipidemia in a person with diabetes. Though this may be inferred from the results of the automated laboratory tests, it cannot be relied upon. Traditionally, identification of the excess lipoproteins was achieved by lipid electrophoresis, but this non-quantitative method does little to enhance prognostic information. Tables 1.2, 1.3, and 1.4 present an extension of the use of Apo B levels to provide this information in an alternative and potentially more useful format [35]. The different lipoprotein patterns are presented in approximate order of their prevalence in Type 2 diabetes, but as will be explained, the first four are somewhat interchangeable. The last two are substantially less common.

## Predominant Hypertriglyceridemia and Hyperbetalipoproteinemia

Predominant hypertriglyceridemia due to increased VLDL (accompanied by low HDL-C) is the most common form of dyslipidaemia in people with Type 2 diabetes, but it is by no means universal. It may occur with or without an associated increase in cholesterol due to increased LDL. As a result, the first four profiles listed in Table 1.2 are relatively common in people with Type 2 diabetes. This is supported by the observation that Type 2 diabetes is a common secondary cause of the first three lipoprotein patterns in Table 1.3. Nevertheless, it is also important to note that other diseases may cause or contribute to such patterns of dyslipidaemia, and indeed several, such as renal impairment and medications, are common accompaniments of Type 2 diabetes, whilst others, such as obesity and corticosteroid use, represent secondary causes of Type 2 diabetes itself. Furthermore, the mere presence of diabetes does not exclude the possibility of intercurrent primary causes of dyslipidaemia. It has been argued that LDL-C levels in western society are pathologically high due to gene/environment interactions (referred to as "polygenic hypercholesterolemia"), and hence this pattern, the fourth in Table 1.4, may frequently accompany Type 2 diabetes.

Indeed, the first four patterns in Tables 1.2, 1.3, and 1.4 must be regarded as potentially interchangeable. This is highlighted by the condition