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Cellular Metabolism and Related Disorders

Edited by Jesmine Khan and Po-Shiuan Hsieh

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Meet the editors

Jesmine Khan is affiliated with the Faculty of Medicine, Universiti Teknologi MARA (UiTM), Malaysia as an Associated Professor. She obtained her MBBS degree from Mymensingh Medical College, Dhaka University, Bangladesh. She has special interest in nutrition and acquired her PhD degree in surgical nutrition from Osaka University Graduate School of Medicine, Osaka, Japan. She has vast experience in teaching at home and abroad. She is active in writing, has published numerous articles in scientific journals, works as an editorial board member and reviewer of academic journals and is involved in nutrition related research and activities.

Dr. Po-Shiuan Hsieh is currently a Professor and Director at the Institute of Preventive Medicine, National Defense Medical Center in Taiwan. His research interests focus on obesity and weight control as well as the pathological links between obesity, metabolic syndrome, and type 2 diabetes, especially the causal links between the major characteristics of metabolic syndrome, the role of inflammation in the development of insulin resistance and impairment of pancreatic insulin secretion in type 2 diabetes; the involvement of hepatic inflammation in the pathogenesis of type 2 diabetes; and the potential therapeutic application in prevention and treatment of metabolic syndromes and diabetes. He has served as the President of the Chinese Physiology Society from 2012 to 2016. He is also the editor-in-chief of two international journals: Chinese Journal of Physiology (SCI) and Journal of Medical Science.

Contents

Preface

Cellular metabolism is the process within the cells by which food and nutrients are converted into energy. Metabolic processes in the cells must be under strict regulation to maintain homeostasis in the body. There are multiple levels of metabolic regulation in the body. For effective metabolism, different enzymes, coenzymes, and hormones are required.

Any disorder of metabolism, either due to imbalance of the regulating enzymes or the hormones, is characterized by the inability to properly utilize and/or store energy. Metabolic disorders affect the ability of the cells to perform critical biochemical reactions that involve the processing or transport of carbohydrates (sugars and starches), proteins (amino acids), or lipids (fatty acids). Disorders of metabolism can be either congenital or acquired. Congenital metabolic disorders are usually due to mutation of genes resulting in deficiency or absence of particular enzymes. Acquired disorders are usually complex and both genes as well as environmental factors are responsible for them.

The objective of this book was to collect and compile articles on the metabolism of fuels in the brown adipose tissue and brain as well as updates on several disorders of metabolism ranging from diabetes mellitus, insulin resistance, hypothyroidism, and metabolic syndrome to some congenital disorders such as glycogen storage disease and urea cycle disorder.

This book is divided into nine sections. The Introductory section includes the prologue entitled energy metabolism and weight control by Prof. Hsieh Po-Shiuan.

The second section, 'Energy Metabolism', includes two chapters. The first chapter, 'Brown Adipose Tissue Energy Metabolism', is written by Prof. Lu Yuan. This chapter describes the mechanism underlying brown fat energy metabolism and the therapeutic potential in metabolic disorders, especially obesity-related metabolic diseases.

The second chapter, 'Cerebral Energy Metabolism: Measuring and Understanding its Rate', by Avital Schurr summarizes the history of the science behind the current knowledge of the biochemical processes responsible for the production of adenosine triphosphate (ATP) in the brain. It also briefly reviews the various techniques used to measure cerebral metabolic rates of oxygen and glucose, two of the substrates involved in ATP production and elaborates on the potential of measuring the cerebral metabolic rate of lactate to improve understanding of brain energy metabolism.

The third section, 'Diabetes Mellitus', includes the chapter entitled 'Diabetes Mellitus: A Group of Genetic-based Metabolic Diseases' written by Lilian Sanhueza. This chapter discusses the overall picture of different types of diabetes mellitus including the main clinical presentations with the proven genetic basis. In this chapter, emphasis is given to the candidate genes for Diabetes Mellitus type 1

(DM1), Diabetes Mellitus type 2 (DM2), and Monogenic Diabetes and Latent Autoimmune Diabetes in Adults (LADA). Subtypes of maturity onset diabetes in the young (MODY) and their genetic basis is also elaborated in this chapter.

The next chapter, 'Pathogenesis of Insulin Resistance', written by Dr. Zaman Gaffar discusses the risk factors, pathogenesis, and molecular mechanism of insulin resistance in muscle and adipose tissue and the methods for diagnosis of insulin resistance. Understanding the cellular mechanisms might help the researchers to develop novel targets for various treatment modalities in the future.

The fourth section, 'Metabolic Syndrome', includes the chapter 'Metabolic Syndrome', which reviews the pathophysiology of metabolic syndrome (MS) and the relationship between its different components. This chapter mainly discusses the historical evolution, prevalence, relationship of MS with obesity, insulin resistance, and its effects on the body.

The next chapter of this section is 'Metabolic Syndrome: Impact of Dietary Therapy' by Suzanne Fouad. In addition to the general features of MS, this chapter discusses the management of MS through lifestyle modification and the role of different diets in the management of MS.

Thyroid hormones regulate the basal metabolic rate in the body. A low level of thyroid hormones in hypothyroidism leads to reduced metabolism and related effects in the body. The next section contains the chapter 'Hypothyroidism' written by Mauricio Alvarez Andrade and Oscar Rosero Olarte. In this chapter, the authors discuss the physiology and regulation of thyroid hormone production, different types, manifestations, diagnosis, differential diagnosis, and treatment of hypothyroidism.

Chronic uremia causes profound alteration in lipoprotein metabolism, promoting the development of atherosclerosis and cardiovascular disease. The next section contains the chapter 'Lipid Disorders in Uremia' by Valdete Topçiu-Shufta and Valdete Haxhibeqiri, and discusses lipid disorders in chronic uremia.

McArdle disease (glycogen storage disease type V) is an inborn error of energy metabolism in the muscle. Usually the patients regard physical activity as painful and possibly dangerous. The next section contains the chapter 'Sports and McArdle Disease (Glycogen Storage Disease Type V) - Danger or Therapy?' by Georg Bollig, who provided an overview of this disease and the advantages and possible risks of sports for patients with McArdle disease.

The next section contains the chapter 'Emerging Knowledge from Noninvasive Imaging Studies: Is Ammonia Control Enough?' written by Prof. Gropman Andrea, which focuses on the use of noninvasive neuroimaging coupled with neuropsychological tests to understand the complex relationship between ammonia, glutamine, cognitive function, seizures, and specifically the impact on development of working memory in patients with urea cycle disorders (UCD), one of the most common groups of inborn errors of metabolism.

The last section, 'Toxicity of Associated Drug', contains the chapter 'Dipeptidyl Peptidase-4 Inhibitor-Associated Bullous Pemphigoid' by Ágnes Kinyó, which discusses the side effects of Dipeptidyl peptidase 4 inhibitors (DPP-4 inhibitors,

also called gliptins). They are widely used drugs in the treatment of type 2 diabetes mellitus. There is an increased risk of bullous pemphigoid (BP) in patients during DPP-4 inhibitor treatment. An overview of BP during gliptin treatment is provided in this chapter.

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Section 1

Introductory Section

Chapter 1

Prologue: Energy Metabolism and Weight Control

Po-Shiuan Hsieh

1. Introduction

The prevalence of overweight and obesity has increased remarkably over the past decades and become a global epidemic and health threat. Obesity not only has strong genetic determinants but also results from an imbalance between energy storage and energy expenditure. Control of energy homeostasis involved in multiple complicated processes is essential for the maintenance of body weight and life. It becomes extremely important to understand the underlying mechanisms since obesity due to energy excess represents a major threat to health and quality of life.

2. Main subjects

For instance, the progress regarding neuronal circuits that control food intake could extend our understanding of energy homeostasis. In particular, the brain has been considered to play a crucial role in the central regulation of energy intake and also energy expenditure. There are many candidate genes in the central nervous system associated with obesity. In traditional view of homeostatic regulation of the body, weight is mainly by the hypothalamus. However, the recent report showed that the hedonic control of appetite by cortical and subcortical brain areas interacts with homeostatic controls to regulate body weight in a flexible manner to respond to the environmental changes [1]. This new concept has several important implications for the therapeutic strategies of obesity.

On the other hand, the role of adipocytes including brown and white adipocytes has been considered to substantially contribute to the integration of the endocrine and metabolic signaling in energy metabolism regulation. Brown adipose tissue (BAT) thermogenesis is one of the key homeostatic mechanisms for energy expenditure. It is around 60% of "non-shivering" thermogenesis in small mammals attributing to the BAT [2, 3] to sustain their body temperature and survival in the cold [4, 5]. In addition, BAT is currently considered a promising target for the treatment of obesity and T2D [6–10]. Accordingly, there were a number of studies focusing on the related drug development and the underlying mechanism and the several factors implicated in BAT and WAT "browning" such as immune cell-mediated modulation of adipose tissue sympathetic innervation [11]. Nevertheless, although the functional role of BAT in the regulation of energy expenditure, especially thermogenesis and substrate utilization, is dominant in rodent models, the contribution of BAT to energy metabolism and homeostasis in humans is more controversial.

The regulation of mitochondrial metabolism and their consequence also crucially participate in the maintenance of energy homeostasis at the cellular and physiological level. Mitochondria play a central role in the regulation of cellular metabolic homeostasis, which is under the control of the balance between nutrient

supply and energy demand [12]. Metabolic oversupply is followed by fragmentation of mitochondrial network, which leads to a decrease of mitochondrial bioenergetic efficiency that, in association with an increase in nutrient storage, will avoid energy waste. Conversely, under metabolic undersupply, mitochondria elongate in order to increase mitochondrial bioenergetic efficiency and sustain the energy need. Thereby, the mitochondrial function is crucial for the regulation of energy metabolism and weight control.

Even at rest, we need energy for all of the vital functions known as basal metabolic rate (BMR). The determinant factors such as thyroid hormones T3, T4 [13, 14], and sarcolipin [15] and their impact on energy homeostasis will be the other important issue. Thyroid hormones have been well documented as the key regulator of energy metabolism (calorigenic effect), especially the basal metabolic rate for decades. However, the cellular and molecular mechanisms underlying the regulatory role of thyroid hormones are still not fully understood. For instance, recent investigation showed that T4 has been speculated to have more rapid effect on the regulation of basal metabolic rate than T3 in animals [14]. Sarcolipin (SLN) is a novel regulator of sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) in muscle and has been speculated as an important determinant of the BMR in animal with diet-induced obesity [15].

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Section 2

Energy Metabolism

Chapter 2

Brown Adipose Tissue Energy Metabolism

Yuan Lu

Abstract

The brown adipose tissue (BAT) evolved as a specialized thermogenic organ in mammals. Nutrients (i.e., fatty acids and glucose) from the intracellular storage and peripheral tissues are critical to the BAT thermogenic function. The BAT converts the chemical energy stored in nutrients to thermo energy through UCP1-mediated nonshivering thermogenesis (NST). Activated BAT contributes significantly to the whole body energy substrate homeostasis. It is now well-recognized that adult humans possess BAT with functional thermoactivity. Thus, BAT energy metabolism has a significant therapeutic potential in the management of metabolic disorders, such as obesity, insulin resistance, type 2 diabetes, and lipid abnormality in humans.

Keywords: brown adipose tissue, brown adipocyte, metabolism, fatty acid, glucose, metabolic disorders

1. Introduction

Brown adipose tissue (BAT) evolved as a specialized thermogenic organ in the modern eutherian mammals, including *Homo sapiens* [1–7]. The main function of BAT is mediating adaptive thermogenesis or nonshivering thermogenesis (NST), when mammals are challenged in the cold environment. The NST function is a critical adaptation, which helps to maintain the homeothermy in mammals and gives them the evolutionary advantage to survive in the cold habitat, and to thrive from the Artic to the Antarctic region [3, 4]. During the cold challenge, BAT metabolizes nutrients (i.e., fatty acids and glucose) and converts the chemical energy to thermo energy through NST. In addition to its thermogenic function, activated BAT also contributes significantly to whole body energy substrate homeostasis. When activated, BAT presents physiologically significant benefit in fatty acids and glucose homeostasis as well as insulin sensitivity in mammals [8–24]. It is now well-recognized that adult humans possess BAT, which has functional thermoactivity [8–10, 25]. Cold challenge-activated BAT is detected mainly in the supraclavicular, paravertebral, and cervical regions in adult humans [9, 10, 25, 26]. Accumulating evidences indicate that BAT function is inversely associated with age, body mass index (BMI), and diabetic status in adult humans, which indicates that the activation of BAT has potential translational implication in the management of metabolic disorders, such as diabetes and obesity in humans [8–14, 16, 17, 21, 27].

Brown adipocyte, which is endowed with mitochondria, is the most important thermogenic functional unit of BAT [4, 28]. In the mitochondrion of brown

adipocyte, energy generated from nutrients is initially stored as proton gradient membrane potential across the mitochondrial inner membrane, and then converted directly to thermo energy by the uncoupling protein 1 (UCP1)-mediated proton flow [4, 29]. In BAT, brown adipocytes are surrounded by abundant capillaries. The heat generated in the brown adipocyte mitochondria can be quickly distributed by the blood flow to maintain the steady core body temperature in mammals [4]. In addition to classical brown adipocytes, beige/brite adipocytes can be induced from the white adipocytes to conduct thermogenesis upon the cold challenge or catecholamine stimulation [26, 30, 31]. This process is termed as "browning" [26, 30, 31]. Thermogenesis in beige/brite adipocytes can also contribute to mammals' body temperature maintenance and whole body metabolism [26, 30, 31]. Beige/brite adipose tissue generates heat through both UCP1-independent thermogenesis, including calcium cycling in and out of endoplasmic reticulum, futile cycle between creatine and phosphocreatine, and UCP1-dependent thermogenesis in mitochondria [26, 30–33]. The energy metabolism is essential for the optimal UCP1-mediated mitochondrial thermogenic function of brown and beige/brite adipocytes [4, 34, 35]. In this chapter, we discuss the importance of energy metabolism in maintaining brown adipocyte thermogenic function and the proceeding of targeting metabolic disorder through BAT activation in human studies.

2. Fatty acid metabolism in brown adipocytes

Thermogenic brown adipocyte possesses a high capacity for fatty acid β-oxidation that has been reported in both rodent and humans [4, 12, 14, 36]. Fatty acids serve as the activator for UCP1, which is a fatty acid/H^+ symporter directly mediating proton flow and thermogenesis [37]. Fatty acids also serve as the main energy substrate mediating the uncoupling and thermogenic function in brown adipocytes [2, 36–41]. In addition, fatty acids can enhance brown adipocyte thermogenic capacity through the nuclear receptor peroxisome proliferator-activated receptors (PPARs), which are the master transcription regulators for the expression of genes involved in lipid metabolism, oxidative phosphorylation, and the key thermogenic protein UCP1 in brown adipocytes [42, 43].

Intracellular fatty acids are stored in the format of triglyceride in the heterogeneous multilocular lipid droplets in the brown adipocyte [4]. Upon the cold challenge, triglycerides stored in the brown adipocyte lipid droplet are lipolysed. Triglyceride lipolysis is a sequential process that involves different enzymes, resulting in the liberation of glycerol and fatty acids for heat production [44]. The lipid droplet is composed of triglycerides and cholesterol esters, which are surrounded by a monolayer of phospholipids [44]. Important proteins with regulatory and enzymatic functions, including perilipin and CGI58, coexist on the phospholipid monolayer to regulate the lipid trafficking and other functions of the lipid droplet [44–47]. Perilipin stabilizes the lipid droplet and prevents it from lipolysis under basal condition. Upon cold challenge, β-adrenergic stimulation leads to the activation of G-protein-coupled receptor (GPCR) and adenylate cyclase, which subsequently increases the cAMP level in brown adipocyte [48]. cAMP then activates protein kinase A (PKA), which phosphorylates perilipin at its serine residues [49–52]. The phosphorylated perilipin releases CGI-58, an adipose triglyceride lipase (ATGL)-activating protein. CGI-58, subsequently, binds and actives ATGL. Activated ATGL hydrolyzes triglycerides and generates free fatty acids and diglycerides [50, 53–55]. Upon the cold challenge, PKA also phosphorylates serine residues on another key lipolysis enzyme hormone sensitive lipase (HSL) [56]. Although HSL is capable of hydrolyzing triglycerides, diglycerides, monoglycerides,

and a broad array of other lipid substrate, it is the rate-limiting enzyme for hydrolyzing the diglycerides *in vivo* [56]. The phosphorylated HSL catalyzes the diglycerides and generates free fatty acids and monoglycerides [56]. As the last step of lipolysis, monoglycerides is hydrolyzed by monoacylglycerol lipase (MGL) to form free fatty acids and glycerol [57].

Given the importance of fatty acid in brown adipocyte thermogenic function, it is reasonable to predict that the deficiency of the key lipolysis enzymes and fatty acid transportation proteins in brown adipocytes can lead to a defected BAT thermogenic function. Human and rodent *in vivo* studies using both pharmacological and genetic approaches to manipulate triglyceride lipolysis processes were reported [36, 39, 58–60]. In the initial studies, nicotinic acid (NiAc) was used to inhibit intracellular triglyceride lipolysis through acting on metabolite-sensing Gi-protein-coupled GPR109A and subsequent PKA activation [36, 58, 61]. A study in rats suggested the brown adipocyte intracellular lipid lipolysis played a major role in thermogenesis in rodents [36]. It showed that the intracellular triglyceride lipolysis contributes to 84% of thermogenesis during an acute cold challenge (10°C, 2–6 hours) and 74% of thermogenesis during a chronic cold challenge (10°C, 21 days) [36]. The importance of the brown adipocyte intracellular triglyceride-derived fatty acids was also reported in a human study [58]. In this study, administrating intracellular triglyceride lipolysis inhibitor NiAc significantly blunted BAT oxidative metabolism in cold-challenged young healthy humans (average 30 years of age with an average BMI of 24.5 kg/m^2). During a 3-hour cold challenge at 10°C, NiAc administration suppressed BAT intracellular triglyceride lipolysis by about 50% and BAT oxidative metabolism by 70%, despite of the increased blood flow in the BAT [58].

One caveat from both the aforementioned human and rodent *in vivo* studies is that NiAc-mediated lipolysis inhibition took effect in both brown and white adipocytes in addition to other tissues, which makes it hard to delineate the contribution of lipolysis from each cell type. An *in vitro* study nicely confirmed the importance of intracellular lipolysis in brown adipocytes [2]. In cultured primary mouse brown adipocytes, the inhibition of both of the key lipolysis enzymes adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) led to a 97% decrease in isopropanol-induced brown adipocyte respiration, indicating the imperative requirement of intracellular lipolysis in activated brown adipocytes [2]. These studies suggested that fatty acids liberated from the intracellular triglyceride storage serve as a critical fuel resource and contribute significantly to BAT thermogenesis in brown adipocytes upon activation.

Further studies in genetically manipulated mice showed similar results. The ATGL-knockout mouse presented defective triglyceride lipolysis function with increased BAT weight (8.5-fold), enlarged lipid droplet (20-fold), and decreased BAT explant lipid hydrolysis activity (−85%) [38]. The impaired triglyceride lipolysis activity in the *ATGL-knockout* mouse led to a defective thermogenic function. Upon a 5-hour 4°C challenge, mouse body temperature dropped to a critical low point at around 25°C [38]. Another study using mice with CGI-58 deficiency in both white and brown adipocytes also showed decreased BAT thermogenic function. The *adipose-CGI-58-knockout* mice were cold-sensitive, but only under fasted state [60]. The *HSL-null* mouse adipose tissue also presented defected triglyceride lipolysis function. Upon catecholamine-stimulation, *HSL-null* adipose tissue explants exhibited significantly reduced fatty acid and almost blunted glycerol release into the culture medium, parallel with diglycerides accumulation in both white and brown adipose tissue [56, 62]. However, it seems that HSL-mediated lipolysis function is not as critical as ATGL-mediated lipolysis function in brown adipocytes. An in vitro study showed that isopropanol-induced UCP1 activity was

largely dependent on ATGL function (80%) compared to HSL function (35%) in cultured primary brown adipocytes, and the combined inhibition of both ATGL and HSL functions led to an almost complete block of UCP1-mediated thermogenic function (97%) [2].

These studies highlight the cardinal role of intracellular triglyceride liberation in the brown adipocyte thermogenic function, but raise the question if the brown adipocyte intracellular lipolysis is essential for BAT to maintain the adaptive thermogenesis *in vivo*. Interestingly, recent studies using *UCP1-Cre*-mediated knockout of either ATGL or CGI-58 gene in mice showed an unexpected insignificant impact of brown adipocyte intracellular lipolysis and suggested that the circulating fatty acids mobilized from peripheral tissues may play more important roles in BAT thermogenesis in mice [59, 60]. These studies demonstrated that the loss of either ATGL or CGI58-mediated triglycerides lipolysis in brown adipocytes did not compromise the cold challenge-induced thermogenic response in mice [59, 60]. The phenotypic discrepancy between the systemic and BAT-specific *ATGL* and *CGI-58* knockout mice brings very important insights to *in vivo* BAT fatty acid metabolism, and indicates that the brown adipocyte intracellular lipolysis is not the only energy resource for *in vivo* adaptive thermogenesis during cold challenge. Although it is not clear about the exact partition of intracellular and circulating fatty acids contribution to the BAT thermogenic function, it is reasonable to speculate that the core body temperature maintenance is vital for mammals to maintain their optimal function during the cold challenge, when the intracellular lipolysis function is impaired or insufficient, circulating energy substrates from other metabolic tissues, that is, white adipose tissue and liver, are mobilized to provide energy substrates for BAT-mediated adaptive thermogenesis to maintain the adequate core body temperature and ensure the optimal functionality of mammals.

Long-chain fatty acids (LCFAs) are the most abundant format of fatty acid energy substrate in mammals [63–66]. The liberated LCFAs from intracellular lipid droplet are facilitated and transported to mitochondrion and nucleus by fatty acid-binding proteins (FABPs) to conduct their functions [67]. Of the six FABP isoforms, FABP4 (also termed adipocyte p2) and FABP5 are the major FABP isoforms in brown adipocytes [68–74]. Mice mutated in both *FABP4* and *FABP5* gene developed severe hypothermia during fasting after an acute cold challenge (1–3 hours), indicating that fatty acids transportation plays an indispensable role in BAT thermogenic function [74].

The LCFAs-mediated mitochondrial oxidation and UCP-1 activation function require sequential carnitine acyltransferases in order to translocate the LCFA into mitochondrial matrix. Carnitine palmitoyltransferase 1 (CPT1), located on the mitochondrial outer membrane, is the rate-limiting enzyme that mediates LCFAs inward translocation into mitochondrial matrix [63–66]. CPT1 exists in tissue-specific isoforms, and CPT1b is the major isoform expressed in brown adipocytes [75–77]. Mouse embryos-carried homozygous knockout of *Cpt1b* gene were lethal before embryonic day 9.5–11.5 and a normal percentage of $CPT1b^{+/-}$ mice was born from the $CPT1b^{+/-}$ and wild type breeding pairs [78]. However, more than 50% of the $CPT1b^{+/-}$ pups were lost before weaning [78]. The detailed experiment showed that ~7% $CPT1b^{+/-}$ mice developed fatal hypothermia following a 3-hour cold challenge (4°C) and ~52% $CPT1b^{+/-}$ mice developed fatal hypothermia following a 6-hour cold challenge (4°C), indicating the critical contribution of CPT1b-mediated LCFAs transportation and thermogenesis during the cold challenge in infant/young mice [78]. Carnitine palmitoyltransferase 2 (CPT2) is located on mitochondrial inner membrane to further medicate LCFAs translocation into mitochondrial matrix. In line with the study using the *CPT1b*-deficient mice, an *adipose-specific CPT2 knockout* mice also presented hypothermic phenotype after 3 hours cold

challenge (4°C) and decreased LCFAs oxidation in isolated MEF cells [39, 79]. These studies indicate that fatty acid transportation is critical for BAT thermogenic function during the cold challenge.

The intramitochondrial LCFAs contribute to the thermogenesis through UCP-1 activation and β-oxidation. A detailed study confirmed that mice with impaired fatty acid β-oxidation are cold-intolerant [80]. The acyl-CoA dehydrogenases, which catalyze the initial steps of fatty acid β-oxidation, are composed of a group of enzymes, including very long-chain acyl CoA dehydrogenase (VLCAD), long-chain acyl CoA dehydrogenase (LCAD), and short-chain acyl CoA dehydrogenase (SCAD) [81, 82]. A detailed experiment showed that fetal hypothermia was presented in all of the homozygous knockout mice ($VLCAD^{-/-}$, $LCAD^{-/-}$, and $SCAD^{-/-}$) after 1-hour 4°C challenge [80]. Although the mice with single heterozygous of VLCAD, LCAD, and SCAD genes were cold tolerate; more than 30% of mice with double heterozygous $VLCAD^{+/-}//LCAD^{+/-}$ *or* $LCAD^{+/-}//SCAD^{+/-}$ and triple heterozygous $VLCAD^{+/-}//LCAD^{+/-}//SCAD^{+/-}$ combinations developed hypothermia upon the cold challenge [80], indicating the essential role of the LCFAs-mediated thermogenic function in mitochondria.

In summary, these studies highlight the importance of the brown adipocyte lipolysis and the liberated intracellular fatty acid transportation and oxidation during thermogenesis. In addition, these studies indicate that brown adipocytes not only use the intracellular lipid storage, but also the circulating energy substrate to maintain the critical thermogenic function for the organismal survival and optimal function in mammals [2, 12, 38, 44, 56, 59, 60, 80]. In modern days, BAT's ability to metabolize fatty acids mobilized from other peripheral storages, including white adipose tissue and liver, makes it a good potential therapeutic target in humans. Studies have shown that BAT mediates significant plasma lipid clearance during the cold challenge in rodent and humans under both physiological and pathological conditions [83–85].

One study showed that the activated BAT is involved in the basal and post-prandial triglyceride metabolism in rodents [83]. In this study, compared to mice kept at 22°C, mice kept at 4°C had significantly lower triglyceride-rich lipoproteins (TRLs)-triglyceride concentration [83]. The study also showed that the activated BAT was involved in the post-prandial triglyceride metabolic process, evidenced by an oral ^{3}H-triolein tolerance test. Under cold challenge condition, the BAT ^{3}H-triolein uptake was significantly higher than that of either liver or skeletal muscle, suggesting the significant triglyceride/triglyceride-rich lipoprotein metabolism in activated BAT. For the triglyceride clearance, circulating triglycerides rose to a peak at 2 hours postprandial and declined subsequently in mice kept at 22°C. In contrast, the triglyceride level reminded persistently low in mice kept at 4°C, suggesting that the BAT possesses a high postprandial triglyceride clearance function [83]. Most interestingly, the cold challenge increased the uptake of radio-labeled lipoprotein in BAT and reduced the uptake in liver [83]. The cold challenge-induced lipid clearance shift suggests that BAT can be targeted for lipid metabolism *in vivo*. In a pathological setting, the genetically manipulated $Apoa5^{-/-}$ mice with severe hyperlipidemia were studied. A 4–24 hours 4°C challenge corrected $Apoa5^{-/-}$ mouse plasma lipid concentration to the values comparable to wild-type mice, indicating the significant impact of BAT on whole body lipid metabolism in the rodent under the pathological condition [83, 86, 87]. Other studies also showed that the BAT preferentially uptook plasma triglycerides through peripheral tissue lipolysis, and the selective fatty acids uptake from triglyceride-rich lipoprotein ameliorated hyperlipidemia in rodents [84, 85].

Although the studies in rodent strongly support the importance of BAT in lipid clearance, the significance of BAT in human lipid metabolism is still unclear. The contribution of activated BAT in human body was studied using a dietary radio-labeled LCFAs tracer 14(R,S)-[^{18}F]-fluoro-6-thia-heptadecanoic acid (^{18}FTHA) [88].

This study showed that a 4-hour mild cold-challenge at 18°C significantly increased dietary fatty acids distribution in BAT in humans [88]. However, given the relative small volume of BAT tissue, the dietary fatty acids clearance contribution from the BAT is less significant compared to other organs including heart, liver, white adipose tissue, or skeletal muscles, and only contributed to ~0.3% of total body dietary fatty acids clearance upon the mild cold challenge [88]. Similarly, another study using fatty acid tracer ^{18}F-fluoro-thiaheptadecanoic acid (^{18}FTHA) showed that a 3-hour cold challenge at 18°C led to four times higher radio-labeled tracer uptake and ~80% metabolic rate increase in the BAT, contributing <1% of total fatty acids clearance rate in human subjects [12]. Nevertheless, these experiments suggest that BAT not only exists, but also is functionally active and can contribute to systemic metabolism in humans. One possible reason for the relatively low BAT contribution in fatty acids metabolism in humans could be due to the short cold challenge time and mild cold challenge conditions. It is possible that during the acute cold challenge, intracellular lipid lipolysis serves as the main energy resource so that it ameliorates the clearance of circulating TRLs or fatty acids.

The circulating TRLs or fatty acids are transported into brown adipocyte by a group of proteins, including lipoprotein lipase (LPL) and fatty acid transport proteins [83, 89–93]. LPL is a multifunctional protein produced by many tissues, including the adipose tissue [94]. LPL serves as a rate-limiting enzyme mediating extracellular lipolysis [94]. It hydrolyzes triglycerides into lipid-rich proteins into fatty acids and monoacylglycerol. It also mediates the cellular uptake of triglyceride and other lipids [94]. Studies have shown that cold challenge or catecholamine-stimulation induce the expression and activity of LPL in brown adipocytes through a cAMP-mediated mechanism [89–91]. After activation, LPL is released from the brown adipocyte, transferred to the capillary endothelium lumen, and serves as the anchor between the endothelium cell surface and the TRLs [95–97]. Next, the LCFAs liberated from LPL channel into brown adipocyte for thermogenic function [98, 99]. A study indicated that the local LPL activity is required for TRLs uptake into the BAT [83]. In this study, it is shown that either LPL-specific inhibitor tetrahydrolipstatin pretreatment or releasing LPL from endothelium by heparin significantly blocked the uptake of TRL and fatty acids in BAT [83].

The liberated LCFAs in circulation can be transported into cells and activated by both transmembrane fatty acid transporter proteins (FATPs) and scavenger receptor CD36 [92, 93]. FATPs are composed by a family of six proteins mediating circulating LCFAs uptake and distribution in cells [83, 92, 93, 100]. Among the six isoforms, FATP1 (SLC27A1) is the major isoform in brown and white adipose tissue [83, 92, 93, 100]. Studies showed that postprandial lipid uptake is highly dependent on the adipose tissue FATP1 [101]. The FATP1-knockout mice have decreased lipid uptake in adipose tissue and a compensatory lipid redistribution in liver and heart, where FATP1-mediated lipid uptake function is not required under normal conditions [101]. The cold challenge can induce FATP1 expression in BAT [100]. In line with this, the isolated FATP1-null brown adipocytes showed significantly less fatty acids uptake upon catecholamine stimulation [100]. *In vivo* studies showed that in cold challenged FATP1-knockout mice, BAT triglyceride storage was decreased and circulating serum free fatty acids was increased, indicating the importance of FATP1-mediated fatty acid uptake in BAT [100]. The LCFAs uptake can also be mediated by the transmembrane class B scavenger receptor CD36 [83, 102, 103]. Upon the cold challenge, CD36 is significantly upregulated in adipocytes [83, 102]. Other studies using *CD36*$^{-/-}$ mice clearly indicated the importance of CD36-mediated LCFAs transportation during thermogenesis. Around 60% of *Cd36*$^{-/-}$ mice died during a 24-hour cold challenge, which is paralleled with drastically increased plasma free fatty acids concentration [83, 102]. A recent

study indicated that CD36-medicated coenzyme Q (CoQ) uptake is required for the BAT mitochondrial thermogenic function [104].

In summary, fatty acid is indispensable for the brown adipocyte thermogenic function. Fatty acid mediates brown adipocyte thermogenesis through mitochondrial β-oxidation, UCP1-activation, and fatty acid-mediated thermogenic gene regulation. Both the intracellular fatty acids from brown adipocyte lipolysis and the liberated fatty acids from peripheral tissues play essential roles mediating the critical BAT adaptive thermogenic function to main the adequate core body temperature in mammals. In modern days, activating BAT thermogenic function to increase fatty acids uptake and utilization may offer new therapeutics to treat human metabolic disorders.

3. Glucose metabolism in BAT

The radio-labeled glucose analog ^{18}F-fluorodeoxyglucose (^{18}F-FDG), in combination with positron emission tomography (PET) and computed tomography (CT), provides a reliable method for the *in vivo* tissue glucose uptake study [105, 106]. Based on studies using this method, it is now well-recognized that BAT in both rodents and humans possesses a significant glucose uptake capacity upon the cold challenge [4, 9–12, 25, 26, 107–112].

Glucose uptake is regulated in brown adipocytes. *In vivo* studies showed that cold challenge significantly enhanced insulin sensitivity and subsequent glucose uptake in the BAT [9, 10, 25, 26, 107, 112, 113]. Interestingly, other studies showed that starved rats with low insulin level also had increased BAT glucose uptake during the cold challenge, indicating that the enhanced glucose uptake is not completely insulin-dependent [114]. Additional studies also indicated that the enhanced glucose uptake in brown adipocytes can be mediated by different pathways in addition to insulin stimulation, including β-adrenergic receptor activation, AMP-activated protein kinase (AMPK) activation, and mTOR activation [115–117].

The importance of glucose metabolism in BAT is a long-standing question. Glucose transporters, which facilitate glucose across the cell plasma membrane is the first rate-limiting step of the glucose metabolism [34, 118, 119]. Intracellular glucose is subsequently phosphorylated to glucose-6-phosphate (G6P) by the enzyme hexokinase (HK). Glucose-6-phosphate serves as the substrate into different pathways, including glycolysis, glycogen synthesis, and the pentose phosphate pathway (PPP) [34, 118, 119]. Glycolysis breaks down glucose to pyruvate to generate small amount of ATP and NADH [34, 118, 119]. The pyruvate is then transported into mitochondria for oxidation and energy production. Under hypoxia condition, pyruvate is disposed in the form of lactate [34, 118, 119].

Early study indicates that glucose and its metabolites contribute to the brown adipocyte thermogenesis by showing that catecholamine-induced glucose uptake was decreased when mitochondrial β-oxidation was inhibited in brown adipocytes [120]. Other studies in brown adipocyte glucose transportation also indicate the importance of the glucose in brown adipocyte metabolism [6, 112, 121, 122]. Both glucose transporter 1 (Glut1) and glucose transporter 4 (Glut4) are abundantly expressed in brown adipocytes and the insulin sensitive Glut4 is the major isoform [112, 122]. The *in vitro* study showed that knock-down of Glut1 and/or Glut4 gene in cultured brown adipocytes impaired the catecholamine-induced cell oxygen consumption by 30–50% [6, 121]. Other studies indicate the importance of glycolysis in brown adipocyte thermogenesis [115, 123, 124]. An *in vitro* study showed that the knockdown of two glycolysis enzymes, HK2 or pyruvate kinase M (PKM), decreased glucose uptake and catecholamine-induced cell oxygen consumption by 67% or

34% respectively, in brown adipocytes [6]. These studies suggested the importance of glucose metabolism in brown adipocyte function. However, another detailed study indicated that glucose oxidation does not play a major role in brown adipocyte metabolism and thermogenesis, by showing that the rate of $^{14}CO_2$ formation from the ^{14}C glucose was relatively small compared with the maximum rate of oxygen consumption in activated brown adipocytes [125]. Studies using radio-labeled glucose also suggested that glucose uptake was only sufficient to fuel maximally ~15% of the thermogenic capacity in activated rodent brown adipocytes, suggesting that the significantly upregulated glucose uptake in activated brown adipocytes is disassociated with the relative low glucose-mediated thermogenic capacity [123, 125, 126]. A more recent study indicates that the brown adipocyte energy production from glucose depends on the state of the cell: glucose and fatty acid contribute equally to brown adipocyte oxygen consumption under the basal condition; upon catecholamine-activation, oxygen consumption is mainly fueled by fatty acids [6].

The dissociation between high glucose uptake and low glucose-mediated thermogenesis in activated BAT raises an important question: what is the function of the intracellular glucose in brown adipocytes? The importance of glucose in the *de novo* lipogenesis in brown adipocytes has been reported [127–129]. Studies indicate that glucose uptake is an independent process of thermogenesis in both cold-challenged and catecholamine-activated BAT, which further supports that glucose uptake might play other role as energy substrate in activated brown adipocytes [116, 117, 130–132]. One detailed *in vitro* study using rat brown adipocytes showed that norepinephrine significantly enhanced glucose oxidation by sevenfold, while it also inhibited lipogenesis at the same time. On the other hand, insulin stimulation increased the lipogenesis by sevenfold in brown adipocytes whereas glucose oxidation remained very low. Most interestingly, the addition of insulin to the norepinephrine only potentiated the enhanced glucose oxidation, but do not enhance the lipogenesis [115]. On the other hand, another study showed that brown adipocytes converted a greater proportion of metabolized glucose into lactate and pyruvate, but only a smaller proportion into fatty acids through insulin-mediated pathway [125]. These studies suggest that glucose metabolism is involved in two different states in brown adipocytes, the thermogenic state and nonthermogenic state. It is plausible that during the thermogenic state, glucose contributes to both the energy production and lipogenesis; and during the nonthermogenic state, glucose contributes mainly to the lipogenesis and energy storage process in brown adipocytes, which explains the disassociation of glucose uptake and glucose metabolism in brown adipocytes. In addition to glucose, other energy substrates, including glycerol and amino acid (glutamate), might also contribute to BAT metabolism and thermogenesis in human and rodent brown adipocytes. However, the relative contribution and partition of these energy substrates are unclear [132–135].

4. Energy storage in BAT

The cold challenge not only enhances catabolic processes mediating energy substrate metabolism and heat generation, but also induces anabolic processes mediating fatty acid synthesis and lipogenesis, as well as glycogenesis [36, 111, 136–139].

Glucose can be stored as glycogen in brown adipocytes [36, 139]. Studies showed that glycogen synthases (GStot) and uridine diphosphate glucose pyrophosphorylase (Udgp), which mediates glycogenesis, were upregulated upon the cold challenge in BAT [36, 139]. Interestingly, glycogen hydrolysis enzyme, glycogen phosphorylase (Pygl), was also upregulated after cold challenge [36]. Although cold-challenge upregulates both glycogen synthesis and degradation, it is reported

that the stored glycogen is used up shortly after the cold challenge [139]. Indicating that glycogen is not an efficient format for energy storage and a sustainable energy resource in brown adipocytes.

The glucose uptaken by brown adipocyte can also be converted to fatty acid through the *de novo* lipogenesis. Carbohydrate response element-binding protein (ChREBP) is the major transcription factor mediating fatty acid synthesis in adipocytes. There are two isoforms of ChREBP, ChREBPα and ChREBPβ. ChREBPα is abundantly expressed in BAT from rodents and humans [137, 140, 141]. *De novo* lipogenesis involves a series of enzymes mediating the sequential reactions converting glucose-derived citrate into fatty acids, including ATP-citrate lyase (ACLY), acetyl-CoA carboxylases 1 (ACC1), fatty acid synthase (FASN), and stearoyl-CoA desaturase-1 (SCD1). ChREBPα, coordinates with another transcription factor Max-like protein (MLX), directly binds to the carbohydrate response elements (ChoRE) and upregulates these *de novo* lipogenic genes expressions. ChREBPα can also increase the expression of ChREBPβ to further activate the *de novo* lipogenesis enzymes [142, 143]. It has been reported that lipogenic genes and AKT2-ChREBP pathways are upregulated to optimize fuel storage and thermogenesis upon cold stimulation in BAT [136]. In accordance with these studies, *ChREBP*$^{-/-}$ mice presented less BAT weight [142], and adipose-specific *ChREBP-knockout* mice had decreased carbohydrate-induced lipogenesis in BAT [144]. These studies indicate that ChREBP plays important roles in brown adipocyte's *de novo* lipogenesis and energy storage.

Sterol regulatory element-binding protein-1 (SREBP-1) is another transcription factor mediating the *de novo* lipogenesis [145]. Of the three different SREBP isoforms, SREBP1c is more abundant and SREBP1a is less abundant in the adipose tissue [137, 145, 146]. *In vitro* study showed that SREBP1c is sufficient to regulate lipogenic enzymes in cultured adipocytes [147]. In the adipose-specific *ap2-SREBP1c* transgenic mice, lipogenic enzyme Acc1, FASN and Scd1 expression as well as fatty acids synthesis rate were significantly upregulated in brown adipocytes [148]. In line with this study, *ap2-SREBP1a* transgenic mice also developed adipose tissue hypertrophy in accordance with an increased lipogenic enzyme profile and enhance *de novo* lipogenesis in the BAT [148]. However, some *in vivo* studies indicated that SREBP1c's role in the *de novo* lipogenesis in adipocytes is dispensable, as evidenced by *SREBP1-knockout* mice have normal lipogenic enzymes gene expression profile and normal lipid storage in their adipose tissue [149, 150]. These studies suggest that SREBP-1 is involved in the brown adipocyte lipogenesis and triglyceride storage when the excessive energy resources are available; however, SREBP-1's function can be compensated by other factors when it is absent.

In summary, these studies suggest that in activated brown adipocytes, glycogenesis and lipogenesis are upregulated to store/restore energy substrates, which is parallel with energy substrate metabolism and thermogenesis. These coordinated anabolic and catabolic processes are important to maintain the brown adipocyte energy homeostasis.

5. The proceeding of targeting BAT in human metabolic disorders

The understating of BAT energy homeostasis and the discovery of the functional BAT in humans lead to significant interests in targeting BAT for metabolic disorders, for example, obesity, insulin resistance, type 2 diabetes, and lipid profile abnormality. The ability of BAT metabolizing fatty acid and glucose from the intracellular storage, the peripheral tissues liberation, and teh dietary nutrition absorption makes it a good potential therapeutic target for combating metabolic disorders in humans. In addition to the BAT, beige/brite fat, which is coexisted in white adipose tissue,

can be recruited and activated (browning) in respond to cold challenge or pharmacological stimulation and serves as a target for metabolic disorders [151–154].

It has been reported that the BAT ^{18}F-FDG uptake in humans correlates inversely with aging, adiposity, diabetic status, and BMI, indicating that the manipulation of BAT function is a possible approach for combating metabolic disorders [8–14, 16, 21–24, 155, 156]. The studies in mouse models and humans provide evidences for the metabolic benefit of BAT. Mice with genetic ablation of BAT and the *UCP1-knockout* mice under thermoneutrality, both developed obesity [18–20, 85]. In addition, BAT activation reduced hypercholesterolemia and protected mice from atherosclerosis development and liver steatosis [18–20, 85]. It is well-recognized that BAT can be activated upon seasonal temperature changes or short time period (several hours) mild cold challenge (16-19°C) in humans [7, 9, 10, 12, 21, 22, 24–27, 157]. However, the significance of BAT's contribution in human metabolism has not been clearly elucidated. Human studies indicated that BAT activity is inversely correlated with body fat deposition, suggests BAT can serve as a target for obesity [9, 10, 16, 21–24]. Studies also showed that fasting glucose was lower in the human subjects with higher BAT prevalence and the BAT activity were blunted in subjects with obesity [11, 16, 155]. More interestingly, in the same patients with multiple PET scans, BAT was more detectable when fasting glucose in the subjects were lower [16]. Other studies showed that a mild cold challenge significantly increased whole body glucose disposal, glucose oxidation, insulin sensitivity, and whole body energy expenditure in human subjects [13, 17, 158, 159]. Additional study showed that moderate cold challenge (18.06°C) significantly improved the peripheral glucose uptake and insulin sensitivity by 20%, but did not impact the pancreatic insulin secretion [17]. These studies strongly indicate the therapeutic potentials of targeting BAT for glucose metabolism in humans, but leave the question of the relative contribution of activated BAT in whole body glucose metabolism to be answered.

Cold challenge can also enhance BAT lipid metabolism. Studies showed that cold challenge led to significantly enhanced lipid mobilization, increased plasma fatty acid levels, as well as upregulated genes for lipid metabolism in human BAT [12, 58, 156, 160]. It is reported that circulating fatty acids were uptaken by BAT in cold-challenged humans by using the ^{18}FTHA tracing method [88]. In addition, it has been shown that cold-activated BAT significantly contributed to whole body fatty acid utilization in healthy humans [17]. The fatty acid uptake is significantly lower in overweight human subjects compare to healthy humans [156]. Importantly, BAT activation upon mild cold challenge significantly increased systemic lipid metabolism, whole body lipolysis, triglyceride-fatty acid cycling, and fatty acid oxidation in overweight/obese subjects [14]. These studies suggest that BAT contributes to whole body lipid metabolism and homeostasis in healthy humans as well as in humans with metabolic disorders, suggesting that BAT can serve as a target for lipid abnormality in humans.

The significance of BAT/beige adipose tissue in human whole body metabolism has been studied and remarkable progress has been made in recent years. The acknowledgment that BAT can be activated and subsequently contributes to the human whole body energy expenditure is encouraging. Although the capacity and relative significance of BAT's contribution to whole body energy substrate metabolism has not been elucidated, it should be noticed that majority of the studies in humans were conducted for relative short time period with mild cold challenge conditions. Given, humans usually live under thermoneutrality, we can assume that their BAT functions are repressed under this condition. In addition, the prevalence of BAT in humans varies significantly, which depends on individual's life style, physical activity, and health conditions, which makes it harder to evaluate the contribution of BAT in whole body metabolism [7, 9–12, 21, 22, 24–27, 88, 156, 157].

Hence, a sustainable chronic mild cold challenge strategy aiming to recruit more BAT/beige adipose tissue and enhance their oxidative capacity might provide more significant therapeutic potential in humans over time, especially the human subjects with metabolic disorders. Future studies should also delineate the relative contribution from glucose and fatty acid in human BAT under physiological and pathological conditions; as well as compare the glucose and fatty acid partitioning in different tissues and organs, including skeletal muscle, heart, liver and BAT. These details will guide us to establish better strategies targeting metabolic disorders through BAT activation in the future.

6. Conclusion

The energy metabolism plays critical role in maintaining BAT thermogenic function in mammals. Through the energy metabolism, the chemical energy stored in nutrients (i.e., fatty acids and glucose) can be converted to thermoenergy and dissipated as heat in the BAT. Activated brown adipocytes not only contribute to the intracellular substrate homeostasis, but also contribute significantly to the whole body energy metabolism. BAT with functional thermoactivity is present in adult humans. Thus, BAT activation has remarkable therapeutic implications in human metabolic disorder management.

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Conflict of interest

The author has no conflict of interest to declare.

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Chapter 3

Cerebral Energy Metabolism: Measuring and Understanding Its Rate

Avital Schurr

Abstract

The study of brain energy metabolism has taken second place to that of muscle ever since the dawn of this field of research. Consequently, each new discovery made using muscle tissue that advanced our understanding of the biochemistry of energy metabolic processes was attempted to be duplicated in brain tissue. It was only when the brain's high energy needs were recognized that researchers realized its vulnerability to any mishap in its energy supplies and that this vulnerability may play a role in various brain disorders. Understanding of the mechanisms by which the brain deals with energy shortage is of utmost importance in shedding light on the fundamentals of brain disorders and their potential treatment. To achieve such understanding, accurate measurement of brain energy metabolic rates is necessary. This chapter summarizes the history of the current knowledge of the biochemical processes responsible for the production of adenosine triphosphate (ATP) in the brain. It briefly reviews the various techniques used to measure cerebral metabolic rates of oxygen (CMR_{O_2}) and glucose ($CMR_{glucose}$), and elaborates on the potential of measuring the cerebral metabolic rate of lactate ($CMR_{lactate}$) to improve our understanding of brain energy metabolism.

Keywords: BOLD fMRI, cerebral metabolic rate, glucose, glycolysis, lactate, oxygen, paradigm shift, polarography

1. A short review of brain energy metabolism research

1.1 The first eight decades (1900–1980)

Most human cells produce adenosine triphosphate (ATP) via two, mostly interconnected biochemical pathways, glycolysis and mitochondrial oxidative phosphorylation. Erythrocytes (red blood cells, RBCs) produce their ATP via the glycolytic pathway alone, since they lack mitochondria. Under anaerobic conditions, the less efficient glycolytic pathway is the main source of ATP supply, since without oxygen the oxidative phosphorylation pathway cannot be maintained. Throughout the first half of the twentieth century the majority of the researchers in the field of energy metabolism made muscle their tissue of choice for the study of energy metabolism. While muscle was believed to require a great deal of energy to perform its work, the brain was assumed to be a low consumer of energy, as indicated by the following quote: "*the brain is not a seat of active combustion, and considering the very small increase in CO_2 in the torcular blood it seems to us very improbable that the temperature*

of the brain should be perceptibly greater than that of the blood" [1]. It was Tashiro [2] who was the first to demonstrate that nerve produces CO_2 and ammonia during its metabolism. By 1924, Warburg et al. [3] demonstrated that brain tissue is able to convert large amount of glucose to lactic acid, and in 1927 it was shown that nerve produces a measurable amount of heat, an amount that increases upon electric stimulation [4]. Moreover, this increase in heat production was shown to correlate with the amount of oxygen consumed. According to Holmes [5] the above finding was the necessary proof that nerve impulse is a chemical process. All the major discoveries that have led to the elucidation of the biochemical pathways of energy metabolism were made using the tissue of choice in the field i.e., muscle. Both the tricarboxylic acid cycle and the glycolytic pathways were introduced in 1937 and 1940, respectively (the reader is directed to the many detailed reviews on the topic that are available). While there is a general agreement among biochemists, physiologists and neuroscientists as to the accuracy of the mitochondrial tricarboxylic acid (TCA) cycle and the oxidative phosphorylation pathway, disagreements exist on the accuracy of the glycolytic pathway. Hence, glycolysis is the main focus of this chapter, since the original drawing of the pathway stands in conflict with various research findings of the past three decades (for more detailed reviews of this topic the reader is directed to [6, 7]) . Although some research on brain energy metabolism was performed during 1920s and 1930s, it was limited to very few laboratories. Among them, that of Eric G. Holmes and Barbara E. Holmes pioneered important research in the field. The duo, who later joined by C.A. Ashford, published a series of papers [8–14] to demonstrate brain tissue production of lactate from glucose (similar to muscle metabolism), the involvement of phosphates and glycogen in this metabolism and the ability of brain tissue to oxidize lactate. Unfortunately, the latter never received neither the praise nor the scrutiny it deserved. This point is expanded upon in the next section, although it must be emphasized here that if the importance of that discovery would have been recognized at the time, our understanding of brain energy metabolism would be significantly accelerated and advanced. Nevertheless, Holmes and Ashford interpreted their finding of lactate oxidation simply as a process by which the brain rids itself of a waste product, since lactate was believed until the mid 1980s and even beyond, to be just that, a useless end-product of carbohydrate metabolism. Consequently, the research by Holmes and Ashford quickly became obscure. It would have stayed this way if not for a literature search I carried out in 2005 working on an upcoming paper that dealt with the possible role of lactate as an oxidative brain energy substrate [15]. Spending several weeks in the basement of my university medical school library (at the time most of the old literature has not yet been digitized) was an experience akin to treasure hunt, an experience I still cherish today. Discovering Holmes and Ashford's papers in 75-year old, dust-covered, heavy volumes of the Biochemical Journal was almost as exciting as conducting our own research [16]. Three years prior to the publication of the latter on brain energy metabolism, Brooks published his controversial work on muscle energy metabolism [17]. His proposal that skeletal muscle both produces and consumes lactate met with major objections because such consumption would require lactate to be a mitochondrial substrate, which requires the existence of lactate dehydrogenase in mitochondria (mLDH), an enzyme his detractors strongly insisted does not exist. Schurr and colleagues demonstrated *in vitro* that brain tissue is capable of maintaining normal neuronal function when lactate is the sole oxidizable energy substrate [16]. Skeptics of this finding argued that the phenomenon does not occur *in vivo* and even if it does, lactate cannot replace glucose as the obligatory energy substrate in brain [18]. And thus began a long-lasting debate on the validity of these findings and the potential importance of lactate as an oxidative substrate of energy metabolism in brain and elsewhere. **Figure 1** is an illustration

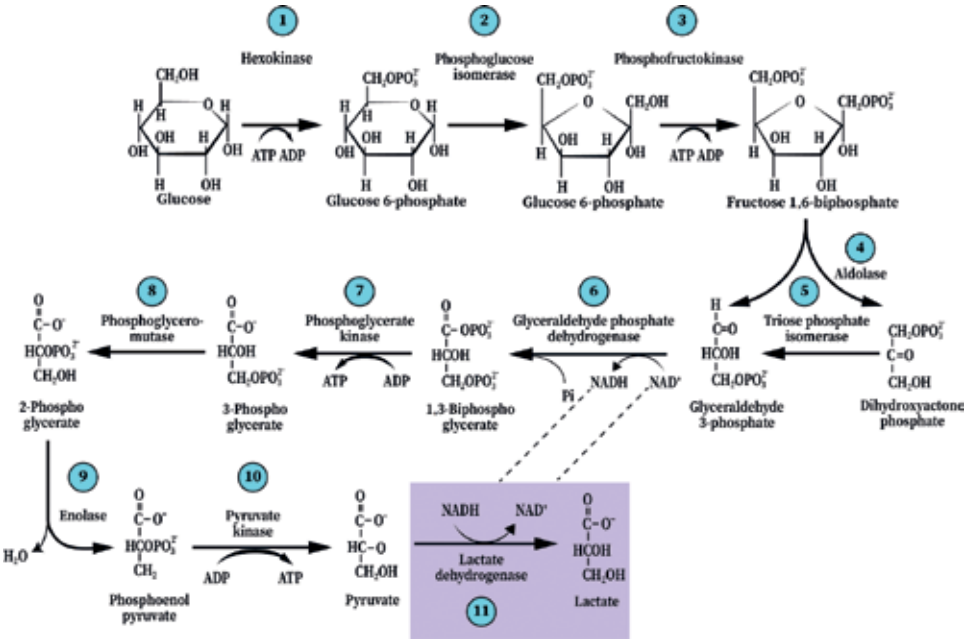

Figure 1.
The classical description of the 10 enzymatic steps of aerobic glycolysis, the pathway that converts glucose to pyruvate with a net production of two molecules of ATP and two molecules of pyruvate, its end-product and the substrate of the mitochondrial tricarboxylic acid (TCA) cycle. Under anaerobic conditions, an eleventh enzymatic step occurs where pyruvate in converted to lactate, which becomes the glycolytic end-product under these conditions.

of the glycolytic pathway as it has been conceived and taught everywhere since 1940. This concept has been based on an initial assumption, made not by those who proposed the original reaction sequence of the glycolytic pathway, but by Krebs and Johnston, who proposed the reaction sequence of the mitochondrial TCA cycle [19]. The latter postulated that pyruvate is the substrate of that cycle and assumed that glycolysis could be its origin. Such an assumption, when made by a leading scientist of the status Krebs had achieved, was persuasive enough to compel Gustav Embden, Otto Meyerhof, and Jakub Karol Parnas to decide that pyruvate is the end-product of aerobic glycolysis and the substrate of the TCA cycle. Hence, for almost eight decades the glycolytic pathway has been described as one that has two possible outcomes, an aerobic one, which ends with pyruvate and an anaerobic one that ends with lactate (**Figure 1**). Every biochemistry textbook published from the 1940s on, every biochemistry, physiology or neuroscience course being taught at any level and every online search, all display this very description of the glycolytic pathway. That, despite ample research data that clearly refute this dogmatic paradigm.

1.2 The last four decades (1980–2018)

As indicated above, questions as to the correctness of the old description of glycolysis began to appear in the late 1980s. The lactic acidosis hypothesis of delayed neuronal damage [20] had a strong following at the time. This hypothesis postulated that lactate accumulation in the ischemic brain is the cause of delayed neuronal damage, damage observed after a recovery from the original ischemic insult had occurred. The popularity of this hypothesis was so strong that any insinuation that lactate could be anything but a menacing factor in cerebral ischemia aroused great skepticism. Consequently, when Fox et al. [21] published

a study, which indicated that neural activation does not require an increase in energy supply and is supported by a mere non-oxidative glucose utilization ("anaerobic" glycolysis), they met with both doubt and a degree of cynicism. Almost simultaneously, Schurr et al. [16] published their findings demonstrating that neuronal function *in vitro* can be supported by lactate as the sole oxidizable energy source. These findings were met with even greater doubt and cynicism, some of which continues to this day. The past 30 years have seen the field of brain energy metabolism grow by leaps and bounds as new technologies and techniques enable scientists to explore, measure and interpret their findings more accurately. Nevertheless, such interpretations depend on the accuracy of our knowledge and understanding of the basic pathways and processes of energy metabolism. The ongoing debate about the correct paradigm of glycolysis, as highlighted by Schurr and Gozal [22] and many of the papers within this Research Topic volume, clearly indicates that such accuracy and understanding are still to be achieved. To illustrate this point consider on one hand, the conclusion of Fox et al. [21] regarding the very low increase in energy demand upon neural stimulation, demand that can be easily answered by non-oxidative glycolysis i.e., glucose consumption unaccompanied by oxygen consumption, while on the other hand, the conclusion by Hyder et al. [23] that activated neural tissue exhibits an increase in energy production, which is fully oxidative i.e., the ratio of oxygen to glucose for this increase is 6:1. Could these completely opposing conclusions be explained by differences in the methodologies used in the two studies (the former made use of ^{18}F-2-fluoro-2-deoxy-D-glucose to measure glucose uptake and $^{15}O_2$ to measure oxygen consumption, while the latter made use of blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI))? Or maybe the measurements by both methods are correct, but their interpretation has relied on assumptions that emanated from older, dogmatic concept?

To answer this question, one must consider the mounting evidence supporting a paradigm shift in our comprehension of the glycolytic pathway [24]. The shift entails redrawing the glycolytic pathway as one consisting of 11 steps, beginning with glucose as its substrate and ending with lactate as its end-product, independent of the presence or absence of oxygen. From its inception, distinguishing between aerobic and anaerobic glycolysis was based not on specific evidence that the two pathways exist and produce two different products, pyruvate and lactate, respectively. That separation was an attempt by the pathway's elucidators to somehow fit it into, what they concluded, is an outcome that produces pyruvate as its main end-product since they accepted Kreb's suggestion that this monocarboxylate is the substrate of the TCA cycle. It must have been relatively easy to accept that suggestion considering lactate's negative reputation [6]. Hence, the glycolytic pathway should be considered one, uninterrupted chain of biochemical reactions that begins with glucose and ends with lactate (**Figure 1**). Accordingly, its last reaction (number 11), the reduction of pyruvate to lactate by the cytosolic lactate dehydrogenase (cLDH), plays a crucial role in keeping this pathway's cyclical nature operational as it provides a continuous supply of NAD^+. If pyruvate was the glycolytic end-product, NAD^+ would have to be imported from other sources and locations, a proposition that has offered a somewhat shaky resolution (see [25] and references within). This, of course, is not the only factor that justifies a paradigm shift. There are numerous studies published over the past two decades demonstrating the presence of lactate dehydrogenase in mitochondria (mLDH), an enzyme that converts lactate to pyruvate [26–32]. Brooks et al. [33] also demonstrated the presence of monocarboxylate transporter 1 (MCT1) in mitochondria, the transporter that is responsible for the transport of lactate along its gradient from the cytosol to the mitochondrion. Havel et al. showed that in blood and in other tissues the

ratio lactate/pyruvate is >10, a value that is not consistent with the assumption that pyruvate is the glycolytic end-product [34]. Moreover, the proposal that aerobic glycolysis ends with pyruvate does not meet the known standard free-energy ($\Delta G^{0'}$) change of the reaction pyruvate → lactate, which is −6.0 kcal/mol, a value indicating that this reaction should proceed independently of the presence or absence of oxygen. In other words, glycolysis, whether aerobic or anaerobic, should always end up with lactate. **Figure 2A** demonstrates the free energy change profile of aerobic glycolysis that ends with the reaction phosphoenolpyruvate → pyruvate, although the potential free-energy change of the conversion pyruvate → lactate (**Figure 2B**, anaerobic glycolysis) determines that glycolysis should end with lactate regardless of the oxygenation condition (**Figure 2C**). Last but not least, the reaction equilibrium of cLDH is tilted heavily in the direction of lactate production, which makes it unlikely for lactate to be converted back to pyruvate by that cytosolic enzyme. In contrast, the reaction equilibrium of mLDH tilts in the direction of lactate oxidation to pyruvate [35, 36].

The above points support the proposed paradigm shift in the glycolytic pathway [24], where lactate, not pyruvate, is its end-product and the oxidative mitochondrial substrate for the TCA cycle. Accordingly, is measuring the cerebral metabolic rates of oxygen (CMR_{O_2}) and glucose ($CMR_{glucose}$) sufficient in providing an accurate picture of brain energy metabolism during rest or activation, in health or disease? If lactate is an oxidative energy substrate, should not $CMR_{lactate}$ also be measured in order to have a more complete account of cerebral energy metabolism? How would the measurement of $CMR_{lactate}$ contribute to our understanding of the brain's ability to handle its energy demands under those conditions?

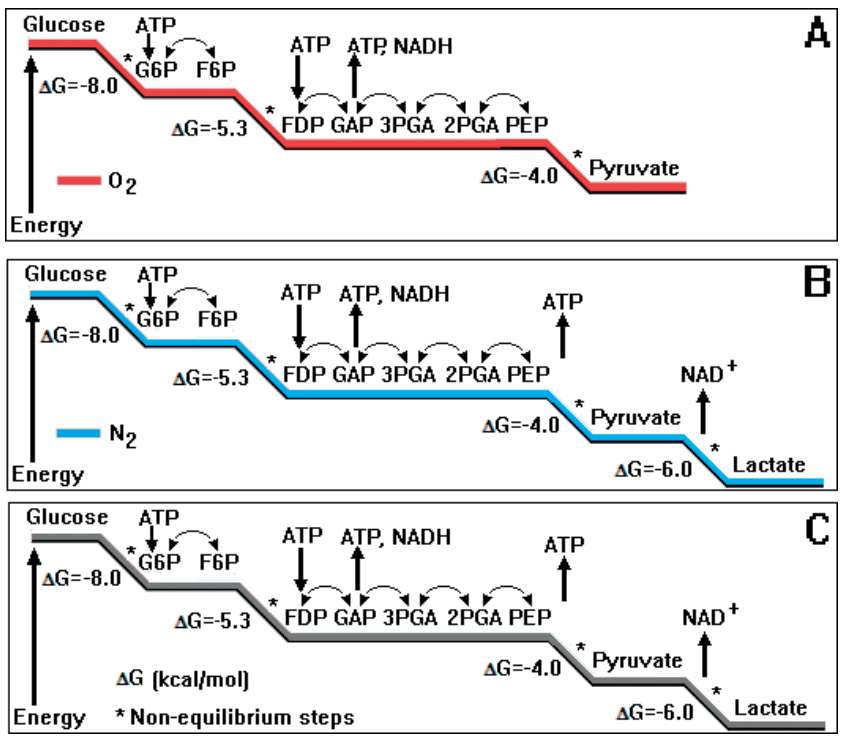

Figure 2.
*A schematic illustration of the potential free-energy change profile of aerobic (A) and anaerobic glycolysis (B). The potential free-energy change of the reaction pyruvate → lactate dictates that it should proceed regardless of the oxygenation conditions (**C**).*

2. Measurement of cerebral energy metabolic rates

At the basis of each technology designed to measure the rate of brain energy metabolism is the idea that measuring the consumption rate of the main two substrates of glycolysis and mitochondrial respiration, glucose and oxygen (O_2), should provide a complete picture of the brain's energy use. Theoretically, under normal conditions, each glucose molecule that enters the glycolytic pathway requires six molecules of oxygen to be fully oxidized via the mitochondrial TCA cycle and the electron transport chain. Thus, simultaneous measurements of glucose and oxygen consumption during rest or activation supposedly produces accurate estimate of the energy needs for the brain region under observation. However, the ratio $CMR_{O_2}/CMR_{glucose}$ values calculated are often significantly smaller than the expected 6/1. Such discrepancies have attributed to other glucose-consuming reactions not accompanies with oxygen consumption. Consequently, it has been a common understanding that a value of $CMR_{O_2}/CMR_{glucose} < 6$ indicates that a partial non-oxidative glucose consumption. The smaller the value of $CMR_{O_2}/CMR_{glucose}$, the greater is the non-oxidative consumption of glucose. This understanding makes sense when one assumes that a fully coupled glycolytic-mitochondrial respiratory apparatus should produce a $CMR_{O_2}/CMR_{glucose}$ value of 6 and an uncoupled apparatus (non-oxidative) should produce a $CMR_{O_2}/CMR_{glucose}$ value of ~0.

As indicated above, myriad techniques and technologies have been developed during the past six decades to measure both CMR_{O_2} and $CMR_{glucose}$. To measure cerebral energy metabolism *in vivo* one can analyze chemical changes in the blood entering and exiting the brain and/or in the cerebrospinal fluid. Of course, brain tissue samples can also be taken for analysis before and after physiological activity, although this approach would lend itself only to experimental animals. The introduction of radioisotopes to the analytical techniques of brain metabolic activity has greatly improved their speed and accuracy. Radioisotopes allow not only the tracing of end-products of cerebral metabolism, but also the detection of intermediates of that metabolism. Nevertheless, these techniques have their own drawbacks, including the need to sacrifice the animal under study only to receive a single measurement which provides mainly a qualitative value. A quantitative measurement is frequently confounded by compartmentation and its misinterpretation thereof. One of the most reliable techniques to measure oxygen consumption is the polarographic technique, which allows the determination of oxygen concentration via the measurement of the partial oxygen pressure (PO_2) locally. Continuous measurements over a period of time when brain activity (EEG) is monitored, demonstrated a correlation between increased activity and decreased tissue oxygen level. The development of oxygen microelectrodes has afforded a more accurate localization of such measurements.

In principle, CMR can be expressed as: $CMR = CBF\ (A - V)$.

where $(A - V)$ is the difference in concentration in arterial and cerebral venous blood, CBF is the rate of cerebral blood flow in volume of blood per unit time, a CMR (cerebral metabolic rate) is the steady state of utilization or production of a substance by the brain [18]. This equation is the foundation on which quantitative CMR studies *in vivo* have been conducted. Since the normal brain consumed approximately 20% of the total body oxygen consumption to maintain its functionality and structure, it is clear that any interruption in this high demand for oxidative energy metabolism could have far reaching survivability consequences. Clearly, a non-oxidative energy metabolism (glycolysis) is incapable of answering the high energy demands of the brain. That a stimulated brain has still higher energy demands than the resting one would be an inevitable conclusion. Hence, when studying the energy demands of a specific activated brain region, such activation is expected to produce

an increase in both CMR_{O_2} and $CMR_{glucose}$. Consequently, when Fox et al. published their study under the title "Nonoxidative glucose consumption during focal physiologic neural activity" [21] they stirred a small tempest among scientists in the community that studies cerebral blood flow and metabolism. These investigators employed ^{18}F-labeled 2-fluoro-2-deoxy-D-glucose to measure $CMR_{glucose}$, a method originally developed over a decade earlier [37], and ^{15}O-labeled molecular O_2 to measure CMR_{O_2}. They demonstrated that transient increases in neural activity elevated glucose tissue uptake in excess of that consumed by oxidative metabolism. They concluded their findings to indicate that stimulated brain activity requires significantly less energy than previously thought. Also, since they measured a corresponding increase in CBF along with the increase in glucose consumption, the investigators argued that this increase is for purposes other than oxidative metabolism. These conclusions stemmed from the prevailing postulate that over 90% of resting brain's glucose consumption is oxidative and less than 5% of that consumption ends in glycolytic lactate production. Since the oxidative consumption of one molecule of glucose produces approximately 36 molecules of ATP, while the glycolytic consumption of one molecule of glucose produces only 2 molecules of ATP, one can easily appreciate how oxidative consumption of glucose is responsible for 90% of the resting brain ATP production. Hence, the finding by [21] that brain stimulation increased glucose consumption without a corresponding increase in oxygen consumption unsettled the established understanding according to which increased brain activity must be accompanied by a corresponding increase in energy supply. This seminal paper was originated from the laboratory of Marcus Raichle, a laboratory that has become a leading center for functional brain imaging [38]. Imaging technologies such as X-ray computed tomography (CT), positron emission tomography (PET), near-infrared spectroscopy (NIRS) and magnetic resonance imaging (MRI) are the main techniques available for the measurement of brain energy metabolism during rest and activity. The most popular technology for this purpose today is the blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI), which was developed by [39]. BOLD fMRI measures changes in blood oxygenation in relation to brain activity, although that relationship is somewhat ambiguous, since it is not accompanied by a direct neural activity measurement such as that allowed by electrophysiology. When the latter is combined with direct oxygen concentration measurements, using an oxygen microelectrode (polarography), a higher resolution than BOLD fMRI can be achieved [40]. Besides tissue oxygen measurements using microelectrodes, tissue glucose and lactate concentrations can also be assessed using specific microelectrodes (sensors). Of course, this approach does not lend itself for regular use in humans, however, for the purpose of *in vivo* studies in experimental animals, the approach proved itself to be very useful and an eye opener.

In this respect, Hu and Wilson [41] studied the coupling of a temporary local energy pool to neuronal activity in the rat brain (**Figure 3**). They were the first to combine the use of three separate rapid response sensors (microelectrodes) to measure tissue oxygen, glucose and lactate concentrations. The investigators placed them in the dentate gyrus of the rat hippocampus, observing how they fluctuate in response to 10 consecutive electrical stimulations of the perforant pathway (each stimulus lasted 5 s and applied every 2 min). Their results were analyzed by Schurr and Gozal [36] (**Figure 3**). A literature search shows that Hu and Wilson's interpretation of their findings has its supporters [15, 42–47] and detractors [48–50]. The former group argued that these findings are strengthening the concept that lactate is the energy substrate that is utilized oxidatively upon neuronal activation. The latter group disagreed with this conclusion. Schurr [24] further analyzed the results of Hu and Wilson [41] beyond an earlier analysis [36]. The more recent analysis was prompted for two reasons. First, two decades have passed since the publication

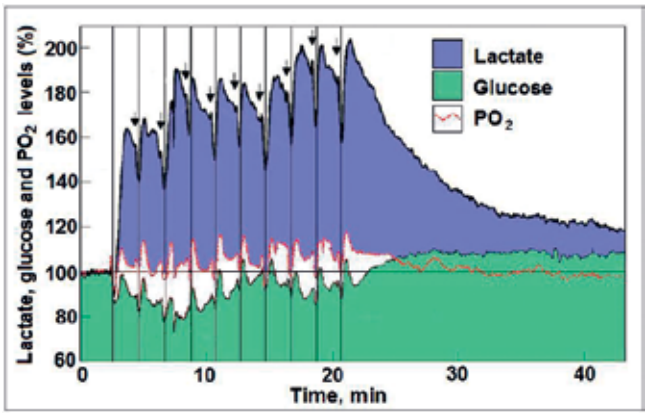

Figure 3.
Profiles of time course and dynamic relationships of local extracellular lactate, glucose, and PO_2 levels in the rat hippocampal dentate gyrus during a series of 5 s electrical stimulations (arrows) of the perforant pathway at 2 min rest intervals (reproduced with permission from Hu and Wilson, copyright 1997, Blackwell, Oxford). The changes in the mean concentration of glucose were always in opposite direction to the changes in mean lactate concentration. The vertical lines were drawn to indicate the simultaneous dip in all three analytes in response to each of the electrical stimulations. For additional details see [41] from where the figure and the legend have been reproduced with permission and [36].

of the paper by Hu and Wilson [41], a period in which numerous studies added much support to the idea that lactate is a mitochondrial oxidative energy substrate. Second, many other studies on cerebral energy metabolism continue to conclude that neural activity is supported by "anaerobic" glycolysis and not by oxidative utilization of glucose, while ignoring the possibility that such activity may be supported by oxidative utilization of lactate.

3. Lactate cerebral metabolic rate and the importance of its measurement

When the results of the study by Hu and Wilson [41] were analyzed before [36], the analysis showed that upon a series of 10 stimulation of the rat hippocampal perforant pathway a steady glucose consumption was accompanied by a gradual increase in lactate consumption. Considering the conclusion of Fox et al. [21] that aerobic glycolytic ATP production is sufficient to answer the energy needs of activated neural tissue, one could assume that it should be sufficient to provide the energy needs of the stimulated hippocampal dentate gyrus. In addition, this analysis points out that if the conclusion of Fox et al. [21] is correct, the energy needs of the activated dentate gyrus declined with each stimulation or stayed the same at a very low level of ATP production (0.8–0.3 mM). However, if lactate oxidative consumption is postulated to be responsible for the ATP production that sustains the energy needs of the stimulated tissue, the increased lactate consumption with each consecutive stimulation signals a concomitant increased ATP production. The calculation shows that the response to the first stimulation produced 3 mM ATP, while the response to the last stimulation produced almost 11 mM (**Figure 4**).

The more recent analysis [24] also indicates that the increased levels of tissue lactate following each stimulation [41] could not be produced from the glycolytically metabolized glucose (**Figure 5**). Hence, this additional lactate had to be originated from other sources i.e., the surrounding tissue or glycogen stores [50]. As was shown by Hu and Wilson [41] (**Figure 3**), a larger amount of lactate was consumed during each consecutive stimulation, while a smaller amount of glucose was

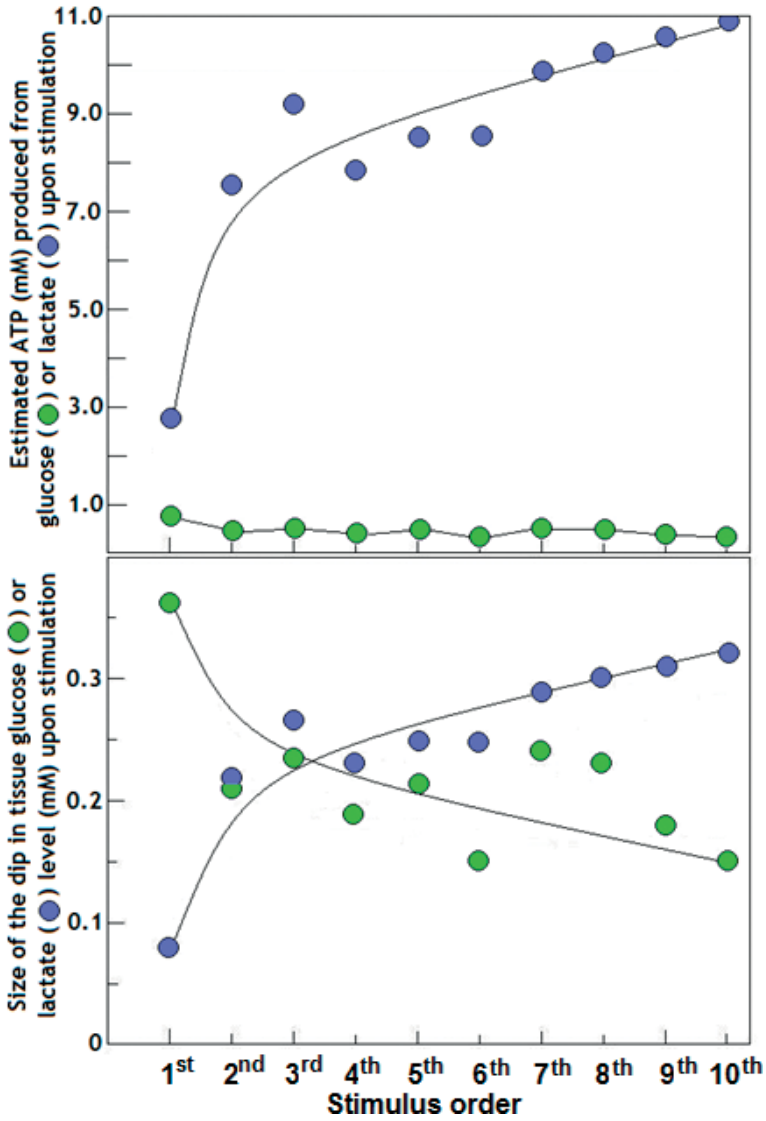

Figure 4.
Time course of changes in the amplitude of the dip in tissue glucose and lactate levels in the rat hippocampal dentate gyrus after each of the 10 electrical stimulations applied to the perforant pathway at intervals of 2 min (bottom panel). The amplitude of each dip (in mM) was calculated from the Hu and Wilson [41] as reproduced in ***Figure 3****. The upper panel represents the estimated ATP amount produced based on the size of the dip (in mM) in tissue glucose and lactate levels as shown in the bottom panel. The estimated ATP levels were calculated as follows: the glucose measured dip (in mM) was multiplied by 2, the net production of 2 moles ATP from each mole of glucose metabolized glycolytically; the lactate measure dip (in mM) was multiplied by 34, the net formation of 34 moles of ATP for every 2 moles of lactate (form glycolytically from 1 mole of glucose) metabolized via the mitochondrial TCA cycle and the oxidative phosphorylation chain.*

consumed. Moreover, following each stimulation, except the first one, the lactate level measured exceeded the level expected from the amount of glucose consumed glycolytically i.e., two moles of lactate from one mole of glucose. Following the second stimulation, the tissue ratio of lactate to glucose was 3.95 and by the 10th stimulation this ratio increased to 8.33 (**Figure 5**). Meanwhile, oxygen tissue levels dipped and rose as expected during and after each stimulation, respectively, signaling that the electrical stimulation evoked an oxidative consumption of substrate. Initially, glucose and lactate were oxidatively consumed at equal amounts however,

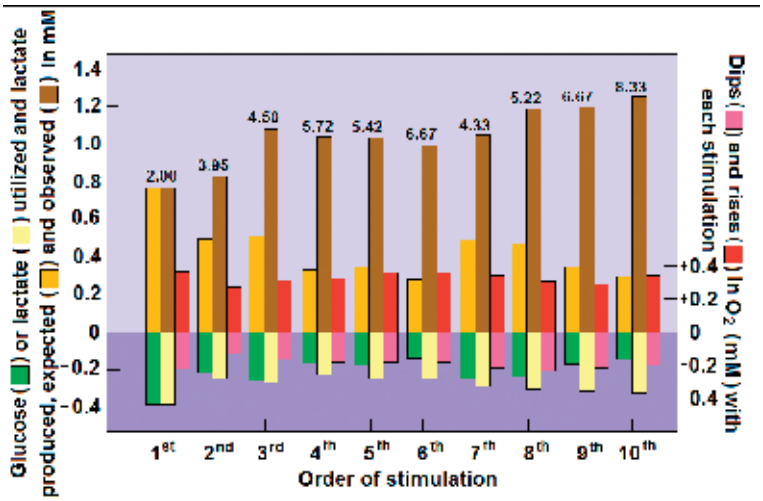

Figure 5.
The local extracellular glucose, lactate and O_2 levels in a rat hippocampal dentate gyrus during a series of 5 s electrical stimulations of the perforant pathway at 2 min rest intervals and the dynamic relationship between them. Glucose, lactate and O_2 concentrations were calculated from their dips and rises as measured by Hu and Wilson [41] using rapid response sensors. The numerical values posted above the columns representing the rises in glucose and lactate post-stimulation are the calculated ratios between the two. For additional details see ***Figure 3*** *and [36].*

from the second stimulus onward more lactate than glucose was consumed (**Figure 5**). The oxygen level as measured by Hu and Wilson [41] fluctuated with a dip upon stimulation and a sharp rise upon its cessation (**Figure 3**). The fast rise can be interpreted as evidence that ample oxygen was available if and when needed. This rise also indicates that the tissue was well oxygenated during the duration of the experiment. Considering that one mole of lactate consumes three moles of oxygen for its full oxidation as compared to six moles of oxygen consumed by glucose for its full oxidation, if lactate, rather than glucose, is the main oxidative energy substrate during neural tissue activation, the expected ratio CMR_{O_2}:$CMR_{lactate}$ should not exceed 3:1. Therefore, it is reasonable to presume that during neural activation, when lactate oxidation is a major supplier of the ATP necessary to support said activation, the ratio CMR_{O_2}:$CMR_{glucose}$ should be considerably lower than 6:1. Obviously, most, if not all, studies aimed at measuring cerebral metabolic rates postulate that the ratio CMR_{O_2}:$CMR_{glucose}$ measured or calculated should approach 6 [23]. The conclusions of Fox et al. [21] are in complete disagreement with the measurements and calculations of Hyder et al. [23]. While the former concludes an almost complete uncoupling between glucose and oxygen consumption by activated neural tissue, the latter asserts the maintenance of full coupling between glucose and oxygen consumption during neural activation. In both studies [21, 23] the interpretation of the results is based entirely on the original, dogmatic paradigm of glycolysis according to which aerobic glycolysis ends with pyruvate, the assumed substrate of the mitochondrial TCA cycle.

The *in vivo* measurements performed by Hu and Wilson [41] of both glucose and lactate concentrations before, during and post electrical stimulation provide much support to the proposal that lactate plays a major role in oxidative energy metabolism of the activated neural tissue. The relatively small dips and rises in O_2 levels in response to the electrical stimulations, as measured polarographically, should be given additional consideration. First, the spatial and temporal resolutions provided by polarographic measurement of O_2 compared to BOLD fMRI measurements allow for a better characterization of the time-course of oxygen responses [40].

Hyder et al. [23] used BOLD fMRI and the measurements used by Fox et al. [21] were even more cumbersome, involving the use of $[^{15}O]H_2O$, $[^{15}O]O_2$ and $[^{15}O]CO_2$.

While BOLD fMRI estimates yielded a CMR_{O_2}:$CMR_{glucose}$ ratio value of 6:1, the ^{15}O measurements produced a ratio value of 0.4:1. These completely opposing outcomes make one wonder whether or not the measurements performed using these two methods and the calculated values of CMR_{O_2} they produced truly reflect the changes in the consumption of molecular oxygen upon neural activation. Could the direct measurements of CMR_{O_2}, $CMR_{glucose}$ and $CMR_{lactate}$ done by Hu and Wilson [41] along with the indirect ones made by Fox et al. [21] and Hyder et al. [23], be reconciled such that a better picture of cerebral metabolic rates of activated neural tissue can be visualized? It is widely agreed that over 90% of the normal brain's energy production originates from glucose oxidation [21, 51]. The normal glucose concentration in the brain is ~2 mM and its normal lactate concentration is about half of that of glucose. Thus, it is safe to postulate that the normal resting brain is supplied with ample amounts of oxygen to continuously oxidize more than 90% of the brain glucose. However, glucose supplies to the normal brain are limited (only 40% of normal blood glucose level). Consequently, the increased rate of CBF along with the increased consumption of glucose upon activation [21, 23, 52] should supply all the oxygen necessary to match the increased demand, in contrast to the limited supplies of glucose. Low resolution techniques for the measurements of oxygen concentrations are unable to detect local fluctuations accurately if at all, which could explain why Fox et al. [21] reached the conclusion regarding the very low oxygen consumption during neural activation. Nonetheless, their conclusion that the energy demands of activated neural tissue are being met through glycolytic ATP production is most likely incorrect. In other words, undetectable or slightly detectable dip in tissue oxygen level upon activation is not necessarily an indication that oxygen is not consumed. The higher resolution of oxygen measurement afforded by polarography exemplifies the fact that local oxygen levels dipped upon stimulation and overshot upon its cessation ([41]; **Figures 3** and **4**). Although local fluctuations in tissue oxygen levels were evident, its overall tissue concentration did not significantly change and may even have risen somewhat above its baseline level. In contrast, both glucose and lactate levels were changed significantly from their baseline levels [24, 36, 41] (**Figures 3**–**5**). The fluctuations between lactate and oxygen were highly synchronized, indicating that lactate is being oxidized upon tissue activation. During the 20 min following the 10th stimulation, the tissue level of both oxygen and glucose climbed above the baseline level, while the high level of lactate gradually declined ([41]; **Figure 3**). These shifts seem to indicate that upon cessation of stimulation, as the tissue is recovering from activation and high energy demands, lactate becomes the preferred oxidative energy substrate, sparing glucose. That the cerebral tissue would prefer lactate over glucose, especially when the former is abundant, is reasonable, considering the fact that lactate oxidative mitochondria. Consequently, the increased rate of CBF along with the increased consumption of glucose upon activation [21, 23, 52] should supply all the oxygen necessary to match the increased demand, in contrast to the limited supplies of glucose. Low resolution techniques for the measurements of oxygen concentrations are unable to detect local fluctuations accurately if at all, which could explain why Fox et al. [21] reached the conclusion regarding the very low oxygen consumption during neural activation. Nonetheless, their conclusion that the energy demands of activated neural tissue are being met through glycolytic ATP production is most likely incorrect. In other words, undetectable or slightly detectable dip in tissue oxygen level upon activation is not necessarily an indication that oxygen is not consumed. The higher resolution of oxygen measurement afforded by polarography exemplifies the fact that local oxygen levels dipped upon stimulation and overshot upon its cessation (**Figures 3** and **4** and [41]). Although local fluctuations in tissue

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4. CMRs measurements and their possible implications in brain disorders

Energy metabolic interruptions are at the basis of several brain disorders and measuring CMRs of patients inflicted by such brain disorders can offer a potentially better diagnosis and treatment. Measurement of CMR_{O_2} and $CMR_{glucose}$ have been performed regularly in numerous studies of cerebral ischemia in an effort to better understand the mechanisms of neuronal ischemic damage. $CMR_{glucose}$ measurement has been used in studying obsessive-compulsive disorder, mood disorder and depression, where the main aim is to follow changes in glucose metabolism in specific brain regions believed to be involved in these disorders. Other brain disorders where glucose metabolic rate has been measured in include amyotrophic lateral sclerosis, Alzheimer's disease, epilepsy, Parkinson's disease and Huntington's disease. The purpose behind the measurement of glucose cerebral metabolic rate when investigating diseases and disorders is usually to identify brain regions that are involved in a given disorder or disease, not to investigate how energy metabolism is being affected by the disease or the disorder. Also, the energy metabolic rates of brain tumors have received great attention due to the unique energy requirements of these tumors. Nevertheless, cerebral ischemia and traumatic brain injury (TBI) are the two disorders for which measurements of CMR_{O_2} and $CMR_{glucose}$ are most abundant. The results of these measurements prompted proposals both for treatments and mechanisms of neuronal damage due to these insults. The most heralded hypothesis attempting to explain delayed neuronal cerebral ischemic damage [20] known as the lactic acidosis hypothesis, postulated the accumulation of lactic acid as the cause of that damage. Consequently, physicians dealing with stroke patients were encouraged to control blood glucose levels in these patients, based on the assumption that the higher the glucose level during cerebral ischemia, the higher the level of lactic acid produced and the damage it causes. The lactic acidosis hypothesis was discarded, although the practice of controlling the blood glucose level of stroke patients remained. To this end, lactate was shown to support neuronal recovery post-ischemia *in vitro* [53–55]. Moreover, higher glucose level pre-ischemia (hypoxia) appear to improve neuronal recovery post-ischemia *in vitro* [56] and any exacerbation of neuronal damage due to pre-ischemic hyperglycemia was shown to be induced by increased levels of stress hormone [57]. Similarly, experimental [58] and clinical studies [59–66] over the past two decades indicate that lactate supplementation after TBI improves post-injury outcome. Measurement of $CMR_{lactate}$ could greatly enhance our understanding of the role that this monocarboxylate plays in these two brain disorders.

5. Conclusions

A paradigm shift of a scientific model should, naturally, result in reconsideration of hypotheses and concepts that have been formulated on its foundation prior to its shift. The understanding of cerebral metabolic rates of energy substrates during rest and activation of neural tissue, the use of the method best suited for the measurement of these rates and the interpretation of the results have always relied on two fundamental assumptions. First, cerebral energy metabolism includes the obligatory glycolytic breakdown of glucose to pyruvate and the utilization of the latter by the mitochondrial TCA cycle and the electron transport chain with oxygen as its final receptor. Second, the activation of cerebral tissue is sustained by an increase in ATP production and therefore an increase in the consumption of glucose and oxygen. Two seminal papers that were published almost simultaneously [16, 21] have forced biochemists, and especially neuroscientists, to reassess these two basic postulates. The paper by Fox et al. [21] has perplexed many with its conclusion that the energy requirements of activated neural tissue are minimal and can be fulfilled by the glycolytic pathway alone (glucose → lactate + 2ATP). The paper by Schurr et al. [16] provoked great skepticism upon demonstrating that neural tissue can function and be activated when lactate is its sole oxidative energy substrate (lactate + $3O_2$ + mitochondria → pyruvate → TCA cycle → $3CO_2$ + $3H_2O$ + 17ATP). While the proposal that lactate is a suitable oxidative energy substrate had faced strong skepticism for many years, it has gained greater support over the past three decades. The proposal that glycolysis could be served as the sole supplier of energy for the activated neural tissue still divides scientists working in this field. By accepting the proposed paradigm shift of glycolysis [24] and its application in the interpretation of the results obtained by Fox et al. [21], Hyder et al. [23, 67], Hu and Wilson [41] and many others, a scenario can be drawn where lactate is supplementing most if not all the energy requirements of activated neural tissue. The data and the line of reasoning presented here strongly argue against the conclusion that these energy requirements are solely fulfilled by glycolysis. Future studies of activated cerebral metabolic rates should include, along with the measurements of CMR_{O_2} and $CMR_{glucose}$, the measurement of $CMR_{lactate}$. Resolving the existing debated issues of cerebral energy metabolism is paramount for our better understanding the many brain diseases and disorders. Hopefully, this chapter provides a possible resolution of some of these issues.

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Section 3

Diabetes Mellitus

Chapter 4

Diabetes Mellitus: A Group of Genetic-Based Metabolic Diseases

Lilian Sanhueza, Pilar Durruty, Cecilia Vargas, Paulina Vignolo and Karina Elgueta

Abstract

Diabetes mellitus (DM) is a disease characterized by defects in action and/or secretion of insulin that results in chronic hyperglycemia and long-term severe vascular complications. The main clinical presentations with the proven genetic base are covered. Type 1 diabetes (DM1) is an autoimmune, heterogeneous, multifactorial, and polygenic-based disease. Selectively destroys 90% of beta cells of the pancreas, mediated by activated T lymphocytes in patients with haplotypes linked to major histocompatibility complex (MHC). Genetic and genomic studies have been carried out in family groups, demonstrating up to 15 affected chromosomal regions. Type 2 diabetes (DM2) presents genes with various polymorphisms which, together with post-genomic and environmental factors, make it more complex to understand the pathogenesis. Monogenic diabetes comprises neonatal diabetes (ND), maturity onset diabetes in young (MODY), an autosomal dominant transmission which is inherited directly in three successive generations, and the very rare mitochondrial diabetes. Latent autoimmune diabetes in adults (LADA) mainly affects patients with normal weight and initially diagnosed as DM2. Its characteristics are low levels of C-peptide in both fasting and post-glucagon tests. They present MHC alleles of susceptibility and positive autoantibodies: Anti-decarboxylase glutamic acid.

Keywords: diabetes mellitus type 1, diabetes mellitus type 2, monogenic diabetes, LADA

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases of different etiologies characterized by chronic hyperglycemia resulting from a deficit in both the secretion and the action of insulin hormone. Now-a-days, there is a genetic basis of these clinical manifestations. In this chapter, we describe the most important ones such as diabetes mellitus type 1 (DM1), diabetes mellitus type 2 (DM2), monogenic diabetes, and latent autoimmune diabetes in adults (LADA).

2. Diabetes mellitus type 1

DM1 is characterized by autoimmune destruction of the beta cells of the pancreatic islets, which leads to an extreme insulinopenia. The character of autoimmunity confirm the presence of islet cell antibodies (ICA), insulin auto antibodies (IAA),

glutamic acid decarboxylase auto antibodies (GAD65), protein tyrosine phosphatase 2 (IA-2), and zinc transporter gene ZnT8 [1]. There is an interaction between genetic and environmental factors in the development of DM1 (**Figure 1**).

2.1 Genetic factors of DM1

DM1 is a polygenic disease, with at least 15 associated chromosomal regions. The leading group of genes that predispose to type 1 diabetes is located on human chromosome 6 specifically at 6p21, and this chromosomal region contains a group of genes called major histocompatibility complex (MHC), responsible for the immune response and the antigen presentation of the beta cell to T lymphocytes [2]. The classic histocompatibility genes are extremely polymorphic (amino acid sequence differs among individuals) and include MHC-A, B, and C molecules (class I histocompatibility antigens) and the immune-response genes DP, DQ, and DR (class II histocompatibility antigens). Numbers (DR3, DR4; A1, A2; B1, B8) are given to distinguish different alleles of any given gene. The designation w (workshop) with numbers is given for provisionally named alleles (DQw8, DQw7) [3]. DM1 has been associated mainly with allelic variants of MHC-DR (DR3/DR4). The MHC locus is a genetic factor of great importance in DM1, and it was first shown in an association study that revealed that about 95% of all patients with DM1 were heterozygous for MHC-DR3/DR4. The majority of type 1 diabetics have the MHC-DR3, MHC-DR4 haplotype, or both [4]. Susceptibility to DM type 1 is associated with these linked DQ alleles that are often in linkage disequilibrium with DR. The closest association in DM1 occurs with the haplotypes DQA1*0301, DQB1*0302, DQA1*501, and DQB1*0201. It has been shown that the beta DQ chains of those affected have valine, alanine, or serine at position 57; near the peptide-binding gap, presence of aspartic acid is normal [5] Factors involved in the pathophysiology of DM1 are shown in (**Figures 2** and **3**).

However, there are exceptions to this association, which indicates that amino acids other than Asp57 at position 57 of the beta chain play an essential but not exclusive role in the susceptibility to DM1 [5]. There is an interaction between genetic and environmental factors in the development of DM1.

2.2 Heritage in type 1 DM

The inheritance of DM1 is unknown. Several hypotheses have been suggested, such as that of dominant inheritance, but it is ruled out by the rarity of DM1 in relatives, children, and descendants. The possibility of recessive inheritance was also

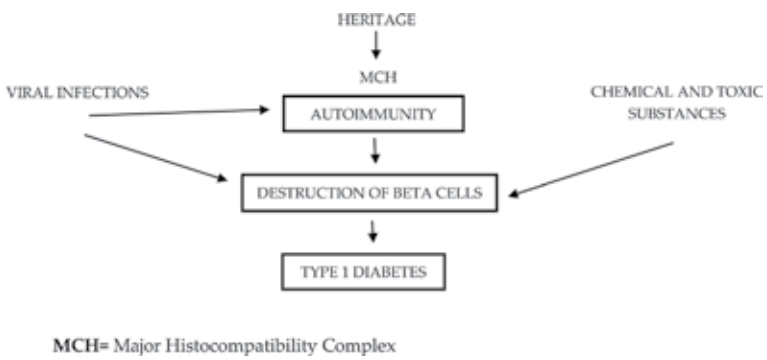


Figure 1.
Pathogenesis of type 1 diabetes.

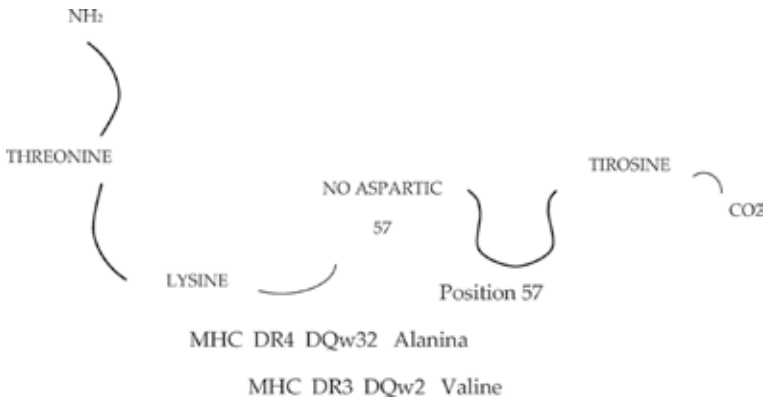


Figure 2.
Etiology of type 1 diabetes. MHC-DQ B antigen amino acid sequence.

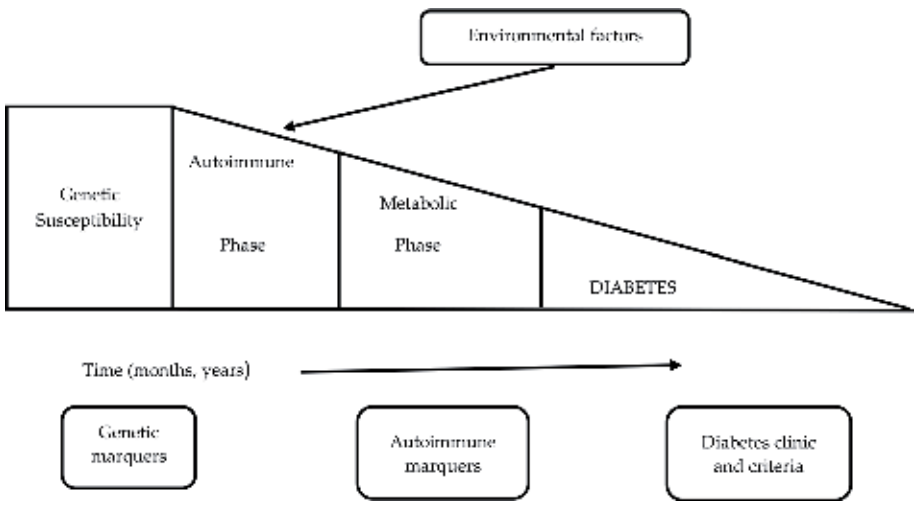


Figure 3.
Pathophysiology of type 1 diabetes.

considered, however, invalidated because in homozygotes for the DR3 or DR4 alleles, the susceptibility to the disease is not increased. The observation that heterozygosis DR3/DR4 increases the risk for diabetes, compared with that presented by homozygotes from other high-risk alleles, suggests a polygenic way of inheritance. The diabetogenic MHC haplotype is necessary for the susceptibility to DM1, but must be positively or negatively influenced by genes not linked to MHC, such as the gene located close to the repeated DNA sequence minisatellite, in the promoter region of the gene of the insulin (chromosome 11p15); one gene on chromosome 11q and another on chromosome 6q [6]. Some genes seem to confer protection against the development of the disease. For example, the DQA1*0102 and DQB1*0602 haplotypes are present in 20% of the United States population but is extremely rare in individuals with DM1 (<1%). This situation indicates that several genes are interacting to determine the DM1 phenotype, so this disease presents genetic heterogeneity (**Figure 4**).

DM1 is uncommon in Chile and usually does not occur in native Chilean families. A study of a family with an affected female child was carried out in a Mapuche community in the Southern (VIII region). This case is a unique and sporadic DM1 case with Mapuche heritage. Genetic analysis by PCR was done

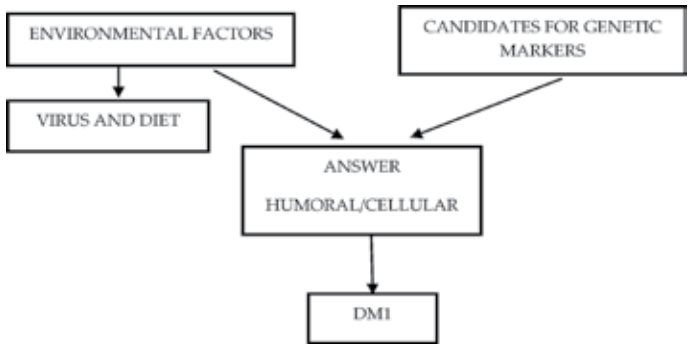


Figure 4.
Interaction of genetic and environmental factors about the immune response in type 1 diabetes.

to detect class I and II HLA genes by reverse dot blot. The proband, her mother, and sister had positive islet cell antibodies (ICA). Her father and brother were negative. All the family was positive for anti-glutamic decarboxylase antibodies (GAD65). All subjects had HLA-DRB1 0407/0407 and HLA-DQB1 0302/0302 alleles. The index case and her father were homozygotes for the HLA-A1: A*68012/A*68012 allele. No evidence of viral infections such as rubella, mumps, or measles was found in this family. All genotypes were compared with the European population, where the diabetogenic combination DR4/DQB1*0302 is the most prevalent [5]. Finally, despite of the high relative risk of DM1 in subjects with certain MHC class II alleles, it does not develop in the majority of people who inherit these alleles, which also suggests that environmental factors influence development of the disease [1] (**Figure 4**).

2.3 Clinical manifestations of type 1 diabetes

The onset of DM1 is usually abrupt, with severe symptoms attributable to hyperglycemia maintained for days or weeks, such as polyuria, polydipsia, polyphagia, asthenia, and progressive weight loss, and manifests as diabetic ketoacidosis. Two pathophysiological situations must be present to establish this condition: extreme insulinopenia and increase of counterregulatory hormones, principally glucagon. DM1 is observed mainly in children, adolescents, and young adults, generally under 30 years, although it can also appear in individuals of more advanced ages.

3. Diabetes mellitus type 2

DM2 has an important genetic component in its pathogenesis. There are multiple genes involved in different metabolic pathways that contribute to the pathogenesis of the disease, in addition to environmental factors such as obesity, an unhealthy diet, and a sedentary lifestyle. These genes have various polymorphisms, which added to post-genomic factors related to their expression and inhibition are responsible for this complex disease, as seen in **Figure 5**.

3.1 Heritage in type 2 diabetes

The genetic nature of DM2 has been based on high heritability, estimated at 30–70% and high prevalence in some ethnic groups; 39% of patients with DM2 have at least one relative with the disease. There are 1.5–3 times higher risk of presenting

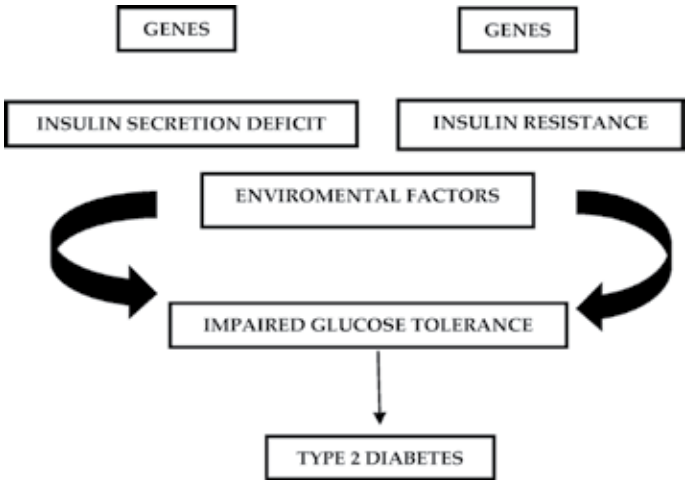


Figure 5.
Pathogenesis of type 2 diabetes.

DM2 if there is a history of the disease in the family; if the affected one is the mother or the brother, the risk is of 2 and 3 times greater, respectively. The heritability described is mainly in middle-aged people (35–60 years) decreasing markedly in larger groups [6]. First-degree relatives of people with DM2 show early defects in insulin withdrawal and action [7]. If other factors such as obesity and impaired fasting glycemia are added to the family history, the risk of presenting the disease is 16 times higher. The presentation of DM2 involves genes that code for proteins or related enzymes in the process of pancreatic formation, synthesis, sectioning, and insulin action. Genome-wide association studies (GWAS) have shown more than 100 locus susceptible to DM2, most related to insulin sequestration, suggesting that this alteration, essential for the presentation of the disease, is more strongly determined by genetic factors.

The genetic susceptibility given by the presence of risk variants is responsible for about 10% of the family aggregation of the disease. There is a considerable percentage of "missing heritability" that could be explained by: frequent variants of low power, others of low frequency but powerful effect, interaction between gene–gene, gene–environment interaction, epigenetic factors, and others.

3.2 Genetic defects in the action of insulin

The genetic variants associated with the action of insulin and DM2 are related to the transcription of the intracellular signal of insulin. Due directly to protein mutations of the signaling cascade intracellularly or indirectly due to mutations in genes associated with metabolic syndrome, such as those related to obesity and lipid metabolism. Yaghootkar et al. evolved cluster of 17 genetic variables associated with insulin resistance related to the development of DM2 [8]. Some of the genes that are most related to DM2 appear in **Table 1** [9].

3.2.1 Insulin receptor substrate-1 gene (IRS-1)

Located on chromosome 2, it encodes peptides related to the insulin signaling cascade. Arg972Gly mutation, a common variant of IRS-1, is more prevalent in Caucasian DM2 than in non DM2, and in obese adults, it has been associated with increased insulin resistance.

Insulin resistance genes
• Insulin receptor substrate gene-1 (IRS-1)
• Peroxisome proliferator-activating receptor gene (PPARϒ)
• Protein tyrosine phosphatase receptor type D (PTPRD)
• β-3 adrenergic receptor gene
• Adiponectin gene
• Leptin gene

Table 1.
Candidate genes of DM2 for insulin resistance.

3.2.2 Peroxisome proliferator-activated receptor gamma 2 gene (PPARG)

Located on chromosome 3, it codes for the peroxisome proliferator-activated receptor. It has a key role in adipocyte differentiation. The presence of a type of polymorphism is associated with 1.25 odds ratio (OR) for DM2.

3.2.3 Protein tyrosine phosphatase receptor type D gene (PTPRD)

Located on chromosome 9, it is encoded for PTPRD. Its overexpression in the skeletal muscle generates insulin resistance. Diabetes-related polymorphism has been evidenced in Chinese with an OR 1.57.

3.2.4 β-3 adrenergic receptor gene

It regulates lipolysis of visceral fat and is related to thermogenesis. It is associated with risk of obesity and early presentation of DM2.

3.2.5 Adiponectin gene

It is located in chromosome 3q27. Low levels of adiponectin have a role in the pathogenesis of insulin resistance and obesity. Insulinosensitivity is a consistent and independent predictor factor of DM2. Variants in the genes that code for adiponectin receptor have proven to be a risk factor for presenting DM2 in some populations.

3.2.6 Leptin gene

Mutations related to this gene are involved with the pathogenesis of obesity and glucose metabolism, thereby decreasing insulin sensitivity and inhibiting the expression of the pre-proinsulin gene in the pancreatic β-cells. Recent evidence suggests that high circulating levels of leptin probably independent of adiposity are associated with an increased risk of type 2 diabetes in men.

3.3 Genetic defects in insulin secretion

There are multiple loci associated with this defect that have been found in GWAS studies. Among them most relevant are those presented in **Table 2** [9].

3.3.1 Calpain 10 gene (CAPN10)

Encodes a family of calpain enzymes, it was one of the first to study in linkage, but it is currently known that the risk of this association is low OR 1.17 [10].

Insulin secretion genes
• Calpain 10 gene (CAPN10)
• Transcription factor 7-like 2 (TCF7L2)
• Potassium voltage gated channel subfamily
• Q member 1 (KCNQ1)
• J member 11 (KCNJ11)

Table 2.
Candidate genes of DM2 for insulin secretion.

3.3.2 Transcription factor 7-like 2 gene (TCF7L2)

It has appeared to be more relevant in the genetic susceptibility to DM2, since a polymorphism of this gene has been found in several ethnic groups of DM2 patients. The increased expression of the gene in the pancreatic beta cell causes secretion alteration due to a decrease in the incretin effect. In liver and adipose tissue, it generates insulin resistance. The risk of DM is consistent with an OR up to 2.5 for homozygous variable [11].

3.3.3 Potassium voltage-gated channel subfamily

Q member 1 (KCNQ1) located on chromosome 11, it codes for the same name channel present in the cell membrane. There are four variants associated with DM2 in various populations. Studies suggest that the effect linked to DM2 is related to epigenetic modifications. J Member 11 (KCNJ11): code for Kir6.2 ATP-sensitive potassium channel. Variant E23K increases the risk of DM2 by 1.2 times associated with decreased insulin sequestration [12].

3.4 Epigenetics in DM2

Epigenetics or genetic modifications not associated to nucleotide mutations that influence the expression of a gene play a key role in the pathogenesis and T2DM complications. There are prenatal factors that induce epigenetic changes that increase the risk of T2DM by altering the secretion and sensitivity of insulin, hepatic glucose production, and the release of hormones involved in glucose metabolism.

The sustained activation of inflammatory-related genes in T2DM patients by epigenetic mechanisms contribute to the progression of vascular complications, arteriosclerosis, and retinopathy.

Types of epigenetic modifications and relation to DM2 are shown in **Figure 6**.

3.4.1 Methylation and histone-modification

These are the epigenetic modifications most associated to vascular complications related to DM2. Both hypomethylations and hypermethylations generate persistent activation of proaterogenic genes such as NF-kB-dependent oxidative and inflammatory signaling pathway.

3.4.2 Non-coding RNAs (ncRNAs) and chromatin remodeling

Non-coding nRNAs play an essential role in post-transcriptional regulation of gene expression.

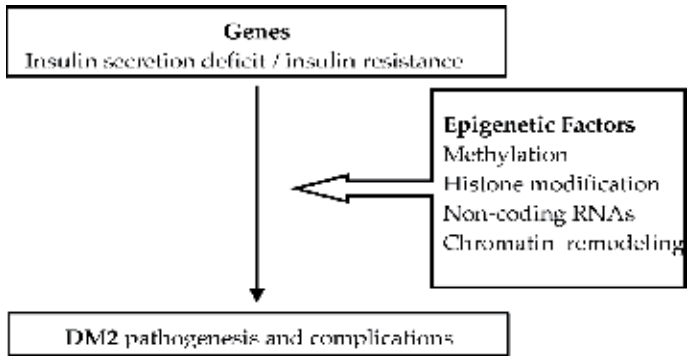


Figure 6.
DM2 epigenetic factors.

The most extensively studied are short nucleotide sequences (18–25) called MicroRNA (miRNA) and represent the principal epigenetic regulators of gene expression.

Deregulation in epithelial cells is correlated to the risk of developing vascular complications in DM2.

There are miRNAs associated with inflammation; for example, miR-155, which is associated to the progression of kidney disease in DM2 patients and miR-126, that when inhibited in pre-diabetes patients is correlated with the increase of activation of the NF-kb pathway in endothelial cells.

Long non-coding RNAs (lncRNAs) have been associated with pancreatic B cell damage, increase of inflammatory processes, alterations in the immune response, and insulin resistance in TSDM. Chromatin remodeling that regulates gene expression, such as p66Shc, has been linked with insulin resistance, increase of vascular risk in DM2, and obesity [13].

DM2 is a polygenic disease, of high heritability, which involves genes related to insulin and action, in addition to those that code for the components of the metabolic syndrome. What has been discovered so far is broad but only accounts for a part of the complex relationship of genetics and its phenotypic expression in DM2. The study of epigenetics in DM2 has opened the possibility to find pathogenetic markers at the onset of the disease and during the development of chronic complications, which will allow early screening and individualized treatment in the near future.

4. Monogenic diabetes

It is caused by one or more defects in a single gene. The disease can be inherited within a family by the genetic transmission of a dominant or recessive nature and not Mendelian. It can also be presented as a spontaneous case due to a de novo mutation [14]. Monogenic diabetes includes neonatal diabetes, maturity onset diabetes young (MODY), and mitochondrial diabetes (**Figure 7**).

4.1 Neonatal diabetes

It is defined as diabetes that appears before 6 months of age and is subdivided into transitory (TNDM) and permanent (PNDM). TNDM develops in the first weeks of life and resolves within a few months, but 50% have a relapse in adolescence or adulthood. TNDM is most frequently caused by abnormalities

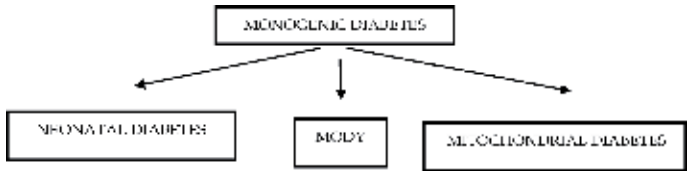


Figure 7.
Subtypes of monogenic diabetes.

in the imprinted region of chromosome 6q24 (spanning two candidate genes PLAGL1 and HYMAI), thereby leading to overexpression of paternally derived genes. Activating mutations in either the KCNJ11 or ABCC8 genes encoding the two subunits (Kir6.2 and SUR1, respectively) of the adenosine triphosphate-sensitive potassium channel on the beta-cell membrane prevent insulin secretion in response to hyperglycemia and can cause both PNDM and TNDM. Diabetes caused by mutations in KCNJ11 and ABCC8 often responds to sulfonylureas. Mutations in other genes critical to beta-cell function and regulation, and in the insulin gene itself, also cause PNDM. Heterozygous coding mutations in the pre-proinsulin gene (INS) are the second common cause of PNDM after K_{ATP} channel mutations [15, 16].

4.2 MODY

Described in 1975 by Tattersall and named in 1976 by Fajans, MODY is recognized as a form of mild-presenting family diabetes that is diagnosed during adolescence or early adulthood. Currently, other types are identified with a less classic presentation [4, 5]. It is estimated that its prevalence is underestimated, and it would correspond about 5% of DM2 and a similar percentage in DM1.

It is presented in 3.6% of the population with diabetes under 30 years [4]. MODY is a heterogeneous group of disorders caused by mutations in genes essential for beta-cell development, function and regulation, glucose sensing, and in the insulin gene itself. Although at least 14 genes are associated with MODY, we describe the four most frequent types (**Table 3**). Mutations in HNF-1α, HNF-4α, HNF-1β, and GCK genes account for over 80% of all known MODY cases.

Heterozygous mutations in three of them are responsible for the majority of cases of this type of diabetes: glucokinase gene (GCK); two genes encoding hepatocyte nuclear factor (HNF) transcription factors HNF-1α and HNF-4α. Most MODY-causative genes, except GCK, encode transcription factors expressed in pancreatic beta-cells (**Table 4**). The majority of patients with MODY exhibit isolated diabetes or stable mild fasting hyperglycemia, but some MODY subtypes have additional features, such as renal abnormalities (MODY 5) and pancreatic exocrine dysfunction (MODY 6) [17–20].

4.2.1 MODY 2 (GCK)

GCK, a glucose sensor expressed in pancreatic beta-cells, is a key enzyme in glucose metabolism that catalyzes the conversion of glucose to glucose-6-phosphate and thus controls glucose-mediated insulin secretion. As such, GCK serves to facilitate insulin release that is both appropriate and proportional to the blood glucose concentration. Heterozygous inactivating mutations in GCK (MODY 2) increase the set point for insulin secretion in response to increased blood sugar, thereby causing stable, mild fasting hyperglycemia. More than 600 mutations have been

	Gene	Frequency %	Physiopathology
MODY 2	GCK	15–20	Glucose sensing defect
MODY 3	HNF-1α	30–50	Insulin secretion deficit
MODY 1	HNF-4α	5	Insulin secretion deficit
MODY 5	HNF-1β	5	Beta cell dysfunction

GCK, glucokinase; HNF, hepatocyte nuclear factor.

Table 3.
Frequent types of MODY and its pathophysiological alteration.

MODY subtypes	Age at the onset of diabetes (years)	Hyperglycemia	Other clinical features	Possible treatment
1	17 (5–18)	High	Neonatal diabetes, neonatal hyperinsulinemic hypoglycemia, low triglycerides	Sensitive to sulphonylureas
2	10 (0–18)	Mild	Mild fasting long-term stability asymptomatic	Diet no medication
3	|4 (4–18)	High	Glycosuria—very low C-reactive protein of <0.5 mg/dl	Sensitive to sulphonylureas
5	<25	Variable	Renal malformations, genital anomalies, pancreatic hypoplasia, low birth weight	Insulin

Table 4.
Clinical characteristics of most common MODY subtypes.

reported. Patients with MODY 2 are usually asymptomatic, and they do not require treatment. In pregnancy, insulin may be required to prevent fetal complications, such as high birth weight and neonatal hypoglycemia. These neonatal complications are dependent on whether the mutation is inherited.

In 2018, a MODY family study was published in Chile [21]. The case is about a 17-year-old woman with DM, fasting blood glucose 130 mg/dl, without ketosis or weight loss, and BMI 18 kg/m^2. No signs of insulin resistance were seen, C-peptide 2.3 ng/ml (normal) and negative DM1 autoantibodies. In a family study, diabetic father and brother with impaired fasting blood glucose (**Figure 8**). The genetic-molecular analysis of the GKC gene, the patient, the father, and the brother presented a mutation at position 1343 of exon 10 corresponding to a heterozygous exchange of guanine for adenine (1343 G > A). The change is not synonymous and determines that at position 448 of the GKC enzyme, the amino acid glycine is substituted by aspartic acid. Diagnosis of MODY 2 was confirmed, and it was established that the mutation was by paternal line.

4.2.2 MODY 3 (HNF-1α)

The transcription factor HNF-1α is expressed in the liver, kidney, intestine, and pancreatic beta-cells. Heterozygous HNF-1α mutations result in progressive beta-cell dysfunction that leads to diabetes in early adult life.

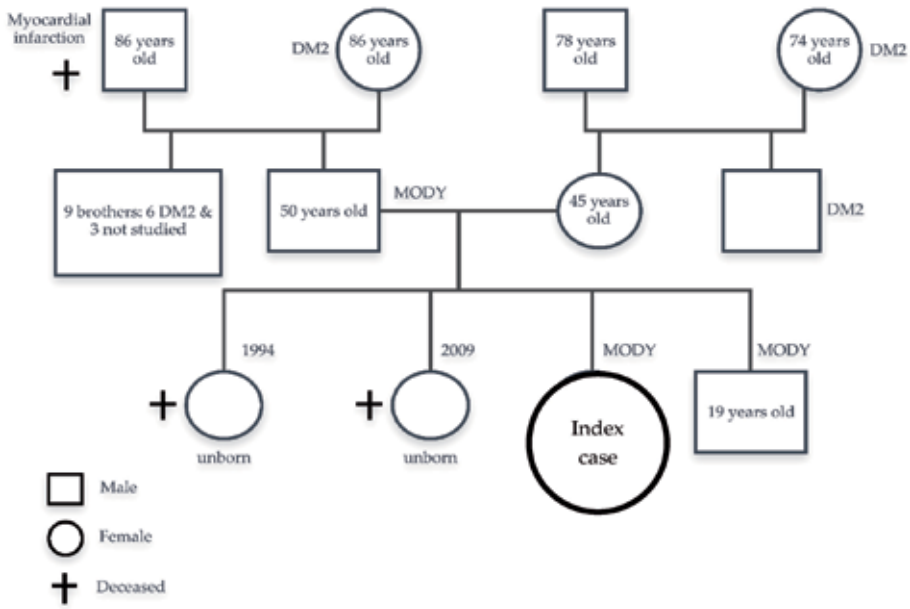


Figure 8.
Chilean family inheritance pattern MODY 2.

According to studies, a total of 414 different HNF-1α mutations were identified in 1200 families, where a mutation (P291fsinsC) in exon 4 was the most common. Hyperglycemia associated with MODY 3 may be severe, and the risk of microvascular and macrovascular complications is similar to DM1 and DM2. Because of this, patients require strict glycemic control and close monitoring of possible complications. There is a defect in the renal resorption of glucose, characterized by a decreased glucose threshold for glycosuria and reduced tubular reabsorption of glucose. Patients are sensitive to sulfonylurea therapy, but most of them eventually progress to insulin treatment. This subtype of MODY is the most frequent in Europe and the US.

4.2.3 MODY 1 (HNF-4α)

This MODY was the first described. The transcription factor HNF-4α is expressed in the liver, kidney, and pancreatic beta-cells. HNF-4α gene encodes a transcription factor important for pancreatic development and beta-cell differentiation and function. Heterozygous HNF-4α mutations cause a similar clinical phenotype observed in MODY 3. Most patients have a progressive insulin deficiency, diabetes onset before age 25 years, and a response to relative low-dose sulfonylurea therapy. Fetal HNF-4α heterozygosity results in macrosomia due to hyperinsulinemia in utero and subsequent neonatal hyperinsulinemic hypoglycemia, which is responsive to diazoxide. MODY 1 is associated with triglyceride metabolism, and mutation carriers may exhibit reduced levels of apoproteins (apoAII, apoCIII, and apoB).

4.2.4 MODY 5 (HNF-1β)

The transcription factor HNF-1β is involved in the organogenesis of the kidney, genitourinary tract, liver, lungs, gut, and pancreas. Patients with heterozygous

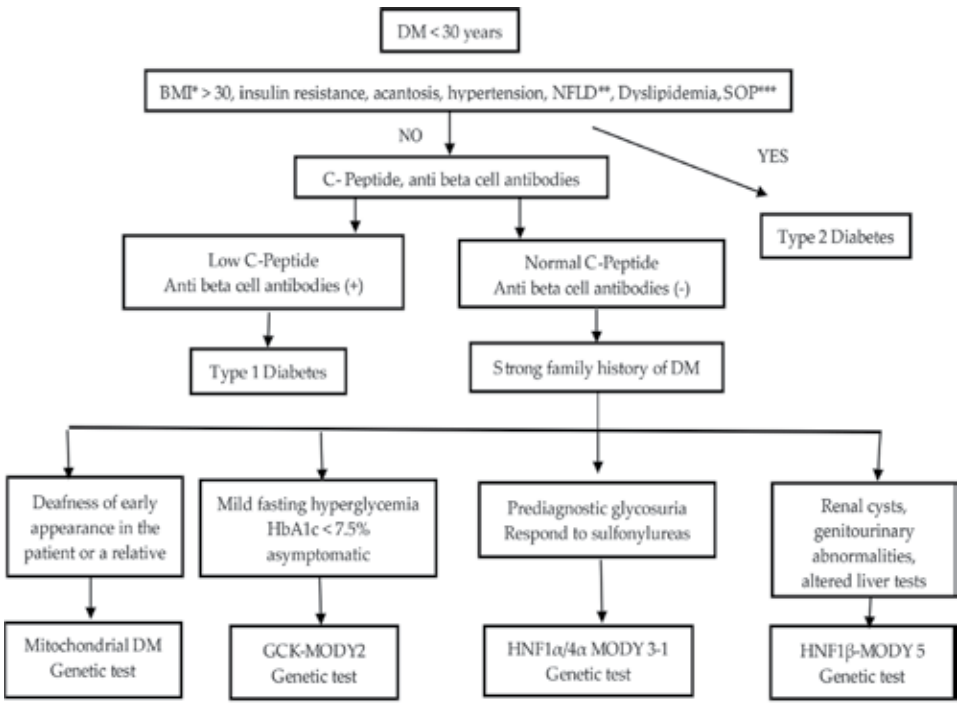
mutations in HNF-1β rarely present with isolated diabetes. By contrast, patients usually have renal disorders (especially renal cyst and renal dysplasia). Urogenital tract abnormalities and atrophy of pancreas may also occur. The sensitivity to sulphonylureas is absent, and early insulin therapy is required. At least 50% of HNF-1β MODY cases are due to microdeletion of chromosome 17 (17q12) involving between 15 and 29 genes, including HNF1β. De novo mutations are frequent (up to 50% of cases) and hence family may be absent.

4.3 Mitochondrial diabetes

This disease is a mitochondrial disorder characterized by maternally transmitted diabetes and sensorineural deafness. The most common form is caused by an exchange between an adenine for guanine (3243A/G) in DNA. This mutation also causes a severe neuromuscular disease syndrome called MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke). Diabetes onset is usually insidious, but 20% of patients have an acute presentation, even in diabetic ketoacidosis. It usually occurs in the third to fourth decades of life in non-obese individuals [14].

5. Diagnostic algorithm of diabetes in young adult

Figure 9 shows a diagnostic algorithm for diabetes in patients under 30 years of age.



*BMI = Body mass index (kg/m²), **NFLD = Non-alcoholic fatty liver disease, ***POS = polycystic ovary syndrome

Figure 9.
Diagnostic algorithm of diabetes in young adult.

6. LADA: Latent autoimmune diabetes adult

Diabetes is a complex disease, which makes its classification difficult. DM1 is caused by autoimmune destruction of the beta cell, which leads to absolute insulin deficiency. DM2 is secondary to the progressive loss of insulin secretion by the beta cell, in the context of insulin resistance. Other types of diabetes are gestational diabetes, MODY, post-transplant, and exocrine, among others [22]. DM1 is a heterogeneous disease, the incidence of which is higher in children and adolescents, characterized by the presence of specific immunological markers. However, no less than a percentage of adults experience the disease; so, the term latent adult diabetes (LADA) has been coined. In this case, the disease is even more heterogeneous, since there is a variable proportion of destruction of the beta cell, with the presence of immunological markers, but zero or deficient initial insulin requirements, so they can initially be misclassified as DM2 [23]. These patients probably have pathophysiological processes similar to DM1, but with differences in genetic penetrance and immune factors. The term LADA was introduced in the 90s, to define a subgroup of patients with diabetes initially not requiring insulin, but with immunological markers of DM1 detectable in the serum [24]. In 2015, the Immunology of Diabetes Society proposed three diagnostic criteria for LADA: age of onset>30 years, presence of any DM1 marker antibody, and lack of need for insulin treatment for at least 6 months from diagnosis [25].

Currently, the American Association of Diabetes (ADA) does not recognize this entity, but instead classifies it within the group of patients with DM1, but every day there is more information about its clinical and pathophysiological characteristics, which keep the debate open.

6.1 Epidemiology

The available data show that the prevalence of LADA is higher than previously recognized. About 40% of cases of DM1 occur in adults over 30 years [26]. Scandinavian studies show that 7.5–10% of the population with apparent DM2 have circulating antibodies against the beta cell (ICA or GAD 65) [27]. The Action LADA study, conducted in Europe, which evaluated 6000 adults attended in primary and secondary care centers, reported a frequency of 9.7% of LADA [28]. A Chinese study, LADA China study reported a 5.9% positive antibody in adults previously diagnosed with DM2 [29]. The prevalence of LADA is, therefore generally underestimated, due to the lack of study with antibodies in adult patients; so, the level of clinical suspicion should be high.

6.2 Pathophysiology

6.2.1 Genetics

In genotype analysis, patients with LADA have been shown to share genetic characteristics with DM1 (HLA, INS VNTR, CTLA4, and PTPN22) and DM2 (TCF7L2) [30], which might suggest that LADA is a spectrum of insulin deficiency between DM1 and DM2. The HLA-DRB1*04-DQB1*0302 and HLA-DRB1*0301DQB1*0201 haplotypes, which confer high susceptibility to DM1, and decrease progressively with increasing age, have been further diminished in elderly DM1 patients and have been described less frequently even in patients with LADA [31].

6.3 Autoimmunity

DM1 is a known autoimmune disease, mediated by cells. The presence of T lymphocytes that are reactive to islet cells, in LADA, gives us some evidence that there is a cell-mediated immune response as well [32]. Adult autoimmune diabetes has a "lower genetic load," characterized by lower circulating antibodies than early-onset DM1 in childhood or adolescence, which correlates with less intense beta cell destruction and lower HLA genetic susceptibility. Many studies have compared circulating antibodies in early DM1 in childhood with LADA, finding ICA, AAI, IA-2, and anti ZnT8 more frequently in children than in adults, while anti GAD and IA-2 were found with similar frequency in both ages [33]. Anti-GAD is the antibody most frequently found in patients with LADA, up to 90% positive, also being the most persistent over time. The beta cell function in early DM1 in childhood and adolescence is severely compromised since diagnosis, a difference in LADA in that the deficit is less severe. It has also been found that there is a correlation between age of diagnosis and fasting C-peptide levels, which is related to the latest age of LADA. To explain the later and less aggressive presentation compared to DM1, several theories are postulated, among others: intermittent crisis of autoimmune aggression (**Figure 10**) or greater capacity to regenerate beta cells and protection against the apoptotic process.

6.4 Clinical characteristics

Patients with LADA are a heterogeneous group, with antibody titers and body mass index (BMI). In general, the appearance of the condition is 35 years later, with cases described since the age of 25. Patients with LADA tend to have a BMI higher than DM1, but less than DM2. The existence of other autoimmune comorbidity or their family history is common, mainly thyroid disease. A higher frequency of anti-peroxidase antibodies (TPO) has been seen, in up to 27% of patients, compared to those with anti-GAD negative, which makes it necessary to monitor thyroid function and perform screening for other autoimmune diseases. The initial response to oral therapy is satisfactory, progressing in varying degrees to insulin requirements, from 6 months to several years, depending mainly on antibody titers (**Table 5**).

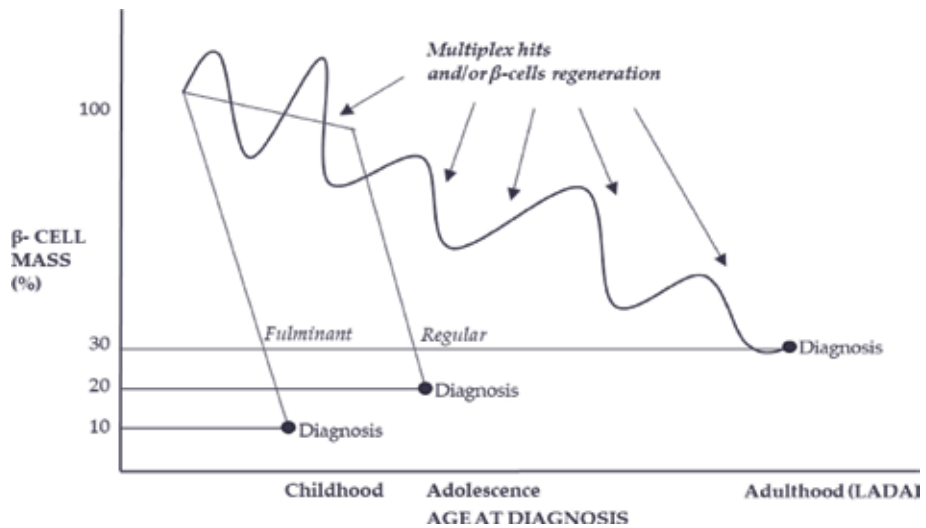


Figure 10.
The destruction of beta cell and the appearance of DM1 according to the age of onset and the putative pathogenetic mechanisms.

Clinical features	DM1	LADA	DM2
Age at diagnosis	Childhood and adolescence rare adulthood	>30 years	Adulthood, rare childhood, and adolescence
Start	Acute	Rare acute	Slow
Autoimmunity	Greatly increased	Increased	No modifications
Ketosis	Frequently	Rare	Rare
Insulin resistance	No modifications	Increased or without changes	Severely increased
Beta cell function	Very reduced	Reduced	Increased or unchanged
Insulin requirements	From the diagnostic	>6 months from the diagnostic	Years from the diagnostic
Body mass index	Low or normal	Normal or overweight	Overweight or obesity
MHC susceptibility	Severely increased	Increased	No changes

Adapted to [35].

Table 5.
LADA, DM1, and DM2 clinical features.

High titers of anti-GAD compared to low ones, have lower BMI, less endogenous insulin secretion, and faster progression to insulin-dependence. The presence of anti-GAD antibodies (or ICA) may be useful to identify patients with a previous diagnosis of DM2, who respond partially to treatment with oral antidiabetics and who quickly require insulin therapy. Regarding the metabolic profile, patients with LADA have advantages regarding DM2 with a better profile, that is, lower triglyceride levels, higher levels of HDL, lower BMI, and lower waist circumference. There are no specific guidelines for the treatment of patients with LADA. However, the metabolic goals are the same as for DM1 and DM2 patients, so you should try to achieve HbA1c <7%. The diet and exercise recommendations do not show differences with the classic presentations. Despite the extensive use of oral antidiabetics in DM2, especially metformin, there are no studies of this drug in patients with LADA. Glibenclamide and insulin were compared in LADA patients, finding that the group that used GBC had worse metabolic control and faster deterioration of CP secretion at a follow-up of 30 months. Therefore, the use of sulfonylureas as a first-line drug in this type of diabetes is not recommended. TZD combined with insulin show preservation of beta cell function in a small group of Chinese patients. The use of other agents such as insulin sensitizers could be used in combination with insulin in patients who share characteristics with DM2, that is, BMI >30 kg/m^2 and signs of insulin resistance. The role of the iDPP4 is not established. Patients with LADA treated with insulin glargine, the effect of adding sitagliptin or placebo was compared, the group with sitagliptin had a minimal decrease in C-peptide at one-year follow-up, compared to placebo. However, more studies support this evidence. We should keep in mind, like DM1 and DM2, patients.

Today, evidence indicates that early insulin therapy, along with changes in lifestyle, is the therapy of choice in patients with LADA when metabolic control is impaired primarily in young patients with elevated antibody titers since this treatment slows the deterioration of beta cell function, controls hyperglycemia, and prevents glucotoxicity [34]. TZD combined with insulin shows preservation of beta cell function in a small group of Chinese patients. The use of other agents such as insulin sensitizers could be used in combination with insulin in patients who share

characteristics with DM2, that is, BMI >30 kg/m^2 and signs of insulin resistance. The role of the iDPP4 is not established. Patients with LADA treated with insulin glargine, the effect of adding sitagliptin or placebo was compared, the group with sitagliptin had a minimal decrease in C-peptide at one-year follow-up, compared to placebo. However, more studies support this evidence. We should keep in mind, like DM1 and DM2, patients with LADA require a multidisciplinary approach to proper treatment.

6.5 Clinical features of DM1, LADA, and DM2

This Table summarizes major clinical features of DM1, LADA, and DM2.

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Chapter 5

Pathogenesis of Insulin Resistance

Gaffar S. Zaman

Abstract

Insulin resistance is interpreted as being a normal or raised insulin level giving rise to a biological reaction which is attenuated in effect; classically this cites to the weakened sensitivity to the disposal of insulin arbitrate glucose. *Compensatory hyperinsulinemia* eventuates when the secretion of the β cells of pancreas gets elevated to sustain the level of blood glucose in normal levels. The term *insulin resistance syndrome* is used to refer to a group of abnormalities and interconnected physical consequences that eventuate in long-standing insulin-resistant persons. Under standard situations of insulin reactivity, the response of insulin triggers the intake of glucose into the body cells, for utilization as energy, and impedes the utilization of fat for energy, as a result of which, the concentration of glucose circulating in the blood decreases. There are a number of risk factors for insulin resistance. Four major metabolic abnormalities characterize type 2 diabetes mellitus (T2DM): impaired insulin action, obesity, increased endogenous glucose output, and insulin secretory dysfunction. The evolution (and subsequent progression) of type 2 diabetes mellitus is delineated by a gradual deterioration of glucose tolerance over several years. Glucose tolerance testing, hyperinsulinemic euglycemic clamp, modified insulin suppression test, homeostatic model assessment (HOMA), and quantitative insulin sensitivity check index (QUICKI) method for insulin assessment are some of the methods by which insulin resistance can be measured. Moreover, longer-term effective researches as well are essential to preferably ascertain the significance of the glycemic index in the blood glucose regulation and to prevent the complications of diabetes, particularly in relations to insulin resistance risk factors. The possible role of insulin resistance in the glycemic index in depleting oxidative stress postprandially and related pro-inflammatory situations also merits further appraisal.

Keywords: insulin resistance, compensatory hyperinsulinemia, type 2 diabetes mellitus, obesity

1. Introduction

One of the most renowned hormones of our body is insulin which enables glucose to go inside the cells which additionally decreases blood glucose. The hormone insulin is secreted by the pancreas in response to glucose entering the bloodstream after a meal. Insulin resistance (IR) is contemplated as a pathological situation in which our body cells decline to react normally to insulin hormone [1]. To avert hyperglycemia and apparent damage to our body organs in the future [2], insulin production by the body starts when glucose enters into the bloodstream, predominantly from the dietary carbohydrate digestion and absorption. Under

standard situations of insulin reactivity, the response of insulin triggers the intake of glucose into the body cells, for utilization as energy, and impedes the utilization of fat for energy, as a result of which, the concentration of glucose circulating in the blood decreases, which results in glucose remaining within the normal range in case of consumption of a substantial amount of carbohydrates. Carbohydrates contains sugars, i.e., from only one glucose containing monosaccharides, such as fructose and glucose; two glucose containing disaccharides, like cane sugar; and many glucose containing polysaccharides (e.g., starches) and glycoprotein, glycolipids, etc. Fructose, ultimately metabolized into triglycerides inside the liver, stimulates the production of insulin and is seen to have a more impressive sequel than other carbohydrates. A customarily increased intake of carbohydrates, and specifically fructose, imparts to insulin resistance and has been connected to gain of weight and obesity [3–5]. If surplus blood glucose is not adequately transported into the cells even in the insulin's presence, the augmented level of blood glucose can elicit in the classic hyperglycemic triad of polydipsia (increased thirst), polyphagia (increased appetite), and polyuria (increased urination). Circumventing carbohydrates, a zero-carbohydrate diet or conditions of fasting can counteract insulin resistance [6, 7]. The first narration of insulin resistance is found historically in the 1960s, soon after the invention of radioimmunoassays helped in making serum insulin quantification possible and subsequently revealed that people having late-onset diabetes mellitus had high levels of insulin [8, 9]. Drs. Yalow and Berson [8, 9] defined insulin resistance as "a state in which a greater than normal amount of insulin is required to elicit a quantitatively normal response." The next milestone discovery in this context was the detection and ascertaining of the insulin receptor and also the discovery that insulin resistance led to hyperinsulinemia and the fact that this was interconnected very much with atypical/unconventional binding of insulin hormone with its receptor in various rodent models [10, 11]. The succeeding milestone discovery in the history of insulin resistance came with the pioneering recognition of the receptor for insulin and the observation that high insulin in blood, secondary to the insulin resistance, was interconnected with atypical binding of insulin hormone to its receptor [10, 11]. It was not before the year 1976 when the evidence came that defects in receptor for insulin could be interlinked with resistance to the insulin hormone in humans, provided genetic translational verification for the significance of insulin resistance [10]. Researchers like Kahn et al. [10] delineated two syndromes that were distinguished by virilization, acanthosis nigricans, hirsutism, anovulation, acne, and flawed binding of insulin on the insulin receptor of lymphocytes circulating in the blood. This initial syndrome was designated as type A insulin resistance when it took place without the existence of anti-insulin antibodies and the corresponding accountability of the insulin receptor was the main factor, and in contrast it was designated as the type B when it occurred with the clinical characteristics of various autoimmune ailments and it always occurred when neutralizing anti-insulin antibodies were present [10]. After this specification of type A and type B syndrome, all rare and extreme levels of insulin resistance like acanthosis nigricans syndrome, hyperandrogenism, the Rabson-Mendenhall syndrome, lipodystrophy, and leprechaunism were discovered [12, 13]. To defend the idea by Himsworth that some cases of diabetes were not secondary to absolute insulin deficiency, it was imperative to prove the truth that insulin circulating in the blood was present in the insensitive form in those patients as classified by Himsworth. This was put to rest with the publication by Yalow and Berson in 1960 of an immunoassay research of endogenous blood insulin in humans. During this novel innovative work, Berson and Yalow delineated an excellent immunological technique for measuring the amount of insulin that integrated the degree of specificity with sensitivity required to take the measurement of even the smallest

concentrations of insulin contained in the body circulation. Utilizing this novel technique to categorize immunoreactive insulin amounts present in plasma in normal people to those particular patients having maturity-onset type diabetes, it was conceived that the amounts of insulin were on the average elevated in the patients with diabetes. Putting stress on the rationale of these particular outcomes, both of them summarized that the tissues of a person with maturity-onset diabetes do not have a good response to the level of insulin; on the contrary, the tissues of a nondiabetic person respond very well to his level of insulin. In the words or terminology of Himsworth, patients with this character in diabetes can be considered as "insulin insensitive." In spite of the fact these results of the novel publication by Yalow and Berson were afterwards authenticated by various research groups, it became evident that the interconnection between insulin concentrations and plasma glucose in patients having type 2 diabetes was not such a simple one. To be precise, in a person with comparatively slight increments of fasting plasma glucose concentration, the responses of plasma insulin to oral glucose were equivalent or prominently higher than the normal but with elevated proportions of glucose intolerance and with the appearance of noteworthy hyperglycemia during fasting [14–17]. It was obligatory to evolve an exploratory perspective that would quantify in an unequivocal way the capability of an individual person to get rid of fixed glucose load under the influence of identical insulin stimuli during steady-state conditions [18].

2. Risk factors for insulin resistance

A number of risk factors are found for insulin resistance, together with being overweight or obese or pursuing a sedentary lifestyle [19]. Numerous genetic constituents can elevate the chances for the same, and there are some particular medical circumstances correlated with insulin resistance [19].

The National Institute of Diabetes and Digestive and Kidney Diseases has specified several hazard factors:

1. Age of 45 or more.

2. Native Alaskan, Asian American, American Indian, African American, Native Hawaiian, or Latino/Hispanic ethnicity.

3. Having abnormal health states such as increased systolic/diastolic pressure and increased levels of cholesterol.

4. Having gestational diabetes history.

5. Having a history stroke or heart disease [19].

6. In addition, some medications and other health conditions can raise the risk [19].

2.1 Types of people more likely to develop insulin resistance

Individuals who have hereditary factors or lifestyle-related factors are bound to have in their later life insulin resistance or prediabetes [20]. Hazard factors incorporate:

- Overweight or obesity.

- Age 45 or more.

- Having a parent, sibling, or sister with diabetes.
- African American, Alaskan Native, American Indian, Asian American, Hispanic/Latino, Native Hawaiian, or Pacific Islander American ethnicity.
- Physical idleness.
- Health conditions, for example, hypertension and high cholesterol levels.
- A history of gestational diabetes.
- A history of coronary illness or stroke.
- Polycystic ovary disorder, also known as PCOS.
- Individuals who have metabolic disorder—hypertension, irregular cholesterol levels, and enormous waist size—are bound to have prediabetes.
- Hormonal imbalances, for example, Cushing's disorder and acromegaly.
- Sleep issues, particularly rest apnea.

In spite of the fact that you cannot change hazard factors, for example, family ancestry, age, or ethnicity, you can change lifestyle factors such as eating, physical activity, and weight. These ways of life changes can bring down your odds of creating insulin resistance or prediabetes [21].

There are a number of other hazard factors that are firmly connected to insulin resistance; however, these factors are yet to give clear answers about how much these variables might be a reason for the same.

The variables include:

- Abundance of fat
- Having hypertension or hypercholesterolemia
- Having a nearby relative with type 2 diabetes
- History of gestational diabetes
- Having potential to develop type 2 diabetes

2.2 Diet

The food that we take are regularly seen as a conspicuous reason for diabetes and frequently supposed as a reason.

Many studies have shown that our diet can have an effect in type 2 diabetes; however, it is one factor among numerous others, and speculations ought not be drawn without the thought of other contributing variables.

2.3 Contributions of genetics

Research has revealed various factors, which are related with an elevated danger of diabetes. There are various elements which can impact our plasma glucose, as

well as taking into consideration where we disperse fat in our body and how efficiently our muscles allow glucose to enter from the blood.

It is a well-known fact that genes help control and regulate every metabolic activity in the body, and mutations, in genes, which have influence in the digestion and absorption process can cause problem with controlling blood glucose level. To date scientists have distinguished more than 60 genes related with type 2 diabetes mellitus (T2DM).

2.4 Medication

Corticosteroids treat mainly inflammatory disorders, as rheumatoid joint inflammation, lupus, and hypersensitivities. Normal steroids incorporate hydrocortisone and prednisone. Be that as it may, steroid creams (for a rash) or inhalers (for asthma) aren't an issue.

Medications that treat nervousness, attention deficit hyperactivity disorder (ADHD), sorrow, and other emotional well-being issues can incorporate clozapine, olanzapine, risperidone, and quetiapine:

- Contraception pills
- Medications that treat hypertension, for example, beta-blockers and thiazide diuretics
- Statins to bring down cholesterol
- Adrenaline for serious hypersensitive responses
- High doses of asthma medications or medications that you infuse for asthma treatment
- Isotretinoin for skin breakout
- Tacrolimus, which you get after an organ transplant
- A few meds that treat *human immunodeficiency virus* (HIV) and hepatitis
- Pseudoephedrine, a decongestant in some cold and influenza prescriptions
- Niacin or vitamin B3

Alongside these hazard factors, different things that may add to insulin resistance incorporate:

- Antihypertensive agents such as β-blockers, diuretics, oral contraceptives, corticosteroids, nicotinic acid, and antipsychotic agents are said to increase insulin resistance [22, 23]; in addition, many anti-retroviral protease inhibitors utilized to treat human immunodeficiency virus infection also cause insulin resistance. The mechanisms of actions vary: β-blockers impede the secretion of insulin from the pancreas by blocking the β-adrenoceptors, depletion of the blood levels of potassium is the main action of thiazide diuretics, counter-regulatory hormonal activity is the main action of oral contraceptives and corticosteroids, and loss of peripheral subcutaneous fat with partial

lipodystrophy (with resultant accumulation of truncal adipose tissue) is the main action of HIV-1 protease inhibitors. All these ultimately lead to insulin resistance [24].

2.5 Lifestyle factors

Many hormones can instigate insulin resistance, prominent among them being human placental lactogen, growth hormone, and cortisol [25]. Counteraction of insulin is done by cortisol and can cause elevated hepatic gluconeogenesis, decreasing peripheral use of glucose and elevating insulin resistance [26]. Cortisol does this by diminishing the translocation of glucose transporters (especially GLUT4) to the respective cell membrane [27, 28]. This is based on the noteworthy augmentation in the sensitivity of insulin in humans after doing bariatric surgery and surgical removal of the duodenum in rats [29]; it has been speculated that some substance is manufactured in the mucosa of duodenum, which gives a signal to the body cells to become insulin resistant. With removal of the producing tissue, cessation of the signal occurs, and reverting back of the body cells to normal insulin sensitivity is seen. No such particular substance has been discovered as yet, and the certainty of such a substance remains speculative. Leptin is a hormone derived from the adipocytes and ob gene [30] whose role is the regulation of hunger by forewarning the body when it is full [31]. Researches have depicted that dearth of leptin leads to severe obesity and is intensely associated with insulin resistance [32].

3. Pathogenesis

Four major metabolic abnormalities characterize type 2 diabetes mellitus: impaired insulin action, obesity, increased endogenous glucose output, and insulin secretory dysfunction [33–35]. In spite of the fact that there is considerable authentication that three of these idiosyncrasies exist in most people before the commencement of diabetes, the concatenation with which they evolve and their corresponding contributions to the advancement from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT), and ultimately to type 2 diabetes [36–38], cannot be confirmed for sure even though some detailed longitudinal studies have provided some information [39–44]. Contemporaneous comprehension of the pathogenesis of type 2 diabetes is established on a wide-reaching number of cross-sectional [45–57] and prospective [58–72] studies. The evolution (and subsequent progress) of type 2 diabetes mellitus is delineated by a gradual degeneration of glucose tolerance over several years [33–38]. Prospective and cross-sectional data demonstrate that defects in insulin secretion, body weight gain, insulin action, and an elevation in endogenous glucose output are cardinal in this decline [45–56]. The pathogenetic history of diabetes—the corporeal chain of events with which these metabolic aberrations evolve in relation to one another throughout the various stages of the illness—remains unrevealed. Many authors have suggested that a flaw in insulin activity is the principal aberration in the premature stages of the evolution of type 2 diabetes and that secretory dysfunction of insulin takes place only at a later stage.

3.1 Molecular mechanism of insulin resistance in the muscle

Cellular contents of lipids inside myocytes (of muscles) are referred to as intramyocellular lipid (IMCL) and basically reflect intramyocellular triglyceride content. Although IMCL emphatically relates with muscle insulin resistance in

inactive people, triglycerides themselves have been disassociated from insulin resistance, recommending that other lipids (e.g., diacylglycerols, ceramides, and so on) intervene insulin resistance [73]. Various investigations have depicted the interrelationship insulin resistance in muscles and between diacylglycerol (DAG) content. Insulin-animated tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) and IRS-1-related phospho-inositide 3-kinase (PI3K) actuation were intensely debilitated in skeletal muscle of lipid-injected people [74] and rodents [75, 76]. In rodents, lipids and high-fat intake bring about transient increments in muscle DAG content [75], bringing about continued appearance of protein kinase C-theta (PKCθ) that constrained phosphorylation of IRS-1 by insulin receptor substrate 1 (IRTK). Lipid mixtures in normal human volunteers correspondingly elevated skeletal muscle DAG [77, 78] and caused muscle insulin resistance. The improvement of muscle insulin resistance can prompt metabolic ailment. This has been seen in hereditary mouse models of particular muscle insulin resistance [79], which are inclined to hepatic steatosis [75] and increased adiposity [77]. In young, lean, and people with skeletal muscle insulin resistance, ingested glucose is not taken up by muscle and gets occupied to the liver, where it becomes substrate for liver once more by lipogenesis, increasing liver triglyceride; furthermore, plasma triglyceride increase results in decreasing plasma high-density lipoprotein (HDL) levels [80]. Nonalcoholic fatty liver disease (NAFLD) is unequivocally connected with hepatic insulin resistance. In patients with lipodystrophy, ectopic lipid accumulation in the liver and skeletal muscle was related with extreme hepatic and muscle insulin resistance [81]. Leptin treatment diminished the consumption of calorie, settled hepatic steatosis, and improved insulin activity [82]. Lipodystrophic mice have a comparable phenotype, and fat transplantation protected these mice by permitting redistribution of lipids from ectopic destinations to transplanted fat tissue and standardization of insulin activity [83]. Mice overexpressing lipoprotein lipase in the liver cause hepatic steatosis and liver-explicit insulin resistance [84]. In rodents and mice, high-fat eating regimens lead to hepatic steatosis and hepatic insulin resistance. In this way, these considerations show that ectopic lipid in the liver is explicitly related with hepatic insulin resistance. Magkos et al. showed that hepatic DAG content (not hepatic ceramide content) was the best indicator of hepatic insulin resistance in obese people [85]. There are a few conditions wherein hepatic steatosis shows up disassociated from hepatic insulin resistance. A typical single-nucleotide polymorphism (rs738409, I48M) in the lipid bead protein-like phospholipase domain-containing protein 3 [patatin-like phospholipase domain-containing A3 gene (PNPLA3), likewise called adiponutrin (ADPN)] has been related with expanded hepatic steatosis, but not insulin resistance [86–88]. Along these lines, occasions of obvious disassociation of hepatic steatosis and hepatic insulin opposition might be clarified by a superior comprehension of the subcellular conveyance of DAG [89]. Ceramides are additionally bioactive lipid particles that are embroiled in the advancement of insulin resistance. Increments in hepatic and muscle ceramide content have been related with insulin resistance in rodents, and inhibitors of ceramide blend can forestall insulin resistance [90, 91]. In any case, a disassociation between ceramide substance and tissue insulin resistance has been revealed in numerous investigations [85, 92, 93], and the fundamental component connecting ceramides to insulin resistance has not been completely finalized. As of late, some investigations analyzed how a particular ceramide species, C16:0, resists mitochondrial oxidation, permitting triglyceride to accumulate and cause insulin resistance [94, 95]. Despite the fact it has not been concluded, it is conceivable that the equal relationship between C16:0 ceramide and mitochondrial oxidation could additionally cause increments DAG and impede insulin action.

3.2 Insulin resistance in the adipose tissue

Some of the actions of insulin on adipose tissue are (1) stimulation of uptake of glucose and biosynthesis of triglyceride and (2) suppression of triglyceride hydrolysis-cum release of free fatty acids (FFA) and glycerol into the blood [96, 97]. It has been seen that adipose tissue insulin resistance (Adipo-IR), which means diminished suppression of lipolysis when high insulin levels are present, is interlinked with glucose intolerance, and increased plasma FFA amounts have also shown to diminish insulin signaling in muscles, endorse gluconeogenesis in the liver, and diminish glucose-activated insulin response [97–103]. In spite of the fact that the natural history and role of β-cell abnormality (or impairment) and insulin resistance in muscle are firmly established in the evolution of T2DM, the influence of Adipo-IR in the progression from normal glucose tolerance (NGT) to type 2 diabetes mellitus (T2DM) is still not clear. It is possible to quantitate palmitate turnover by the utilization of tracers [104–106] which can also provide the release rate of glycerol [107, 108] to furnish a lipolysis index. Tracer turnover (i.e., labeled palmitate or glycerol) or FFA suppression during insulin infusion (euglycemic-hyperinsulinemic clamp) or oral glucose tolerance test (OGTT) has led to the development of a number of indices of Adipo-IR [109]. Gastaldelli et al. [104] *confirmed that* fasting Adipo-IR index can be considered as a reliable index of insulin resistance in the fat cell when considered over the entire spectrum from NGT to T2DM. There has been consistent demonstration of weakened suppression of plasma FFA and also glycerol and 14C-palmitate turnover with the stepped hyperinsulinemic clamp [106, 110, 111] (i.e., adipocyte insulin resistance) in persons having T2DM. Therefore a decline in insulin secretion/insulin resistance (disposition) index has been seen with progression from lean NGT to obese NGT to IGT [112]. A decline in the secretion from β-cell of insulin is also interlinked with an elevation in the fasting Adipo-IR. Therefore, it can be said that the fasting adipocyte insulin resistance index (fasting FFA × fasting insulin) increases in a forward-looking, innovative manner over the stretch of glucose tolerance, extending from NGT to T2DM, and furnishes a reliable index of fat cell sensitivity to the actions of insulin [113–115]. In contradiction, there is increment of adipocyte insulin resistance index during OGTT (from NGT to IGT) and decrease with advancement of IGT to T2DM; this is due to gradual deficiency of the secretion of insulin in this group having diabetes. In conclusion, the gradual decrease in β-cell function that progresses from NGT is interlinked with a gradual elevation in fasting Adipo-IR and FFA [104].

Fat insulin resistance is the failure of insulin to activate fat glucose transport, advance lipid take-up, and diminish lipolysis. While diminished fat glucose take-up is exhibited in both in vivo and in vitro models, metabolic effect of hindered insulin-intervened glucose take-up in fat tissue is not well explained. For instance, the loss of fat GLUT4 in mice does not modify adiposity or, on the other hand, how weight gain prompts insulin resistance in skeletal muscle and liver [116]. Glucose transport into fat cells initiates starch reaction component restricting protein (ChREBP), which may affect fat lipid digestion [117]. Adipocytes discharge explicit unsaturated fats that are related with increased insulin affectability, as palmitoleate [118, 119] or monomethyl chain unsaturated fats [120].

4. Methods for diagnosis

4.1 Fasting insulin levels

A fasting serum insulin amount of more than 25 mU/L or in other sense 174 pmol/L designates insulin resistance. The same amounts pertain to 3 hours after taking the last meal [121].

4.2 Glucose tolerance testing

While performing a glucose tolerance test (GTT), a patient who is fasting is given a 75 g of glucose orally. Then plasma glucose levels are continuously monitored (along with urine glucose) for a period of 2 hours.

The elucidation of the test is established on the guidelines of the World Health Organization (WHO). After a period of 2 hours, a plasma glucose amount of less than 7.8 mmol/L (140 mg/dL) is regarded as normal, and a plasma glucose amount of between 7.8 and 11.0 mmol/L (140 to 197 mg/dL) is regarded as impaired glucose tolerance (IGT), and a plasma glucose amount of greater than or equal to 11.1 mmol/L (200 mg/dL) is regarded as diabetes mellitus. Extension of the testing (for several more hours) may reveal a hypoglycemic "dip" that is a result of an overshoot in insulin production after the failure of the physiologic postprandial insulin response.

4.3 Using the hyperinsulinemic euglycemic clamp

"Hyperinsulinemic euglycemic clamp" is also known as the gold standard for investigating and quantifying insulin resistance. It is so-called because it calculates the level of glucose obligatory to reimburse for an elevated insulin level without giving rise to hypoglycemia [122]. It is a kind of glucose clamping technique. The test is seldom carried out in clinical settings but is utilized in medical research [123]. The process takes around 2 hours. Insulin is infused through a peripheral vein. The rate is of infusion is 10–120 mU per m^2/minute. With the intention to recompense for the infusion of insulin, 20% glucose is infused to sustain blood glucose levels between 5 and 5.5 mmol/L. The blood sugar levels every 5 to 10 minutes, to determine the rate of infusion of glucose [123]. The determination of insulin sensitivity is made by the rate of glucose infusion in the last 30 minutes of the test. If greater levels (7.5 mg/min or greater) are required, the patient is considered as insulin sensitive. Low levels such as 4.0 mg/min or lower than that designates that the body is resistant to actions of insulin. Levels between 4.0 and 7.5 mg/min are not conclusive and indicate "impaired glucose tolerance," which is a premature gesticulation of insulin resistance [123, 127]. This basic method may be modified significantly by the utilization of glucose tracers.

4.4 Modified insulin suppression test

Gerald Reaven developed the modified insulin suppression test at Stanford University. The test corresponds well with the euglycemic clamp, with minute operator-dependent error. Particularly, this test has been utilized in research correlating to the metabolic syndrome [123]. A 25 μg of octreotide (Sandostatin) is given to the patient in 5 mL of normal saline over a period of 3–5 minutes through intravenous infusion (IV) as a primary bolus. Subsequently, the patient is continuously infused with an IV infusion of somatostatin (0.27 μg/m^2/min) to repress internal glucose and insulin secretion. Next, 20% glucose and insulin are infused at varying rates of 32 and 267 mg/m^2/min. Plasma glucose is monitored at 0, 30, 60, 90 minutes, and lastly at 120 minutes and subsequently after each 10 minutes for the final 30 minutes of the study. The averages of these final four values are utilized to ascertain the steady-state plasma glucose level (SSPG). People having an SSPG greater than 150 mg/dL are contemplated to have insulin resistance [45].

4.5 Homeostatic model assessment (HOMA)

By this method it is possible to quantify insulin resistance. Also, pancreatic beta-cell function can be possibly elucidated:

$$\text{HOMA-IR} = \frac{\text{Glucose} \times \text{Insulin}}{22.5} \tag{1}$$

and

$$\text{HOMA-}\beta = \frac{20 \times \text{Insulin}}{\text{Glucose} - 3.5\%} \tag{2}$$

where glucose is in mmol/L.
Also,

$$\text{HOMA-IR} = \frac{\text{Glucose} \times \text{Insulin}\,\%}{405} \tag{3}$$

and

$$\text{HOMA-}\beta = \frac{360 \times \text{Insulin}\,\%}{\text{Glucose} - 63} \tag{4}$$

where glucose is in mg/dL.

Note: insulin is taken in μU/mL; both glucose and insulin are taken during fasting; IR means insulin resistance; HOMA-β is the percentage of beta cell function [124–128].

4.6 Quantitative insulin sensitivity check index (QUICKI) method for insulin assessment

QUICKI is obtained utilizing the inverse of the addition of the logarithms of the fasting insulin and fasting glucose:

$$1/[\log(\textit{fasting insulin}\ \mu U/mL) + \log(\textit{fasting glucose}\ mg/dL)] \tag{5}$$

The QUICKI method corresponds well with glucose clamp researches (r = 0.78) and is very good for the measurement of insulin sensitivity (IS), which is derived by utilizing the reciprocal quantity of insulin resistance (IR).

5. Conclusions

From the time insulin resistance was discovered, the cellular and molecular mechanisms were the considerations for which drugs were tried for diabetes mellitus. Considering the cellular mechanisms of insulin resistance which are mostly concerned with plasma cell membrane glycoprotein-1 (PC-1), also termed as ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), the mechanism is really complex. The full understanding of the cellular mechanisms will permit the development of novel targets for various treatment modalities. From the therapeutic point of view, we need to have a clear knowledge about the cellular mechanism of insulin resistance in order to treat and also to prevent the occurrence of diabetes from prediabetic stage. From recent studies, it is evident that insulin resistance can be stopped or reversed if the pathophysiology is clear. It is the necessity to implement a huge global strategic plan for identifying and preventing/treatment of insulin resistance in the prediabetic stage.

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Section 4

Metabolic Syndrome

Chapter 6

Metabolic Syndrome

Armindo Miguel de Jesus Sousa de Araújo Ribeiro

Abstract

Metabolic syndrome (MS) is recognized by a set of cardiovascular risk factors (CVRF) that usually coincide with insulin resistance and hyperglycemia. These risk factors include hyperglycemia (fasting glucose > 100 mg/dl), high blood pressure (SAD ≥ 130 mmHg and TAD ≥ 85 mmHg), triglyceride increase (≥150 mg/dl), decreased HDL levels <40 mg/dl, and central obesity (waist circumference ≥102 cm in men and ≥88 cm in women). The purpose of this chapter is to review the natural history of metabolic syndrome and epidemiology and to review risk factors for the appearance of metabolic syndrome, pathophysiology, and biochemistry among the various cardiovascular risk factors and their importance within the metabolic syndrome.

Keywords: diabetes, hypertension, dyslipidemia

1. Introduction

The purpose of this chapter is to provide a review of the pathophysiology of metabolic syndrome (MS) and the relationship between its different components. Because this is a very broad subject, not all aspects will be discussed. Throughout the chapter, a bibliographic reference is left for each topic so that this can be looked into more deeply. This is a non-systematic review of the literature through research on PubMed and Google.

At an epidemiologic study conducted by Armindo Sousa Ribeiro et al, in a Portuguese population, was found that 23.8% of the population has MS more prevalent in 24 males, no smoking, no significant alcohol consumption, sedentary life style, with a high body mass index (BMI) and its prevalence increases with age [1]. Each CVRF has different importance in the metabolic syndrome [2].

In Western societies, cardiovascular diseases (CVD) are the main cause of mortality and morbidity for both sexes. This fact entails very high social and economic costs [3].

CVDs are the biggest cause of mortality worldwide. In the 2010 Global Burden of Disease Study, CVD is estimated to cause 15.6 million deaths per year worldwide, corresponding to 29.6% of all deaths [4]. Although the tendency is to reduce the number of deaths in Europe due to CVD, this remains the main cause of death, corresponding to more than 4 million deaths per year, that is to say 45% of all deaths recorded in Europe [4]. Of the CVD, coronary heart disease remains the most important cause of death in both genders, which corresponds to 19% in the male sex and 20% in the female sex.

2. Metabolic syndrome and cardiovascular risk factors (CVRF)

2.1 Definition

There are several definitions of MS. MS groups a constellation of pathophysiological factors that increase the risk of developing diabetes mellitus (DM) type 2 and CVD [5–10]. At present, attempts have been made to unify criteria to have a consensus on its diagnosis [11].

The World Health Organization (WHO), International Diabetes Federation (IDF), National Cholesterol Education Program Adult Treatment Panel III

	ATP III	WHO	AACE	IDF
Triglycerides ≥ 150 mg / dl	X	X	X	X
HDL <40 mg / dl in men and <50 mg / dl in women	X	X	X	X
Blood pressure ≥ 130/85 mmHg	X	X	X	X
Insulin Resistance (IR)		X		
Fasting glucose >100 mg / dl	X		X	X
Glucose 2h: 140 mg / dl			X	
Abdominal Obesity	X			X
High body mass index		X	X	
Microalbuminuria		X		
Risk factors and or diagnosis	3 plus IR	More than 2	Clinical Criteria	Abdominal Obesity

Figure 1.
Components of the metabolic syndrome considering its definition: according to the National Cholesterol Education Program Adult Treatment Panel III (ATP III), World Health Organization (WHO), American Association of Clinical Endocrinologists (AACE), and International Diabetes Federation (IDF).

(NCEP-ATP III), and the American Association of Clinical Endocrinologists (AACE) have proposed their diagnostic criteria or components of MS (**Figure 1**) [11].

In 2009, representatives of the IDF and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI)-ATP III Guidelines discussed resolving differences between definitions of MS, unifying criteria [6, 11]. The definition of MS according to the unification of criteria (harmonizing the metabolic syndrome) is used for many international works and publications and requires the presence of three of the following five criteria:

1. Elevation of fasting blood glucose (≥100 mg/dl) or receiving antidiabetic treatment with insulin or oral antidiabetics

2. Elevation of systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (PAD) ≥ 85 mmHg or receiving antihypertensive drug treatment

3. High-density lipoprotein (HDL) cholesterol values <40 mg/dl (men) or <50 mg/dl (women)

4. Triglycerides ≥150 mg/dl or under treatment with specific lipid lowering agents

5. Abdominal perimeter ≥102 cm (men) or ≥88 cm (women) [6, 12]

The concept of MS was carried out in a progressive manner. Its clinical importance is enormous due to the fact that it can identify patients with high risk of suffering some CVD and/or DM, allowing a preventive intervention [6, 13, 14].

The notion that CVRF has a tendency to aggregation allows an earlier diagnosis of MS, thus also leading to the early introduction of therapeutic, pharmacological, or non-pharmacological measures.

2.2 Historical evolution

The interest in the MS is not something recent. Its nomenclature has evolved over the years.

About 260 years ago, before the description of MS, the Italian anatomist and doctor, Morgagni, identified the association between visceral obesity, arterial hypertension (AHT), atherosclerosis, hyperuricemia, and frequent episodes of sleep apnea. In the mid-twentieth century, French physicist Vague first identified the relationship between android obesity and the increased prevalence of DM and CVD. In the 1960s Avogaro and Crepaldi attributed the name "plurimetabolic syndrome" to the simultaneous presence of obesity, AHT, and DM. They verified that all these abnormalities were frequent among the population and contributed to an increase in cardiovascular risk [15]. A decade later, Haller related this set of risk factors with atherosclerosis [15].

In 1980, Vague suggested that adipose tissue alone had little effect on the onset of DM in obese individuals. It is currently known that central or android obesity is a predisposing factor for DM and atherosclerosis and affects insulin secretion unlike gynoid obesity [15].

In 1988, Reaven described this phenomenon as syndrome X, the set of hyperglycemia and insulin resistance, obesity, dyslipidemia, and hypertension. Reaven also suggested that insulin resistance and the consequent increase in blood insulin levels were the central pathophysiological features in syndrome X which in itself was a risk factor for CVD [15, 16].

In 1991, Ferrannini and his colleagues referred to this as insulin resistance syndrome [15, 17].

In 1993, Van Gaal attributed for the first time the name MS to all comorbidities associated with visceral obesity and currently this nomenclature is being used [15].

The concept of MS has evolved significantly in the last decade, which resulted in the presentation of multiple clinical definitions by different scientific societies. In general terms as it was widely treated, MS is defined as an aggregation of several CVRF in the same individual. The nuclear elements for the classification and diagnosis of MS are basically obesity, abnormal glycemic metabolism, iatrogenic dyslipidemia, and hypertension.

The metabolic syndrome was defined by the World Health Organization (WHO) in 1998 [13, 15]. In 2001, there was a new revision of the definition through the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III), where glycemia is not considered an essential factor; in this way it only becomes one of the diagnostic components of MS [18].

With the verification of evidence of the relationship between central obesity and cardiovascular risk, there was a tendency to value this diagnostic component for MS more. Thus, in 2004, the International Diabetes Federation (IDF) created a new definition of MS, where central obesity, defined by the value of the abdominal circumference, would be essential for diagnosis [7, 19]. With the adoption of this definition, an increase in the prevalence of MS was observed in a large part of the populations studied, particularly in the elderly [20].

In 2005, a new review of the criteria by the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) maintained the criteria of the NCEP-ATP III [21]. The justification was the fact that in that criterion a single etiology for MS does not stand out and is of simpler application. They modified only the cutoff point of fasting blood glucose from 110 to 100 mg/dl, an adjustment promoted by the American Diabetes Association (ADA) in the diagnosis of DM. However, the first Brazilian Guideline for the Diagnosis of Treatment of MS, of 2005, uses the criteria of the NCEP-ATP III, of 2001, for diagnosis [22, 23]. In the criteria of the IDF, the component of the abdominal circumference becomes essential, using a smaller waist circumference, and categorized more people as having the MS than the ATP III definition. However, higher values of abdominal circumference in older people have been related to lower values of body mass index (BMI), in relation to younger adults [24–28].

2.3 Prevalence of metabolic syndrome

The prevalence of MS varies according to geographic area, with age, race, sex, and classification used for diagnosis [29].

The DARIOS study performs a pooled analysis of 11 studies in 24,670 Spanish individuals aged 35–74, using the criteria of the IDF/NHLBI/AHA-2009, and shows a prevalence of 32% in men and 29% in women [12, 30].

The West of Scotland Coronary Prevention Study (WOSCOPS), one of the largest in Europe, reports a general prevalence of 26.2% [31].

The Third National Health and Nutrition Examination Survey (NHANES III), in the USA showed that 7% of individuals between the ages of 20 and 29, 42% of individuals with ages between 60 and 69 years, and 44% of individuals with ages over 70 years presented MS. The overall prevalence was 23.7% in the 8814 adults who entered the study. This study showed that the incidence increased with age, mainly after 40 years, with significant variations consistent with ethnicity, being the most frequent MS in Hispanics (31.9%) compared with Caucasians (23.8%) and African-Americans (21.6%). A similar prevalence was recorded in both sexes,

although it was more prevalent in males in Caucasians and in females in African-Americans and Hispanics [32].

Based on the Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study of Europe in 2004, the prevalence of MS was 15.7% in male and 14.2% in the female [33]. Epidemiological studies on MS have suggested that the prevalence of MS in Western societies is high and is increasing due to the increase in obesity, especially in younger individuals [34].

In Portugal there are few studies on the prevalence of MS. In 2004, an epidemiological study was carried out in Porto where the prevalence of MS was 23.9%, being more prevalent in females (27%) than in males (19.1%) [35].

In 2008, the first study was conducted on the prevalence of MS and its cardiovascular complications in the Portuguese adult population at the primary care level in continental Portugal, Azores, and Madeira—VALSIM study (epidemiological study of the prevalence of metabolic syndrome in the Portuguese population). In this study, 719 family doctors participated. The study was carried out between April 2006 and November 2007. A total of 16,856 individuals were evaluated between the ages of 18 and 96, with averages of 58.1 ± 15.1 years. This study showed a high prevalence of MS in adults affecting 27.5% of the population analyzed, being more prevalent in females (28.7%) compared with males (26%). This study revealed an increase in the prevalence of MS related to age, body mass index, and abdominal perimeter. This study also demonstrated the association between metabolic risk factors, including MS and the occurrence of cardiac events, stroke, and DM [34]. The prevalence of MS exhibited a regional variation, being more prevalent in Alentejo (30.99%), Madeira (29.38%), Central Region (28.79%), and North Region (28.17%) and less prevalent in Algarve (24.42%), Lisbon and Tagus Valley (25.71%), and Azores (26.05%) [34].

3. Insulin resistance and metabolic syndrome

The pathophysiology of MS is not yet fully understood, but the most accepted hypothesis is insulin resistance, so MS is also known as insulin resistance syndrome. Insulin resistance is defined as a defect in its action at the peripheral level that results in hyperinsulinemia, required to maintain normal blood glucose [36, 37]. Predisposing factors for insulin resistance are central obesity and the release of large concentrations of free fatty acids from adipose tissue. Free fatty acids in the liver will determine the increase in glycogenesis and gluconeogenesis, increased triglyceride production, and the secretion of very low-density lipoproteins (VLDL).

Insulin is a hormone with anti-lipolytic action that is produced in pancreatic beta cells and is released into the bloodstream starting its metabolic effects after binding to its receptor. The insulin receptor is a transmembrane glycoprotein composed of four subunits linked by disulfide bridges, that is, two extracellular alpha subunits that contain the insulin binding domain and two β subunits, which contain an extracellular domain, a transmembrane domain, and a intracellular domain insulin binding to the α subunit and β subunit, and tyrosine kinase activation in the β subunit is the first stage of insulin action on glucose metabolism [38, 39]. The activation of tyrosine kinase, and phosphorylation of the β subunit of the insulin receptor, catalyzes the phosphorylation reaction of several tyrosine residues in a family of proteins called insulin receptor substrate (IRS), which include IRS-1, IRS-2, IRS-3, and IRS-4, which are the most specific in the insulin signaling cascade. Other isoforms are growth factor receptor-bound protein 2 (GRB2)-associated binding protein 1 (Gab-1), Shc, and p62 [40, 41].

Structurally, the four IRS proteins have many similarities, in particular, the phosphotyrosine-binding domain (PTB), whose function is the recognition sequence of asparagine-proline glutamic phosphotyrosine acid (NPEpY) located in the juxta membrane region of the β receptor of insulin. It is involved in the interaction of IRS proteins with the insulin receptor and with the COOH-terminal domain of the proteins, capable of binding to the SH2 domains [39]. In addition to their similarities, the four IRS proteins differ in tissue distribution [39]. The SH2 domain consists of 100 amino acids, and it is the phosphotyrosine binding site [41]. The activation of proteins that contain the SH2 domain is important for the transmission of the insulin signal and thus carry out its functions [39].

The IRS-1 gene located on chromosome 2q36–37 was the first substrate that was identified. IRS-1 is involved in many of the actions of insulin, in the activation of nitric oxide (NO) synthetase, in the activation of the activity of the iN + pump, and in vascular relaxation through activated protein kinases by mitogens (MAP kinase) [39, 42].

The IRS-2 gene is found on chromosome 13q34 [43]. The IRS-2 protein and the IRS-1 are very similar in structure and functions, and the main difference lies in the region between amino acids 591 and 789 of the IRS-2. IRS-2, unlike IRS-1, is more in the cellular cytosol [44].

IRS-3 does not appear to be expressed in human cells [39].

IRS-4 is a protein that consists of 1257 amino acids located in the cell membrane of various organs in which it is expressed. It was discovered initially in embryonic kidney cells. The IRS-4 gene is found on the X chromosome [39].

Insulin causes vascular relaxation through stimulation of NO production in the endothelial cells of blood vessels and by reducing the concentration of intracellular calcium in muscle and smooth muscle cells by decreasing sensitization of the light chains of calcium (Ca^{2+})-myosin. These effects are mediated by the activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway, as well as the stimulation of glucose transport in skeletal muscle and vascular adipose tissue [45]. In the case of insulin resistance, the increase in lipolysis produces greater amounts of fatty acids, promoting greater inhibition of the lipolytic effect of insulin, causing an increase in lipolysis [46, 47].

Insulin resistance is defined as the inability of glucose uptake by skeletal muscle and adipose tissue and the inability of the liver to inhibit gluconeogenesis in response to an increase in insulin [38, 43].

The evidence of the causal relationships between insulin resistance and hypertension is increasing [48]. There are still many uncertainties about the mechanisms that link the two conditions, but in addition to the common genetic predisposition between insulin resistance and AHT, there are several other possible mechanisms that can explain it [48, 49].

The renin-angiotensin-aldosterone system (RAAS) is a therapeutic target for AHT. Changes in the RAAS are also important in insulin resistance, and the common molecular mechanisms of insulin resistance and AHT are not well understood [50, 51].

The RAAS thus plays an important role in MS. Angiotensinogen is a 58 kDa protein produced primarily in the liver under physiological conditions, but it can be produced in smaller amounts in adipocytes, mesangial cells, and epithelial cells of the proximal contoured tubule and in the brain (neurons and glial cells). Angiotensinogen by the action of the renin enzyme produced in the glomerular juxta cells in the kidney is subjected to cleavage in the amino-terminal acid of 10 NH2 to form angiotensin I. Angiotensin I is an inactive peptide that is hydroxylated by the enzyme angiotensin converter, forming an octapeptide designated angiotensin II [50].

The RAAS has autocrine/paracrine activity in various tissues, especially in the skeletal muscle, smooth muscle of blood vessels, kidneys, heart, and pancreas [52]. The angiotensin (AT) II receptor is a transmembrane protein that belongs to the family of receptors coupled to the G protein which is divided into type I (AT1) and type II (AT2) [40, 42, 53]. The genes encoding the AT1 receptor were cloned in 1992, and the genes encoding the AT2 receptor were cloned in 1994 [54]. The binding of angiotensin II to the AT1 receptor leads to the phosphorylation of various proteins such as IRS-1 and IRS-2, making the P110/PI3K subunit the common activation mechanism for the insulin signaling pathway. Resistance to insulin is caused by the inhibition of insulin through the inhibition of the mechanisms involved in glucose transport and vasodilation [42, 45].

Skeletal muscle is important in the development of insulin resistance, which is responsible for 75–95% glucose metabolism [55, 56]. Skeletal muscle constitutes the largest insulin-sensitive tissue in the body and is the primary site for insulin-stimulated glucose utilization [55]. Skeletal muscle resistance to insulin is fundamental to the metabolic dysregulation associated with obesity and physical inactivity and contributes to the development of the MS [55]. Potential mechanisms contributing to reduced insulin signaling and action in skeletal muscle include adipose tissue expansion and increased inflammatory adipokines, increased RAAS activity, decreases in muscle mitochondrial oxidative capacity, increased intramuscular lipid accumulation, and increased reactive oxygen species (ROS) [55].

Angiotensin II has prooxidant and pro-inflammatory effects that regulate apoptosis, inflammation, cell growth, fibrosis, and insulin sensitivity by inducing the formation of oxygen free radicals through the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [56]. ROS are formed by the electron transport chain in the mitochondria, and it increases in situations that require the oxidation of substrates such as glucose [56]. Multiple ROS activate transcription factors such as factor nuclear kappa B (NF-KB) protein-1 and hypoxia-inducible factor-1 HIF-1, which are involved in the mechanism of insulin resistance in skeletal muscle [57].

Hyperinsulinemia can alter some of the components of the RAAS such as profibrotic stimulants and pro-inflammatory actions mediated by angiotensin II and can cause cardiovascular disease development [50].

3.1 Relationship between inflammation and insulin resistance

Obesity is a worldwide epidemic that has generated many scientific publications in recent years. Thus, new paradigms were emerging, while the old challenges are still to be unraveled [58]. Obesity is a chronic disease and has become a major problem in most industrialized countries due to its increased prevalence and association with various diseases and due to its great economic impact [59]. To preserve the energy reserves of body fat during periods of negative energy balance, the body has developed various regulatory mechanisms [60]. This control is achieved by balancing the intestinal absorption of glucose, the production of glucose by the liver, and the absorption and metabolism of glucose by peripheral tissues [61]. Insulin regulates blood glucose due to its action in stimulating glucose uptake in the muscle and liver and inhibiting hepatic gluconeogenesis [61]. Despite the change in tolerance to the action of insulin initially, blood glucose is normal because pancreatic β cells have the ability to increase the ability of insulin secretion to overcome existing insulin resistance [9]. Excess nutrients induce hypertrophy of adipocytes that promote the development of various chronic morbidities such as type 2 DM and insulin resistance, glucose intolerance, and dyslipidemia with the consequent development of cardiovascular diseases [60]. There is also strong evidence to suggest that cell hypoxia may be an important factor in the pathophysiology of adipocytes and may be one of the causes of this dysfunction contributing to the metabolic alteration associated with obesity [62].

Obesity is defined according to the WHO, as an abnormal or excessive accumulation of fat that can cause imbalances in the health of an individual [63]. Males are more likely to accumulate intra-abdominal adipose tissue compared with females [32, 64]. On average, men have twice the amount of visceral fat than women and have a higher prevalence of metabolic diseases associated with obesity and MS [65].

Obesity is associated with a higher incidence of type 2 DM, insulin resistance, AHT, dyslipidemia, some types of cancer, and CVD [66]. Fat tissue is deposited in two main compartments, subcutaneous and central or visceral [63]. Based on this, obesity can be divided into central and peripheral obesity. Central obesity is characterized by hyperplasia and hypertrophy of adipocytes around the intra-abdominal organs and is associated with greater development of MS [65].

Adipocyte hypertrophy is the main consequence of excess nutrient intake, promoting the development of insulin resistance and glucose intolerance [63]. Triglycerides present in fatty tissue are the body's largest energy reserve, so adipose tissue is a very effective energy storage mechanism that allows survival of living beings in times of famine [59].

Recent studies have shown that hypoxia in adipose tissue can play an important role in the cellular mechanisms of chronic inflammation, macrophage infiltration, adiponectin reduction, leptin elevation, adipocyte apoptosis, and reticulum endoplasmic and mitochondrial dysfunction in white adipose tissue in obese [67].

Hypoxia inhibits pre-adipocyte differentiation and stimulates the secretion of leptin and vascular epithelial growth factor (VEGF) of mature adipocytes [68].

There are several genes that regulate the action of hypoxia, such as HIF-1α (hypoxia-inducible factor 1α), vascular endothelial growth factor VEGF, glucose transporter-1 (GLUT-1), heme oxygenase-1, and pyruvate dehydrogenase kinase 1 [67]. It was found that, under conditions of hypoxia in adipose tissue, all genes increased their expression except for VEGF messenger ribonucleic acid (mRNA) [67].

Adipose tissue is thus divided into white adipose tissue and brown adipose tissue, which have different functions. Brown adipose tissue has the function of producing heat. The understanding and vision we currently have in white adipose tissue have changed significantly in the last 15 years [69]. Currently, white adipose tissue is considered, not only as a tissue in which energy is stored in the form of triglycerides but also as the main secretory and endocrine organ of the organism [70]. White adipose tissue produces and secretes various proteins. Initially, these proteins were designated adipocytokines [71]. Currently, it is universally adopted the name of adipokines to the set of proteins synthesized and secreted by adipose tissue, since not all proteins are cytokines [71].

In addition to adipokines and fatty acids, adipose tissue produces other lipid substances such as steroid hormones, prostaglandins and prostanoids, cholesterol, and retinol (cholesterol and retinol are not synthesized by adipocytes but are stored and released from them) [71].

In 1994, Friedman and his colleagues discovered leptin, a hormone produced by white adipose tissue. Leptin, also called OB protein, is a hormone composed of 16 kDa and is produced from a protein with molecular weight of 18 kDa. It is cleaved leading to the production of leptin protein. Leptin is expressed in many tissues including the white and brown adipose tissue, stomach, placenta, mammary gland, ovarian follicles, and others [72]. It is expressed primarily in adipose tissue, but it is also expressed in smaller amounts in the brain and in the cerebrospinal fluid [70].

Leptin acts both in the central nervous system (hypothalamus) and in the peripheral organs. This discovery allowed us to realize that adipose tissue is not only a tissue in which energy storage occurs but also an endocrine organ [73].

White adipose tissue produces various adipokines, many of which are inflammatory mediators such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-β, and IL-6. The production of inflammatory interleukins increases with obesity and is involved in the development of insulin resistance and in the metabolic syndrome [62, 74]. Adipokines have various functions, in particular in the energy balance (e.g., leptin) on insulin sensitivity and glucose metabolism (e.g., adiponectin), inflammation (e.g., TNF-α), immunity (e.g., adipsin), lipid metabolism (e.g., cholesterol ester transfer protein), blood pressure control (e.g., angiotensinogen) hemostasis (e.g., plasminogen activation inhibitor-1), and angiogenesis (e.g., vascular epidermal growth factor) [62, 71].

3.2 Relationship between hypoxia and insulin resistance

Inflammation is an important process in obesity and in type 2 DM, as it promotes insulin resistance by inhibiting the function of IRS-1 and IRS-2 in the pathway insulin signaling [61, 75].

Obesity is characterized by a state of chronic inflammation with increased inflammatory parameters, for example, haptoglobin, IL-6, C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1), and TNF-α [69]. Large concentrations of cytokines produced by adipose tissue, such as TNF-α, IL-6, and IL-1β, and low concentrations of certain adiponectins cause chronic hyperinsulinemia and insulin resistance [63].

3.3 Relationship between endothelial dysfunction and insulin resistance

During periods of positive energy balance, there is an increase in white adipose tissue in order to be able to store excess triglycerides. Consequently, adipose tissue becomes hypoxic due to the decrease in vascularization of the same [62]. The role of hypoxia in adipose tissue in obesity and insulin resistance is still unclear [76]. Regulatory responses mediated by HIF-1 depend on its degree and duration [76].

HIF-1 is a heterodimeric transcription factor induced by hypoxia and composed of two subunits, the α subunit consisting of 120 kDa and the β subunit, consisting of 91 to 94 kDa [77]. The α subunit (HIF-1α) determines the transcriptional activity of HIF-1, and its increase occurs in response to hypoxia. The β (HIF-1β) subunit is constitutively expressed and may also be referred to as an aryl hydrocarbon receptor nuclear translocator (tRNA) [78]. Under normoxia conditions, HIF-1α is hydroxylated in proline and asparaginyl residues, catalyzed by the enzyme prolylhydroxylase (PHD), which promotes binding to an ubiquitin ligase complex. This process leads to proteosomal degradation mediated by HIF-1 [76, 78]. Under conditions of hypoxia, hydroxylation and activation of HIF-1 target genes are inhibited [76].

HIF-1α activates fibrosis of adipose tissue, causing an increase in macrophage infiltration into adipose tissue that mediates a greater increase in inflammation and concomitant sensitivity decrease in insulin [79, 80].

VEGF is a target gene of HIF-1. VEGF promotes angiogenesis, a necessary process for the differentiation of adipocytes and the growth of adipose tissue [81].

4. Obesity and metabolic syndrome

The endothelium is a layer of cells that lines the inner surface of the blood vessel [82]. Endothelial dysfunction is defined as the interruption of the release of vasodilator factors such as NO and prostacyclin (PGI2) and vasoconstrictor factors such as endothelin-1 (ET-1) and angiotensin II [74]. Alterations in vascular endothelial

function at the level of adipose tissue can cause insulin resistance [81]. In insulin resistance, the release rate of free fatty acids from the adipose tissue, which contribute to diabetic dyslipidemia, increased. HDL-cholesterol decreased, increasing low-density lipoprotein (LDL) cholesterol and free fatty acids. This stimulates the inflammatory response, causing the adhesion of monocytes and lymphocytes to the endothelial cells, and increases the flow of glucose and free fatty acids to the cells, causing excessive formation of oxygen free radicals, with an increase in metabolic and hemodynamic degradation. There is an increase in free radicals and nitrites by intramitochondrial enzymatic oxy-reduction which promotes apoptosis and impaired vascular endothelial function [82]. This alteration causes insulin metabolic dysfunction, promoting greater resistance to insulin [74, 82].

4.1 Relationship between insulin resistance and hypertension in obesity

Obesity alone is the cause of AHT in 78% of men and 65% of women [83]. Evidence of the causal relationship between insulin resistance and AHT is increasing [40]. Under physiological circumstances, insulin and insulin growth factor-1 (IGF-1) increase vasodilation by stimulating the production of NO and reducing the concentration of intravascular calcium in vascular smooth muscle cells. It also increases the sensitization of myosin-Ca^{2+} light chains. These actions are mediated through the activation of the PI3K enzyme and the Akt protein. Vascular relaxation in response to activation of PI3K/Akt pathway is mediated by endothelial cells producing NO, which involves phosphorylation of endothelial NO synthetase [41].

Patients with AHT have an increase in fasting and postprandial insulin levels in relation to non-hypertensive patients with the same body mass index [41]. It does not occur with secondary AHT but with essential AHT. This is related in part to the action of insulin that changes at the level of muscle tissue, which is the predominant site of glucose use stimulated by insulin [40].

There is an association between blood pressure and the proportion of type II fibers in skeletal muscle, which are less sensitive to insulin than type I fibers [40]. Under normal physiological conditions, there is a relationship between glucose-mediated insulin availability and increased blood flow in response to insulin. This response decreases in obese people with insulin resistance, which suggests resistance to insulin action in reference to vascular NO production [41]. Due to the difficulty of assimilating the rapid growth of adipose tissue in a hypoxia at an early stage, conditions are created for an increase in the expression of HIF-1α [84–87].

There is evidence that insulin resistance and hyperinsulinemia predispose patients to the development of AHT due to cellular abnormalities in insulin signaling pathways and are associated with metabolic and hemodynamic alterations [84]. Metabolic abnormalities are linked to hypertension caused by pathophysiological processes that involve the sympathetic-adrenergic system, the imbalance of cell cations, the increase in inflammation, the oxidative stress, and the RAAS [40].

RAAS plays an important role in insulin resistance by being more active in visceral adipose tissue compared to peripheral adipose tissue [83]. Insulin resistance in obese individuals is also associated, in part, with an antagonism to the action of angiotensin II (AT II) [83]. This produces a decrease in NO production with an increase in vasoconstriction and a decrease in GLUT-4 in skeletal muscle. It also reduces glucose uptake and decreases vasodilation in tissues [83]. Under normal conditions, the RAAS system is a mechanism for regulating blood pressure [88]. With the increase in the activity of the RAAS, there is a greater production of ATII, which leads to stimulation of the sympathetic nervous system, insulin resistance, sodium retention, and increased intravascular volume. It also contributes to kidney disease, hypertension, insulin resistance, and left ventricular hypertrophy [83].

There is a common genetic predisposition to insulin resistance and hypertension. Genetic defects have been described in people who had insulin resistance and AHT and mutations found on chromosome 7q, where other genes important for glycemic control, blood pressure, and obesity are also found [49].

Hyperinsulinemia can directly stimulate the reabsorption of sodium and water in the kidney, which increases the volume in the extracellular space, causing AHT. Another mechanism by which insulin can cause hypertension involves stimulation of the sympathetic nervous system (SNS) by increasing the concentration of norepinephrine. This increase is directly related to the increase in pulse and blood pressure by direct effect on the reabsorption of sodium in the kidney, peripheral vasoconstriction, and increased cardiac output [9].

5. Conclusion

The correlation of CVRF with the metabolic syndrome differs from one another. The notion that CVRF has a tendency to aggregation allows an earlier diagnosis of MS, thus also leading to the early introduction of therapeutic, pharmacological, or non-pharmacological measures. The prevalence of MS increases with age and is present in people of working age, increasing the risk of cardiovascular diseases, work-related absences, and socioeconomic costs. The concept of MS was carried out in a progressive manner. Its clinical importance is enormous due to the fact that it can identify patients with high risk of suffering some CVD and/or DM, allowing a preventive intervention. This may explain the importance of understanding the pathophysiology of the MS, and the author hopes that at the end of the chapter, the reader can understand it. This chapter is the beginning of an explanation of metabolic syndrome that should be complemented by reading other articles.

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Chapter 7

Metabolic Syndrome: Impact of Dietary Therapy

Suzanne Fouad Soliman

Abstract

Metabolic syndrome refers to the coexistence of insulin resistance (IR) with several risk factors, including abdominal obesity, atherogenic dyslipidemia, and hypertension, which is usually complicated by cardiovascular and/or cerebrovascular diseases. This clustering of risk factors suggests that they are interrelated and not independent of one another and that they share underlying mechanisms, mediators, and pathways. Its prevalence exceeds 40% of those over 40, and it has recently been diagnosed in adolescents and even children. Metabolic syndrome is a pro-inflammatory prothrombotic state with determination of elevated level of cytokines, acute phase reactants, fibrinogen, and plasminogen activator inhibitor-1. A comprehensive definition of metabolic syndrome and its pathogenesis would facilitate research into its causes and disease pathophysiology linking the components of metabolic syndrome with the increased risk of cardiovascular diseases. The management to mitigate these underlying risk factors constitutes a first-line intervention; dietary therapy of metabolic syndrome includes lifestyle modification, hypocaloric diet, and consumption of functional food. Healthy food quantity and time of consumption help restore the normal metabolic profiles. Hopefully, this will lead to new insights into facilitating epidemiological and clinical studies of pharmacological, lifestyle, and preventive treatment approaches.

Keywords: metabolic syndrome, circadian rhythm, insulin resistance, sleep, functional foods, lifestyle modification, hypocaloric diets

1. Introduction

The prevalence of metabolic syndrome (MetS) worldwide varies between 10 and 84% depending on the age, gender, and race of the population [1]. The International Diabetes Federation estimates that one-quarter of the world's population has MetS [2]. Although the prevalence of MetS varies throughout the world and depends on the particular health organization used for its definition, it is clear that the number of people complaining about this syndrome has globally risen [3], due to a more sedentary life, the increase in the number of smokers, unhealthy dietary habits, and the increase in stress [4].

MetS is a cluster of metabolic disturbances that tend to coexist. Different health organizations have suggested diverse definitions for MetS. However, it is clear that MetS is a clinical entity of substantial heterogeneity, commonly represented by the combination of central abdominal obesity, hyperglycemia, dyslipidemia, and/or hypertension [5, 6].

In 1994, the World Health Organization [7] defined MetS as the presence of one of the following: type II diabetes mellitus, insulin resistance, or impaired glucose tolerance, plus at least two of the following—triglycerides (TG) ≥150 mg/dL (≥1.7 mmol/L) and/or HDL cholesterol <40 mg/dL (≤0.9 mmol/L) (males) and <50 mg/dL (≤1.0 mmol/L) (females); urine albumin excretion >20 g/min or albumin/creatinine ratio >30 mg/g; systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or a prescription of hypertension; and central obesity, waist circumference (WC) ≥102 cm (males) and ≥ 88 cm (females), waist/hip ratio > 0.90 (males) and >0.85 (females), and/or body mass index (BMI) >30 kg/m^2 [8].

The International Diabetes Federation defined MetS as encompassing at least three of the following five conditions: (1) male waist circumference ≥ 94 cm and female waist circumference ≥ 80 cm; (2) fasting glucose level ≥ 100 mg/dL; (3) SBP ≥130 and/or DBP ≥85 mmHg or under medical treatment for hypertension; (4) TG ≥150 mg/dL or ≥ 1.7 mmol/L or prescribed pharmacological treatment for hypertriglyceridemia; or (5) male HDL cholesterol <40 mg/dL and female HDL cholesterol <50 mg/dL [9].

Other markers used for diagnosis of metabolic syndrome include high-sensitivity C-reactive protein concentrations (hs-CRP) ≥3 mg/dL [10], uric acid [11], fibrinogen [12], plasminogen activator inhibitor-1 [13, 14], increased homocysteine [15, 16], and decreased adiponectin [17]. Fibrinogen, plasminogen activator inhibitor-1, cytokines, and hs-CRP protein are often elevated in patients with MetS, resulting in a prothrombotic and pro-inflammatory state. These biomarkers are not routinely evaluated in clinical practice. hs-CRP ≥3 mg/dL indicates a state of inflammation and a higher risk of atherosclerotic cardiovascular disease (ASCVD).

2. Characterization and risk assessment

MetS is a link between visceral adiposity, insulin resistance, inflammation, and endothelial dysfunctions [18, 19].

2.1 Visceral adiposity

Visceral adiposity (abdominal fats close to the visceral organs) leads to an imbalance in the secretion of pro-inflammatory and anti-inflammatory factors. Adipocytes produce pro-inflammatory factors in excess, such as IL-6, IL-10, TNF-α, and hs-CRP. These cytokines block the intracellular insulin signaling pathways. Moreover, the adipose tissue inhibits adiponectin [20, 21].

2.2 Insulin resistance

Insulin resistance (IR) rises with increased waist circumference or body fat and is not related to the body mass index. IR results in the diversion of excess non-estrified fatty acids from lipid-overloaded IR muscles to the hepatic cells, resulting in nonalcoholic fatty liver disease and atherogenic dyslipidemia. IR predisposes to glucose intolerance, which may be aggravated by increased hepatic gluconeogenesis with an IR liver [22].

2.3 Pro-inflammatory condition

Inflammation is a response of the immune system to injury. It is a mechanism in the pathogenesis and progression of obesity-related medical disorders and the link between adiposity, IR, MetS, and cardiovascular disease [23]. Oxidative stress is a

condition in which there is an imbalance between the prooxidants and antioxidants in the body [24]. It plays a key role in the pathogenesis of atherosclerosis through different mechanisms such as the oxidation of LDL-c particles [24, 25] or the impairment of HDL-c functions [26].

2.4 High blood pressure

Hypertension is defined as a resting SBP ≥140 mmHg and/or DBP ≥90 mmHg or the use of medical prescription to lower blood pressure [27]. Activation of the sympathetic nervous system together with the activation of the renin-angiotensin system by the hyperglycemia and antinatriuretic effect of insulin, in addition to some obese people being salt sensitive, contributes to the development of hypertension. It is identified as a major cardiovascular and renal risk factor related to heart disease, myocardial infarction, cerebrovascular stroke, and vascular diseases [28].

2.5 Circadian rhythm disruption

Over the last two decades, a deeper comprehension of the molecular mechanisms that control the biological clock and circadian rhythm has been achieved. The 2017 Nobel Prize in Physiology was awarded to Jeffrey C. Hall, Michael Rosbash, and Michael Young for their work on circadian rhythms [29, 30]. There are interconnections between the circadian rhythm and endocrine homeostasis. Biological clock disruption was in fact associated to an increased incidence of metabolic and endocrine diseases. Irregular rhythms have been linked to various chronic health conditions, such as sleep disorders, obesity, type II diabetes mellitus, hypertension, gastrointestinal motility diseases, depression, cardiac diseases, cognitive dysfunction, bipolar disorder, and seasonal affective disorder [31]. The master clock in mammals that controls circadian rhythms consists of a group of nerve cells in the brain called the suprachiasmatic nucleus (SCN) which contains about 20,000 nerve cells. It is located in the hypothalamus superior to the optic chiasm and receives direct light information from the retina via the retinohypothalamic tract. The master clock in the brain coordinates all of the body's peripheral clocks through different neural and humoral signals, so they are in synchrony [32].

The nonstop 24/7 lifestyle has given rise to human clock gene mutation, for which the human biological clock does not have a suitable design for physiological adaptation; this leads to an imbalance of autonomic nervous system function toward the parasympathetic predominance in the abdominal compartment and the sympathetic predominance in the muscular and thoracic compartments, resulting in the impairment of insulin secretion, glucose uptake by the muscle, and increase in blood pressure, intra-abdominal fat, and fatty liver [33].

The timing and nutritional composition of meals can regulate circadian rhythms, particularly in the peripheral tissue. The translation of these findings to human physiology now represents an important goal. In healthy humans, glucose tolerance lowers throughout the day, leading to the term "afternoon diabetes"; a daily rhythm in pancreatic insulin production is observed, with increased insulin production in the morning compared to the evening. Moreover, lipid metabolism exhibits circadian regulation, with elevated plasma concentrations of triacylglycerol during the biological night and an elevated postprandial response following a nighttime meal compared with the same meal consumed during the day [34]. In 2006, Staels stated "When the clock stops ticking, metabolic syndrome explodes" [35]. When to eat is as important as what to eat.

2.5.1 Sleep in relation to melatonin and growth hormone secretions

Melatonin is synthesized by multiple tissues in the body, but the pineal gland is the major contributor to circulating melatonin concentration. The rhythmic activity of the SCN determines the release of melatonin, which directly correlates with day length, and it may in turn modulate other physiological functions through paracrine signaling. Pancreatic hormone insulin, gastric hormone ghrelin, growth hormone (GH) from the pituitary gland, and leptin hormone represent as key factors in metabolic regulation, and now several circadian factors are recognized as regulators of their secretion and activity. Sleep deprivation results in circadian misalignment and increases markers of inflammation and IR. A better understanding of such relationships might prove useful and supportive in designing new therapeutic approaches for metabolic syndrome [36].

Growth hormone is a peptide hormone secreted by the anterior pituitary under control of the hypothalamic-pituitary axis. Actions of GH depend on the age of the individual. The primary role of GH in children and adolescents is to promote growth until the final adult height is achieved, while in adults, the role of GH is the regulation of protein, carbohydrate, and lipid metabolism, insulin resistance, and metabolic homeostasis. Sleep restriction and disturbance have also been shown to negatively impact hormone secretion. In sleep deprivation cases, GH secretion decreases, the appetite-suppressing hormone leptin decreases, and the appetite-stimulating hormone ghrelin increases. The end result is the consumption of additional food and calories. Plasma GH peak appeared with the onset of deep sleep and did not correlate with the changes in plasma glucose and insulin. GH secretion decreased if the onset of sleep was delayed [37].

2.6 Risk assessment

Metabolic syndrome directly promotes the development of atherosclerotic cardiovascular disease (ASCVD) [38] and type II diabetes mellitus [39]. Atherogenic dyslipidemia, high circulating levels of prothrombotic factors, and the increase of inflammatory markers carry a higher risk for acute cardiovascular syndromes [40]. Previous studies on middle-aged persons with metabolic syndrome had concluded that many middle-aged people are at increased absolute risk for cardiovascular diseases in the 10-year risk. Furthermore, in young adults who develop the syndrome, long-term risk for ASCVD is increased even when the 10-year risk is not high. The Framingham risk scoring is used to classify metabolic syndrome patients into three risk categories based on a 10-year risk for coronary heart disease, cerebrovascular disease, peripheral artery disease, and heart failure risk: *low-risk* (10-year risk <10%), *moderate-risk* (10-year risk 10–20%), and *high-risk patients* (10-year risk >20%) [41, 42].

3. Metabolic syndrome, mental and cognitive functions

Obesity and dementia frequently coexist. They share many common pathways such as insulin resistance as well as inflammation and oxidative stress. Midlife obesity is directly related to the risk of developing dementia later in life. Dementia is a syndrome characterized by gradual decline in cognitive functions (memory, attention, behavior, language, mood, and learning). In 2008, Dr. Suzanne de la Monte and Dr. Jack Wands proposed that Alzheimer's disease could be termed type III diabetes, based on the fact that IR within the brain revealed to be a characteristic of Alzheimer's disease [43]. There is no definite cure for dementia, and prevention

is the only option. Prevention may be achieved by early treatment of the risk factors resulting in cognitive impairments (obesity, DM, HTN, smoking, dyslipidemia, anxiety, and depression). Several biomarkers could be involved in the pathogenesis of mild cognitive impairment or early neurodegenerative diseases. Assessment of these biomarkers in obese middle-aged persons could serve as a basis for early management to alleviate the burden of cognitive impairment and dementia in the future.

3.1 Cognitive and mental evaluations

The Mini-Mental State Examination (MMSE) was performed for clinical evaluation of mental and cognitive status. The 30-point questionnaires take between 5 and 10 minutes and examination functions including registration, attention, calculation, recall, language, commands, and orientation [44]. It is a sensitive, valid, and reliable method that is used extensively in clinical and research settings to measure cognitive impairment and to estimate the severity and progression of cognitive impairment. It is also used to follow the course of cognitive changes in an individual over time, thus making it an effective way to document an individual's response to treatment. Sleep quality and the number of sleep hours and their pattern were evaluated. Exposure to sun and length of time, duration, and clothing were recorded. General subjective life stresses, life pattern to evaluate general activity, and history of exercising were recorded.

C-peptide was detected by C-peptide enzyme immunoassay [45]. Modified homeostatic model assessment of insulin resistance (M.HOMA-IR) was calculated by Eq. (1), in which insulin was replaced by C-peptide so as to be applied on diabetic patients using exogenous insulin [46]:

$$\text{M.HOMA} - \text{IR} = 1.5 + \text{Fasting blood glucose} \times \text{Fasting c} - \text{peptide}/2800 \quad (1)$$

Blood sampling and biochemical markers of cognitive function impairments were performed as ceramide kinase enzyme, alpha-synuclein, serum clusterin, amyloid beta, and inflammatory markers (hs-CRP, IL-6, and TNF-α).

Ceramides are agents involved in the pathogenesis of mild cognitive impairment or early neurodegenerative diseases. They are mediated by insulin resistance and inflammatory states. Ceramides are lipid soluble and therefore likely to readily cross the blood-brain barrier resulting in cytotoxic effects in the central nervous system with various dementia-associated diseases, including Alzheimer's disease. It is important to prevent its elevation in the human body via increasing the activity of the ceramide kinase enzyme (CERK enzyme) that converts the ceramide to ceramide-1-phosphate (C1P) via its phosphorylation. C1P is a sphingolipid metabolite that has been implicated in the membrane fusion of brain synaptic vesicles and neutrophil phagolysosome formation. C1P is a key regulator of cell growth and survival, it stimulates DNA synthesis and cell division, and it is a potent inhibitor of apoptosis. Many studies have shown that C1P is important for membrane biology and for the regulation of membrane-bound proteins and the CERK enzyme has appeared to be tightly regulated in order to control both ceramide levels and production of C1P [47]. Improvement of the metabolic profiles including C-peptide concentration and the M.HOMA-IR values was associated with the improvement of the serum level of the enzyme CERK and of cognitive functions [48].

Alpha-synuclein (α-Syn) protein was originally isolated from Alzheimer's disease plaques and was thought to be a presynaptic nerve terminal protein. The α-Syn is a member of the synuclein's family of cytoplasmic, predominantly neuron-specific proteins. Synucleins are small, prone to aggregate proteins associated

with several neurodegenerative diseases. The α-Syn has been found in body fluids, including blood and cerebrospinal fluid, peripheral tissues, and the central nervous system. Previous reports suggest that α-Syn is widely expressed peripherally, including the macrophages. The expression of α-Syn is enhanced in activated macrophages, suggesting that α-Syn may modulate macrophage function and thereby inflammatory processes. Diet-induced obesity may be an environmental risk factor for the development alpha-synucleinopathies [49]. There was a significant positive correlation between serum IL-6 and serum α-Syn. Fighting obesity, dyslipidemia, and the associated complications especially the inflammatory processes improved the deleterious effects on the cognitive functions in obese persons [50].

Clusterin (apolipoprotein J) is a heterodimeric glycoprotein in which α and β chains are interconnected via five disulfide bonds. There is a strong association between the single-nucleotide polymorphisms in the clusterin gene and Alzheimer's disease. Moreover, plasma clusterin is considered a potential peripheral biomarker of cognitive dysfunction and AD. Clusterin may be related to AD pathogenesis through various mechanisms. It could bind amyloid extracellularly and inhibit the aggregation of amyloid beta monomers into toxic oligomers. In addition, the neurotoxicity of the amyloid might be reduced by the interaction of clusterin with the molecules involved in signal transduction, DNA repair, cell cycle, and apoptosis. Brain apolipoprotein clusterin plays an important role in cholesterol transport and neuronal lipid metabolism. Furthermore, it has a role in the inhibition of neuroinflammation which is thought to be a major factor in AD pathogenesis and identified as a key component in cerebrovascular diseases. In addition, it is known that neuroinflammation plays an important role in dementia pathogenesis and neurodegenerative diseases [51, 52]. The improvement of the C-peptide concentration and the M.HOMA values were parallel with improvement of oral cognitive tests and clusterin value; clusterin was presented as a cognitive function parameter [52].

Plasma amyloid beta (Aβ) can be applied as trait, risk, or state biomarker for AD and denote a neuropathologic condition. A previous study has reported the presence of a stronger correlation between plasma Aβ and positron emission tomography or Pittsburgh Compound-B-C11 as radiotracers illustrate fibrillar brain amyloid deposits which is a reliable method to measure brain amyloid plaque accumulation. Reduction of body weight and improving the metabolic profile reduced the level of serum Aβ protein, an effective role in improving the cognitive function. At the same time, previous studies stated that plasma Aβ measures possibly aid in clinical investigations as markers for the pharmacological impacts of medications that influence amyloid protein transformation [53].

Serum hs-CRP level may be used as a marker for cognitive functions in obese middle-aged persons. Peripheral inflammatory markers are elevated in obese patients. Improvement in cognitive functions is recorded after dietary therapy, with decrease in hs-CRP serum levels. Significant inverse correlation is found between cognitive functions and hs-CRP levels, insulin resistance, minimal waist circumference, and BMI [54].

4. Management of metabolic syndrome

As the presence of MetS carries a risk for cardiovascular diseases and diabetes mellitus, the primary goal of clinical management to individuals with metabolic syndrome is directed towards decreasing the major metabolic risk factors: high LDL-C, hypertension, obesity by losing fat percentage and not muscle mass, decreasing insulin resistance, blood glucose, and maintaining normal HDL and

triglyceride levels through lifestyle changes. Moreover, medical drug therapy might be considered in high-risk patients to modify cardiovascular disease risk factors [7]. Bariatric surgery has been indicated to treat morbidly obese persons. The safety and effectiveness of bariatric surgery in patients with metabolic syndrome have been studied and encouraged [55, 56].

Chronotherapy means the timing of drug and dietary treatment to obtain maximum therapeutic effect. It can be achieved through the timing of light exposure, exercise, food consumption, medication uptake, and sleep, with the goal of optimizing any treatment by taking into account the circadian rhythms of the body [57].

4.1 Therapeutic lifestyle modifications

Physical exercise, diet, and adequate sleep are the way to reach the target. Gradual permanent change in the patient's lifestyle can lead to better and easily maintain normal parameters than major food deprivation introduced all at once. Implementing the following changes increases the chances of success: changing a sedentary life through regular sustained physical exercise and eating several small portions of different foods varieties; consuming complex carbohydrates such as barley, oat, corn, quinoa, and brown rice while decreasing the consumption of simple carbohydrates such as white sugar and sweets; eating fresh seasonal fruit and vegetables daily as they are good sources of fiber, vitamins, and minerals noting that dietary fiber helps prevent gastrointestinal problems such as flatulence and constipation; eating foods rich in unsaturated fatty acids such as salmon, mackerel, sardines, tuna, raw nuts, and flaxseed oil; increasing the consumption of black beans, kidney beans, green peas, and lentils; avoiding processed and red meats; limiting the intake of sugar, salt, and carbonated drinks; replacing salt with spices; boiling, baking, or steaming food instead of frying; paying attention to portion size by utilizing smaller plates; and drinking plenty of fluids, of which 30 ml/kg/day water is the best [7].

Avoid light exposure in late evening or at night as artificial light disrupts the circadian rhythm and the production of melatonin and therefore has a negative effect on sleep quality, mood, cognition, and hormonal functions [58, 59]. Sleep early and get at least 6 hours of sleep per night [60], and stop smoking.

4.2 Energy-restricted diets

For our patients, the Harris-Benedict equation (Eq. (2)) was used to calculate the caloric requirements for each individual in order to estimate the caloric needs or basal energy expenditure (BEE) as follows [61]:

$$\begin{aligned}\text{Males: BEE} &= 66.5 + 13.8\,(\text{weight in Kg}) + 5\,(\text{height in cm}) - 6.8\,(\text{age}).\\ \text{Females: BEE} &= 65.5 + 9.6\,(\text{weight in Kg}) + 1.7\,(\text{height in cm}) - 4.7\,(\text{age}).\end{aligned} \tag{2}$$

These equations yield basal energy expenditure that is frequently multiplied by various activities and/or stress factors to generate the patient's estimated resting energy expenditure, and then we subtracted 500 calories per week from the calculated energy requirement, to produce weight loss of 0.5–1 kg per week. A hypocaloric diet goal is to reduce body weight by about 10% over the first 6 months. The menu varied according to the participant's age and eating habits. It was low in fat (20–25%), high in complex carbohydrate (50–60%), and sufficient in protein (25–30%) from both animal and vegetarian sources [54]. Carbohydrates in the

form of complex CHO, which have low glycemic loads and indexes such as whole grains, oats, fresh seasonal fruits, and vegetables, were consumed to increase high fiber content (eight servings of fresh vegetables and fruits/day). On the other hand, they were instructed to decrease foods rich in saturated fats such as fried foods and packaged meats [62].

4.3 The Mediterranean diet

The Mediterranean diet (MedDiet) is based on scientific evidence that inhabitants of Mediterranean countries (Greece, Italy, and Spain), around the Mediterranean Sea adhering to the principles of nutrition traditional for their region, have better health indicators than people in other areas. Originally, it was developed to prevent and treat the symptoms of hypertension and/or heart disease. In this MedDiet, the emphasis is on the use of plant-based foods as the main source of nourishment: fruits, vegetables, cereals, nuts, legumes, seeds, and whole grains. It is also recommended to replace butter and hydrogenated fats with extra-virgin olive oil and salts with spices and herbs. Red meat and sweets should be eaten once a week, and the main sources of protein should be eggs, yoghurt, fish, and poultry. Red wine could be taken in limited amounts (one glass per day for women; two glasses per day for men) [63–65].

4.4 Role of functional foods

There is no universal definition for functional foods, but the easiest one is from the International Food Information Council which described functional foods as "dietary or food components that provide a health benefit beyond providing basic nutrition" [55]. Functional foods have an important role in the management of metabolic syndrome. Functional foods can help in weight loss, regulation of blood pressure, and control of blood glucose and lipid profile levels. Previous studies supported the beneficial effects of using functional foods containing bioactive components in the management of metabolic syndrome. This includes the intake of foods which have a thermogenesis effect (process in which body heat production increases, e.g., ginger, caffeine, red pepper, and green tea); functional foods with anti-inflammatory properties such as curcumin; and foods with the ability to increase insulin sensitivity, e.g., cinnamon, and increase intake of foods rich in plant estrogens such as soymilk, soybeans, flaxseed, sesame, green beans, fennel, peanut, and pumpkin seeds. In addition, soy products are rich in amino acids and can preserve lean body mass, decrease body fat percentage, and increase basal metabolic rate [48, 50, 53, 66–70].

4.4.1 Functional food classification

- Natural functional foods: food products rich in biological active compounds (food rich in dietary fiber such as oat, barley, psyllium, vegetables, and fruits) [66]
- Foods with approved scientific heath nutrients: apple (pectin), garlic (*Allium sativum*), and fish (omega 3 fatty acids) [71]
- Fortified foods with specific nutrients: iron fortified chocolate for iron deficiency anemia, calcium fortified juices, and zinc fortified products
- Fermented products rich in probiotics or synbiotics [72]

4.4.2 Eat to beat metabolic syndrome

The use of functional and prepared designed food would help fight MetS. Three thousand years ago, Hippocrates announced the tenet "Let food be thy medicine and medicine be thy food." Dietary supplements proved to improve the MetS criteria which include Syrian bread prepared mainly from barley flour and wheat germ mixed with either turmeric or ginger; doum biscuits; barley biscuits; bread prepared with soybean flour and enriched with curcumin or ginger; and cookies prepared with whole wheat flour and fennel or chia seeds. The higher fiber content of these products has a satiety effect so as to decrease food consumption. Moreover, fibers decrease digestion and absorption of dietary carbohydrates. Soy flour is a very rich source of essential amino acids except methionine. Preparation of bakery products using bioactive ingredients has positive beneficial effects for obesity, diabetes, and dyslipidemia management. These compounds have different benefits such as anti-atherogenic, antioxidant, and anti-inflammatory effects [48, 50, 53, 67–70].

4.5 Pharmacological management

There are no specific recommendations regarding the pharmacological management of metabolic syndrome; instead, the focus is on the management of the risk factors which need to be aggressively treated in order to prevent cardiovascular disease and type II diabetes mellitus if lifestyle changes aren't enough to reduce the risks. The available evidence of the pharmacological prescriptions is related to their cardiovascular benefits in clinical practice. Pharmacological treatments include statins and/or fibrates for dyslipidemia, angiotensin-converting enzyme inhibitors or renin-angiotensin-aldosterone system inhibitors for hypertension, metformin or sodium/glucose cotransporter 2 inhibitors or glucagon-like peptide 1 receptor agonists (GLP-1RAs) for glucose intolerance, and low-dose aspirin for prevention of arterial thrombosis [73]. Melatonin provides an innovative approach in the management of MetS through its useful effects on circadian rhythmicity, insulin resistance, dyslipidemia, high blood pressure, weight loss, and improving the antioxidative status (melatonin tablet 1 mg/kg) [74, 75]. Melatonin reduces the toxicity of many pharmaceutical agents and has a high safety profile [76].

5. Conclusion

Prevention is the best form of treatment of metabolic syndrome, and it starts from childhood by following a healthy lifestyle based on proper nutrition, exercise, and adequate early night sleep. Timing of food consumption, food quality, food quantity, light exposure, medication intake, and sleep are likely to play an important role in human health. Eat small portion sizes of food, and follow the rhythm of the day to maintain the synchronicity of the biological clock. Consuming natural effective supplements rich in bioactive substances leads toward the optimization of biochemical parameters of patients with metabolic syndrome in favor of a healthy outcome. Bariatric surgery should be considered in individuals with morbid obesity or obesity associated with comorbidity.

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Abbreviations

ASCVD	atherosclerotic cardiovascular disease
α-Syn	alpha-synuclein
AD	Alzheimer's disease
Aβ	amyloid beta
BEE	basal energy expenditure
BMI	body mass index
C1P	cermide-1-phosphate
CERK	ceramide kinase enzyme
CRP	C-reactive protein
DBP	diastolic blood pressure
DM	diabetes mellitus
GLP-1RAs	glucagon-like peptide 1 receptor agonists
GH	growth hormone
HDL-c	high-density lipoprotein cholesterol
hs-CRP	high-sensitivity C-reactive protein
IL-6	interleukin-6
IL-10	interleukin-10
IR	insulin resistance
LDL-c	low-density lipoprotein cholesterol
MedDiet	Mediterranean diet
MetS	metabolic syndrome
M.HOMA-IR	modified homeostatic model assessment of insulin resistance
MMSE	mini-mental state examination
SBP	systolic blood pressure
SCN	suprachiasmatic nucleus
TG	triglycerides
TNF-α	tumor necrosis factor-α
WC	waist circumference

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Section 5

Hypothyroidism

Chapter 8

Hypothyroidism

Mauricio Alvarez Andrade and Oscar Rosero Olarte

Abstract

Hypothyroidism is a condition that results from thyroid hormone deficiency that can range from an asymptomatic condition to a life-threatening disease. The prevalence of hypothyroidism varies according to the population, from up to 3 to 4% in some populations and in the case of subclinical hypothyroidism up to 5–10%. Clinical symptoms of hypothyroidism are diverse, broad, and non-specific and can be related to many systems, reflecting the systemic effects of thyroid hormones. The severity of the symptoms is usually related to the severity of the thyroid hormone deficit. The most common form of hypothyroidism, primary hypothyroidism, is diagnosed when there is elevation of TSH and decrease in the level of free T4 and Subclinical hypothyroidism is diagnosed when there is an elevation of TSH with normal levels of free T4. The most frequent cause of primary hypothyroidism in populations without iodine deficiency is Hashimoto's thyroiditis or chronic lymphocytic thyroiditis. Iodine deficiency is the main cause of hypothyroidism in populations with deficiency of iodine intake. The treatment of choice for hypothyroidism is thyroxine (T4), which has shown efficacy in multiple studies to restore the euthyroid state and improve the symptoms of hypothyroidism. In subclinical hypothyroidism, the treatment depends on the age, functionality, and comorbidities of the patients. The total replacement dose of levothyroxine in adults is approximately 1.6 mcg/kg; however in elderly patients with heart disease or coronary heart disease, the starting dose should be from 0.3 to 0.4 mcg/kg/day with progressive increase of 10% of the dose monthly.

Keywords: hypothyroidism, autoimmune, Hashimoto's disease, thyroxine, thyroid diseases

1. Introduction

Hypothyroidism is a common disease in many populations, and prevalence varies depending on the sex and the age of the population studied, the iodine status of the population, and the cut points used to define overt and subclinical hypothyroidism [1–11].

Hypothyroidism is 10 times more prevalent in women than in men [2]. It is more prevalent in the elderly people, ranging from 2 to 5% of the population. In iodine-sufficient populations, hypothyroidism ranges from 1 to 2%, while in iodine deficient areas, the prevalence can be as high as 3–4% [2–14]. Prevalence in the USA in the Colorado trial can range from 0.3% and 3.7%, while in Europe can be 0.2 and 5.3% [12].

The prevalence of subclinical hypothyroidism is even higher; in one of the largest studies in the USA, it reaches 9% of the population [12].

2. Physiology

Thyroid word comes from the Greek shield because of its shape. The weight is from 10 to 20 g. Changes of thyroid shape and volume are dependent on age and sex. In the adult population, the usual length is 40–60 mm and the diameter 13–18 mm. The volume is 10–15 ml for females and 12–18 ml for males [15, 16].

The thyroid gland is composed of functional secretory units known as follicles where thyroid hormones are synthetized and storage in the follicular cells and the lumen of the follicles where colloid is stored. The colloid contains high amounts of thyroglobulin (Tg), a 660 kDa glycoprotein, where thyroid hormones are stored [17].

Thyroid hormones are composed by an inner ring, the tyrosine molecule, and the outer ring with a phenyl ring. The active forms of thyroid hormones are thyroxine or T4, with four iodine atoms, and triiodothyronine or T3, with three iodine atoms. Under physiologic states, 90% of thyroid gland output is T4 and 10% is T3. The half-life of thyroid hormones is of few hours for T3 and 7 days for T4 [17].

The follicular cells contain a sodium iodide active symporter responsible for iodine transport against a concentration gradient to synthesize thyroid hormones; it can increase iodine concentration in the follicular cell by more than 20 times above the serum concentration. Iodine is then transported by pendrin in the apical membrane to the colloid in a passive manner [17].

Iodine must be organified to be attached to the tyrosine residues of thyroglobulin. The thyroid peroxidase (TPO) is the enzyme of the selenoprotein group, with hydrogen peroxide organifying iodine to thyroglobulin. Once in the colloid, iodine is organifid to thyroglobuline to produce, monoiodothyrosine and diiodothyrosine wich finally are coupled to produce T4 and T3, this process is catalyzed by the TPO [17].

TPO activity is regulated by iodine concentration and can be blocked by an excess of iodine concentration, which is known as the Wolff-Chaikoff effect and can lead to a temporary hypothyroidism with an escape mechanism. On the other side, an iodine-depleted thyroid gland that is exposed to iodine can increase thyroid synthesis, which is known as the Jod-Basedow effect, and lead to hyperthyroidism [17].

TPO is the enzyme related to autoimmune hypothyroidism as most of the patients have positive anti-TPO antibodies [16, 17].

2.1 Regulation of thyroid hormones production

Thyroid hormone production is regulated by the hypothalamus pituitary axis. At the hypothalamus, thyrotropin-releasing hormone (TRH) is produced. TRH stimulates the pituitary to produce thyroid-stimulating hormone (TSH) [17].

What trigger thyroid hormone synthesis and release is the stimulation of the TSH in the basolateral receptor of the follicular cell, which is a receptor of the seven transmembrane domain G protein-coupled receptors proteins. The effects of the TSH receptor in the follicular cells are derived to the increased concentration in intracellular cyclic adenosine monophosphate (cAMP) that results in increased iodine uptake and increased protein synthesis to enhance the production of thyroid hormones as well as produce a trophic effect at the thyroid gland [17].

3. Definitions

Hypothyroidism is a condition that results from thyroid hormone deficiency which can range from an asymptomatic condition to a life-threatening condition of the patient life [17–19].

Hypothyroidism can be primary due to a decrease in the production of thyroid hormones in the thyroid gland, secondary due to deficit in the production of TSH in the pituitary or tertiary due to a deficit in the production of TRH in the hypothalamus.

3.1 Subclinical hypothyroidism

Subclinical hypothyroidism is a biochemical diagnosis in which there is an elevation of TSH with a normal level of free thyroid hormones in plasma.

4. Manifestations of hypothyroidism

4.1 Adults

Clinical symptoms of hypothyroidism are diverse, broad, and neither sensitive nor specific to make the diagnosis of hypothyroidism and can be related to many systems, reflecting the systemic effects of thyroid hormones. The severity of the symptoms is usually related to the severity of the thyroid hormone deficit.

Systemic symptoms include lethargy, cold intolerance, goiter, and weight gain. At the cardiovascular system hypothyroidism can produce bradycardia, cardiac failure, angina, and pericardial effusion. Gastrointestinal symptoms are constipation and ileus, neuromúscular manifestations include myalgia, hoarse voice, slow relaxing reflexes, depression, emotional lability, psychosis an carpal tunnel syndrome. Hematologic changes include macrocytic anemia, pernicious anemia, and iron deficiency anemia. Skin manifestations include, myxoedema, hair loss, and coarse skin. In the reproductive system menorrhagia and infertility and finally, hyperlipidaemia as the main metabolic manifestation [17–21].

4.2 Myxedema

Myxedema coma is a severe state of hypothyroidism and an endocrine emergency. Manifestations are depressed mental state and hypothermia with a hypometabolic state with bradycardia. Decreased myocardial contractility and pericardial effusion lead to hypotension. Other features are anemia, hyponatremia, and renal dysfunction [22].

4.3 Congenital hypothyroidism

Thyroid hormones are necessary to have a normal neurodevelopment and growth. Symptoms and signs of congenital hypothyroidism are goiter, poor feeding, macroglossia, prolonged jaundice, developmental delay hypothermia, bradycardia, edema, large fontanelles, umbilical hernia, and poor growth [23].

5. Etiology

5.1 Diagnosis

The diagnosis of hypothyroidism is made biochemically. The elevation of TSH levels associated with low levels of free T4 confirms the diagnosis of primary hypothyroidism; in this case it is not necessary to measure levels of free T3 or T3 because in the state of hypothyroidism the peripheral conversion of T4 to T3 is increased so that T4 will be more diminished than T3 [18, 19, 24].

In secondary hypothyroidism, decreased T4 is found, and TSH is low or normal (not elevated), which means that the pituitary is not responding adequately to a deficit of thyroid hormones.

Subclinical hypothyroidism is diagnosed in patients with elevated TSH despite having normal levels of free T4. These patients may or may not be symptomatic [18, 19, 24].

Although the differential diagnosis in hypothyroidism involves multiple pathologies, with symptoms and signs related to hypothyroidism such as anemia and hyponatremia, among others, the differential diagnosis must also be among the various pathologies that produce hypothyroidism that will be discussed in the etiology section [25, 26].

However, it is important to take into account the sick euthyroid syndrome that refers to alterations in thyroid function tests that can be found in patients with critical illness and can vary depending on the severity and duration of the disease.

In laboratory alterations, there is a decrease in T3 and a smaller proportion of T4, due to an increase in the activity of reverse T3. Subsequently, there is a progressive decrease in TSH followed by a progressive elevation and finally normalization of all thyroid function tests once the injury is resolved [25, 26].

6. Differential diagnosis

6.1 Primary hypothyroidism

6.1.1 Chronic autoimmune thyroiditis or Hashimoto's thyroiditis

It is the most prevalent cause of hypothyroidism in iodine-sufficient countries.

Chronic autoimmune thyroiditis can be goitrous or atrophic. Goitrous hypothyroidism is called Hashimoto thyroiditis [27].

More than 90% of patients have elevated anti-thyroglobulin or anti-peroxidase (microsomal antigen) or anti-sodium iodine transporter. Antibodies against thyroid gland produce chronic autoimmune thyroiditis with lymphocytic infiltration and fibrosis, leading to goiter or atrophy of the thyroid gland [27–29].

Women are five times more affected than men. After the age of 45, the rates of hypothyroidism increase [27].

Based on one of the most representative studies of the population of the USA that included 17,353 people, it found a prevalence of 4.6% of hypothyroidism, 0.3% frank hypothyroidism and 4.3% subclinical hypothyroidism [30].

The course of chronic autoimmune thyroiditis is a gradual loss of thyroid function. The spectrum ranges from subclinical hypothyroidism with positive antibodies to frank hypothyroidism, a process that affects approximately 5% patients per year.

The majority of patients present hypothyroidism for life; however it may be transient [28].

The risk factors for Hashimoto's thyroiditis are multiple; the female gender and the older age are two risk factors. There is a genetic factor associated with multiple polymorphisms in human leukocyte antigen (HLA) genes, T cell receptors, and immunomodulatory molecules. Patients with Down or Turner syndrome have a higher prevalence. Chronic autonomic thyroiditis can be part of the autoimmune type 2 polyglandular syndrome, and affected patients are more likely to develop other autoimmune diseases such as diabetes and adrenal insufficiency [27, 31, 32].

6.1.2 Iodine deficiency

Iodine deficiency, defined as the daily intake of less than 100 mcg of iodine, is the most common cause of hypothyroidism worldwide and the most prevalent cause of hypothyroidism in populations with iodine deficiency. As previously described, excess iodine can produce hypothyroidism due to Wolff-Chaikoff effect; however this effect is usually transient [33, 34].

6.1.3 Iatrogenic disease

Post-thyroidectomy state, radioactive iodine therapy for thyroid cancer, or hyperthyroidism and neck radiation at doses greater than 25 Gy are the main causes of iatrogenic hypothyroidism, and as the use of these therapies increases, it becomes a more prevalent etiology [35, 36].

6.1.4 Drugs

Thionamides, lithium, tetracyclines, thalidomide, ethionamide, and iodine-containing drugs like amiodarone can cause hypothyroidism. Thyrosine kinase inhibitors can cause thyroiditis; sorafenib can produce hypothyroidism by increased type 3 deiodination. Immune therapy including pembrolizumab, nivolumab, ipilimumab, alemtuzumab, interleukin 2, and interferon alfa can produce hypothyroidism [37].

6.2 Thyroiditis

6.2.1 Subacute or granulomatous thyroiditis

Subacute or granulomatous thyroiditis is an acute inflammation of the thyroid gland of viral etiology that presents with hyperthyroidism, followed by hypothyroidism and subsequent recovery of thyroid function. Transient hypothyroidism usually lasts from a few weeks to a maximum of 3–6 months and may be permanent in 5% of patients [38, 39].

The presentation consists of acute pain in the thyroid region that increases when swallowing or moving the head and radiates to the jaw. Symptoms may vary depending on whether the patient is in the hyperthyroid, euthyroid, or hypothyroid category [38, 39].

The findings in thyroid gammagraphy are compatible with thyroiditis due to a decrease in iodine uptake diffusely [38, 39].

6.2.2 Silent thyroiditis or postpartum thyroiditis

Silent thyroiditis or postpartum thyroiditis corresponds to a thyroiditis of autoimmune etiology, which occurs in the first year postpartum. It presents with hyperthyroidism in up to 30% of patients, followed by hypothyroidism in up to 50% of patients; however it can only be present as hypothyroidism or hyperthyroidism [40–42].

It is more frequent in patients with type 1 diabetes mellitus and patients with positive anti-peroxidase antibodies. The prevalence can reach up to 17%. It has been associated with deterioration or onset of postpartum depression. Up to 30% of patients remain hypothyroid [40–42].

6.2.3 Infiltrative diseases

Riedel's thyroiditis is a fibrosclerosing thyroiditis of unknown etiology, with a probable primary anti-immune or fibrotic origin similar to retroperitoneal fibrosis, fibrosing mediastinitis, sclerosing cholangitis, and lacrimal fibrosis, among others [43, 44].

Riedel's thyroiditis is characterized by slow, non-painful growth, sensation of pressure in the neck, dysphagia, dysphonia, and hypoparathyroidism. From 30 to 60% of patients present clinical or subclinical hypothyroidism. It is one of the IgG4-related disease varieties, together with Fibrosing hashimoto thyroiditis, Igg4-related Hashimoto's disease, and Graves' disease associated with IgG4 [43–45].

Other infiltrative diseases like hemochromatosis, scleroderma, leukemia, cystinosis, *M. tuberculosis* infection, and *P. carinii* are less frequent causes of hypothyroidism.

6.3 Secondary hypothyroidism

Central hypothyroidism is a much less frequent form of hypothyroidism, with a prevalence of 1:16,000 to 1:100,000 in the general population. It can be congenital or acquired [46].

The causes of acquired central hypothyroidism are usually related to the causes of hypopituitarism like a pituitary sellar region mass, usually a pituitary adenoma, which produces secondary hypothyroidism by thyrotropic cell compression, or tertiary hypothyroidism with decreased production of TRH by the hypothalamus. Other lesions of the sellar region, such as meningiomas, cysts, abscesses, metastasis, craniopharyngiomas, and dysgerminomas, can produce central hypothyroidism [46, 47].

In addition to space-occupying injuries, radiation with doses greater than 40 Gray performed for brain, orbital, or nasal lesions can produce central hypothyroidism [46, 47].

Other less frequent causes of central hypothyroidism are hypophyseal infiltrative pathologies such as haemochromatosis, sarcoidosis or tuberculosis, cranial trauma, Sheehan syndrome, and the use of drugs as checkpoint inhibitors [46, 47].

7. Treatment

7.1 Clinical hypothyroidism

Since the nineteenth century, levothyroxine has been used for the treatment of hypothyroidism. Usually hypothyroidism requires treatment with lifelong hormone replacement with levothyroxine. In a few cases, transient hormonal substitution due to transient secondary hypothyroidism is required, for example, to subacute thyroiditis or drug-induced hypothyroidism [48–50].

The treatment of choice is thyroxine (T4), which has shown efficacy in multiple studies to restore the euthyroid state and improve the symptoms of hypothyroidism [51–54].

The goal of treatment of primary hypothyroidism is to take the patient to the normal range of TSH. However, the normal range of TSH varies depending on the age and population studied. Most people are in the range of 0.5–4.5 mU/L; however as the age of people increases, the normal range of TSH increases, leading to values of up to 7.0 mU/L in those over 90 years. In contrast, most young and healthy patients are in the range of 0.5–2.5 mU/L [50].

For this reason, in the treatment of hypothyroidism, the dose of levothyroxine and the goal of TSH depend on the age of the patient and the comorbidities [49, 50].

Levothyroxine (T4) is a prohormone and requires deionization to T3 which is the active form of thyroid hormone. Levothyroxine is absorbed in the small intestine. The meal affects the time of maximum concentration, which in normal conditions is 2 h. The bioavailability is from 60 to 80%. The metabolism is catabolized by the thyroid deionidase enzyme that removes the iodine from carbon 5 of the outer ring to transform T4 into T3. Approximately half of T4 is deionized to rT3 (inactive form) and half to T3 (active form). Both T3 and reverse T3 are metabolized to diiodothyronine (T2) and monoiodothyronamine (T1) and T2 and T1 reverses [48].

Multiple medications interact with the function or pharmacokinetics of levothyroxine, amiodarone, androgens, calcium carbonate and citrate, carbamazepine, cholestyramine, ferrous sulfate, glucocorticoids, orlistat, phenytoin, proton pump inhibitors, salicylates, sucralfate, and tamoxifen, which are just some of the medications that alter bioavailability, metabolism, protein binding, or hormone levels [48].

The total replacement dose of levothyroxine in adults is approximately 1.6 mcg/kg, given that the body's requirements for thyroid hormones are proportional to weight. In healthy young patients, the starting dose could be 1.6 mcg/kg/day; however in elderly patients with heart disease or coronary heart disease, the starting dose should be from 0.3 to 0.4 mcg/kg/day with a progressive increase of 10% in the dose every 4–6 weeks [50]. Levothyroxine must be taken with empty stomach 30–60 min before the next meal, usually, breakfast.

The thyroid function is monitored with TSH at 4–6 weeks after starting treatment. If the TSH goal is not achieved, the dose of levothyroxine should be adjusted by increasing or decreasing 10% of the dose ideally, especially in older adults [50].

In the case of secondary hypothyroidism, TSH levels are low or inappropriately normal for a low free T4. Therefore, the follow-up is not done with TSH levels but with free T4 levels, to achieve a normal level for the reference range.

7.2 Subclinical hypothyroidism

In the case of subclinical hypothyroidism, the treatment also depends on the age, functionality, and comorbidities of the patients [50].

For patients younger than 75 years, with TSH greater than 10 mU/L, treatment is recommended. However, in those patients with TSH between 4.5 and 10 mU/L, treatment depends on the presence of symptoms and especially the presence of goiter or anti-TPO antibodies, which predict progression to clinical hypothyroidism [54–56].

In patients older than 75 years, treatment depends on the patient's frailty and should be limited to functional patients with TSH greater than 10 mU/L or patients with TSH of 6–10 mU/L in the presence of antithyroid antibodies, symptoms, and concomitant diseases in that they can be impaired by hypothyroidism such as heart failure. Fragile patients, more than 75 years old, may be advisable to be observed without treatment [50].

8. Conclusion

Hypothyroidism is a highly prevalent chronic disease, widely studied by medical science, with a wide spectrum of severity, ranging from subclinical hypothyroidism to the hypothyroid myxedematous state. In some cases of subclinical hypothyroidism, treatment may not be necessary; however in other cases such as in myxedematous states, the treatment may be lifesaving. There are multiple trials

that evaluate the treatment of hypothyroidism in different populations, and there is still controversy regarding the treatment of subclinical hypothyroidism in some populations. It is very important for the primary care physicians to have a broad knowledge of hypothyroidism since they will face hypothyroid patients in the day-to-day clinical practice.

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Section 6

Uremia and Lipid Disorders

Chapter 9

Lipid Disorders in Uremia

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Abstract

Lipoprotein has important physiologic functions within the human body. Many enzymes, enzyme activators, and protein parts, such as apolipoproteins and specific hepatic and extrahepatic receptors, are involved in their metabolism. Renal failure is associated with an increased risk of cardiovascular disease. One of the main mechanisms underlying this increased cardiovascular risk is dyslipidemia. Abnormal lipoprotein profiles are generally a combination of abnormalities of all fractions. Uremic lipoprotein profile includes increased triglyceride-rich lipoproteins, small dense LDL particles, increased lipoprotein (a), and decreased HDL. Enhanced oxidative stress and uremic environment can strongly modify plasma lipoproteins, changing their interactions with biological functions and especially cardiovascular physiology. This profound lipoprotein disorder has led to the formulation of an accelerated atherogenesis hypothesis and has been commonly linked with their metabolic alteration associated with uremia.

Keywords: chronic uremia, lipoproteins, dyslipidemia, cardiovascular risk

1. Introduction

Urea cycle is one of the most important pathways in the human body. The continuous degradation and synthesis of cellular proteins occur in all forms of life. High rates of protein degradation occur in tissue undergoing structural rearrangements.

Approximately 75% of liberated amino acids are reutilized. Since the excess amino acids are not stored, those not immediately incorporated into new proteins are degraded rapidly. The excess nitrogen from amino acids forms urea. As a hydrosoluble compound, urea is excreted by the kidney. Uremia is a clinical syndrome marked by elevated concentrations of urea in the blood and is associated with many metabolic disorders such as acidosis, abnormalities in lipids, mineral and homocysteine metabolism, oxidative stress, chronic inflammation, insulin and erythropoietin resistance, vitamin D deficiency, and malnutrition. Uremia more commonly develops with chronic kidney disease (CKD), but it also may occur with acute kidney injury if loss of renal function is rapid. Nearly all body organs and systems are affected by the toxicity of uremic compounds retained in the course of renal dysfunction. According to the European Uremic Toxin Work Group, uremic toxins are defined as accumulated solutes, normally excreted by the kidneys, that interact negatively with biological functions [1]. This has shown the need for the search for new uremic compounds, combining them into panels of substances involved in the pathophysiological processes. As example we can mention uridine adenosine, a strong vasoconstrictor, which is considered as a new uremic toxin. It has been demonstrated that uremic patients have increased levels of uridine adenosine, which can influence blood pressure, proliferation rate of vascular smooth

muscle cells, and vascular calcification [2]. All these effects correlated with vascular dysfunctions and development of atherosclerosis. As uremic toxins are considered some components, which concentrations are not directly associated with glomerular filtration, but interacts negatively with vascular physiology. Several acute-phase proteins, IL-1, IL-6, IL-12, α2-macroglobulin, fibrinogen, and myeloperoxidase, together with endothelium-related proteins, such as vascular cell adhesion molecule 1, vascular endothelial growth factor 1, and soluble vascular endothelial growth factor receptor, increased in CKD and play a crucial role in endothelium dysfunction promoting the development of atherosclerosis. Renal failure is associated with an increased risk of cardiovascular disease [3, 4]. One of the main mechanisms underlying this increased cardiovascular risk is dyslipidemia [2]. In uremic environment lipids are affected by oxidative stress. The end products of lipid peroxidation process affect the circulating lipoproteins, lipidic and proteinic, leading to profound alterations of their biological properties, changing their interactions with biological functions and especially cardiovascular physiology. For this reason, lipoproteins, in renal failure, can be also considered as uremic toxins.

In the human body, dietary lipids absorbed from intestine and lipids synthesized by the liver and adipose tissue must be transported between the various tissues and organs for utilization and storage. Since lipids are insoluble in water, the problem on how to transport them in aqueous blood plasma is solved by associating nonpolar lipids (triacylglycerol and cholesterol esters) with amphipathic lipids (phospholipids and cholesterol) and proteins, to form water-soluble particle known as lipoproteins.

The plasma lipoproteins are classified as chylomicrons and very-low-density (VLDL), intermediate-density (IDL), low-density (LDL), and high-density (HDL) lipoproteins, according to their ultracentrifugation characteristics. Chylomicrons and VLDL serve as vehicles to transport triglycerides to the sites of consumption, as myocytes and suprarenal glands or storage in adipocytes. HDL fraction serves as a vehicle to transport surplus cholesterol from peripheral tissues to the liver for disposal. Many enzymes, enzyme activators, and protein parts, such as apolipoproteins and specific hepatic and extrahepatic receptors, are involved in lipoprotein metabolism.

Apolipoproteins (Apo), the protein part of lipoproteins, are present in each lipoprotein and carry out several roles. They can be part of the structure of lipoproteins, serve as an enzyme cofactors or inhibitors, and finally act as ligands for interaction with lipoprotein receptor in tissue. Apolipoproteins of HDL are designated as A (A-I, A-II, A-IV). Apo A-I is an activator of enzyme lecithin-cholesterol acyltransferase (LCAT) and serves as a ligand for HDL binding to specific scavenger receptor B1 (SR-B1). Apo A-II is an inhibitor of enzyme lipoprotein lipase. The main apolipoprotein of LDL and VLDL is Apo B-100, while the chylomicrons contain Apo B-48. Apo B-100 acts as ligand of LDL for LDL receptors in the liver and extrahepatic tissue. Apo B-48 is part of the structure of chylomicrons. Apo E is found in chylomicrons, VLDL, and HDL, and its role is to uptake the remnant of chylomicrons by a receptor specific for apolipoprotein E, in the liver. Apo C-I, Apo C-II, and Apo C-III are transferable between several different lipoproteins. Apo C-II is activator, whereas Apo C-III is an inhibitor for enzyme lipoprotein lipase. The Apo C-I is an inhibitor for enzyme cholesteryl ester transfer protein (CETP).

The main enzymes involved in lipoprotein metabolism are lipoprotein lipase (EC 3.1.1.34), hepatic lipase (EC 3.1.1.3), lecithin-cholesterol acyltransferase (EC 2.3.1.43), and acyl-CoA cholesterol acyltransferase (ACAT) (EC 2.3.1.26).

Lipoprotein lipase is located on the walls of blood capillaries of the heart, adipose tissue, spleen, lung, renal medulla, aorta, lactating mammary gland, and diaphragm. It is abundantly produced as an inactive enzyme by myocytes, adipocytes,

and several other cell types. The inactive enzyme requires sequential glycation and cleavage of a 27-amino acid peptide to become functionally active. The role of lipoprotein lipase is the hydrolysis of triglyceride-rich lipoproteins, as chylomicrons and VLDL. Apo C-II and phospholipids are cofactors for enzyme activity, while Apo A-II and Apo C-III act as inhibitors.

Hepatic lipase is bound to the surface of hepatic cells. Hepatic lipase catalyzes hydrolysis and removal of the triglyceride content of HDL and chylomicron remnant. Accordingly, hepatic lipase plays a central role in the metabolism of chylomicron remnants and HDL.

LCAT is the enzyme of HDL, which is activated by Apo A-I, the structural protein of HDL. The enzyme plays an important role in HDL-mediated cholesterol uptake from the extrahepatic tissues and, as such, serves as a main determinant of HDL maturation and plasma HDL cholesterol level.

The formation of cholesteryl esters from cholesterol and long-chain fatty-acyl-coenzyme A catalyzes the enzyme called ACAT. It is a membrane-bound protein and, at the single-cell level, serves as a regulator of intracellular cholesterol homeostasis. In addition, ACAT supplies cholesteryl esters for lipoprotein assembly in the liver and small intestine.

Cholesteryl ester transfer protein is a hydrophobic glycoprotein that is secreted mainly from the liver and circulates in the plasma, bounded mainly to HDL [5]. It mediates cholesterol ester transfer from HDL to IDL in exchange for triglycerides. CETP promotes the transfer of cholesteryl esters from anti-atherogenic HDLs to pro-atherogenic Apo B-containing lipoproteins, including VLDL, VLDL remnants, IDL, and LDL. In this way CETP transfers lipids from one lipoprotein particle to another in a process that results in equilibration of lipids between lipoprotein fractions.

2. Lipoprotein metabolism

2.1 The metabolism of chylomicrons

The exogenous pathway of lipid metabolism begins with chylomicrons. Chylomicrons are responsible for the transport of all dietary lipids into the circulation. They are produced within the enterocytes containing triglycerides, cholesterol ester, and phospholipids. Apo B-48 is essential for chylomicron formation. The nascent chylomicrons, from the small intestine, are released into the circulation via the lymphatic system. In the circulation, the nascent chylomicrons acquire Apo E and Apo C-II, which are in the surface of HDL. Apo C-II is an activator for enzyme lipoprotein lipase. The endothelium binding accommodates interaction of chylomicrons with the endothelium-bound lipoprotein lipase. Reaction with lipoprotein lipase results in the loss of approximately 90% of triglycerides in chylomicrons. The majority of fatty acids released diffuse into the adjacent myocytes for energy production or into adipocytes for energy storage. After hydrolysis chylomicron remnants are subsequently cleared by the liver and other tissues. Uptake is mediated by a receptor specific for Apo E. Both the LDL (Apo B-100 and Apo E) receptor and LDL receptor-related protein (LRP), specific for Apo E, are believed to take part. Chylomicron remnants return the borrowed Apo C- II and Apo E to HDL before their uptake by the liver and other tissues (**Figure 1**).

2.2 The metabolism of VLDL, IDL and LDL

VLDL particles are produced by the liver and are precursor of IDL and LDL. VLDL serves as the vehicle for delivery of endogenous lipids, endogenous

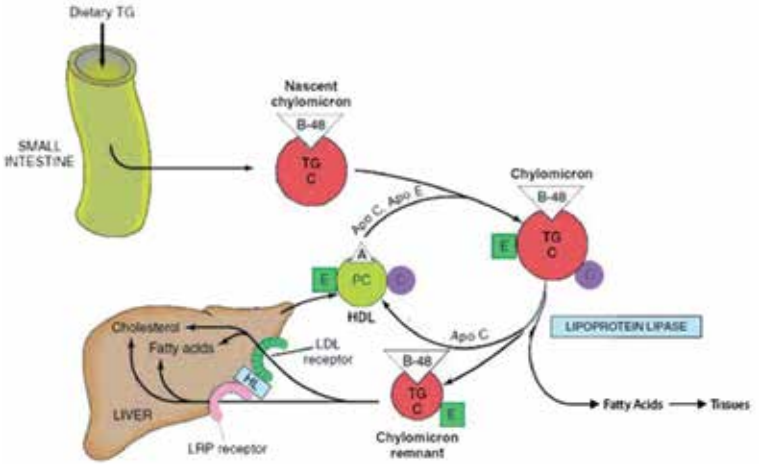

Figure 1.
Chylomicron metabolism. Chylomicrons, from the small intestine, are released into the circulation by apolipoprotein B-48 (B48). Nascent chylomicrons acquire apolipoprotein (Apo) E (green square) and C-II (purple circle), which are in the surface of HDL. Apo A-I (white triangle marked with a) is a main apolipoprotein of HDL. Apo E and Apo C-II are necessary for activation of lipoprotein lipase and for uptakes of remnant chylomicrons by an LDL receptor and LDL receptor-related protein. TG-triglycerides, C-cholesterol, P-phospholipids.

triglycerol, and cholesterol, to the peripheral tissues. Nascent VLDL is formed within the hepatocyte and Apo B-100. Those are triglyceride-rich lipid droplet, followed by the addition of Apo E, Apo A-I, and Apo A-II. The triglycerides and cholesterol ester used by hepatocytes for incorporation into VLDL are generated by the enzymes acyl-CoA diacylglycerol acyltransferase (DGAT; EC 2.3.1.20) and ACAT. Apo C-II and Apo E, borrowed from HDL, are important for subsequent metabolism of VLDL by lipoprotein lipase and the VLDL receptor. Enzyme lipoprotein lipase is activated by the apolipoprotein C-II, and this is followed from the hydrolysis of VLDL triglycerides by the activated enzyme, leading to release fatty acids, which diffuse into the adjacent myocytes or adipocytes for energy production or storage. Lipolysis of VLDL results in reduction in their triglyceride content and detachment and release of a remnant particle, known as IDL. IDL particles may undergo further lipolysis via hepatic triglyceride lipase. Apo E serves as a ligand for remnant VLDL or IDL binding to specific receptors in the liver. This leads to the extraction of nearly all remaining triglycerides from IDL by the liver and formation of cholesterol-rich LDL. LDL particles are then removed via LDL (Apo B-100) receptor by the liver, as well as extrahepatic tissue (**Figure 2**).

2.3 The metabolism of HDL

HDL is synthesized and secreted from the liver and intestine. A major function of HDL is to act as a repository for the Apo C-II and Apo E, for metabolism of triglyceride-rich lipoproteins, chylomicrons, and VLDL. Also, the primary function of HDL is retrieval and transport of cholesterol from the tissue to the liver which is known as reverse cholesterol transport. This cycle is very important for cellular cholesterol homeostasis. The principal apolipoprotein constituents of HDL are Apo A-I and Apo A-II. As the main structural constituent of HDL, Apo A-I is the activator of enzyme LCAT. LCAT system is involved in HDL-mediated removal of excess unesterified cholesterol from triglyceride-rich lipoproteins and tissues. Apo A-II serves as an activator of hepatic lipase, which plays a central role in the removal of HDL triglycerides by the liver. HDL-mediated removal of surplus

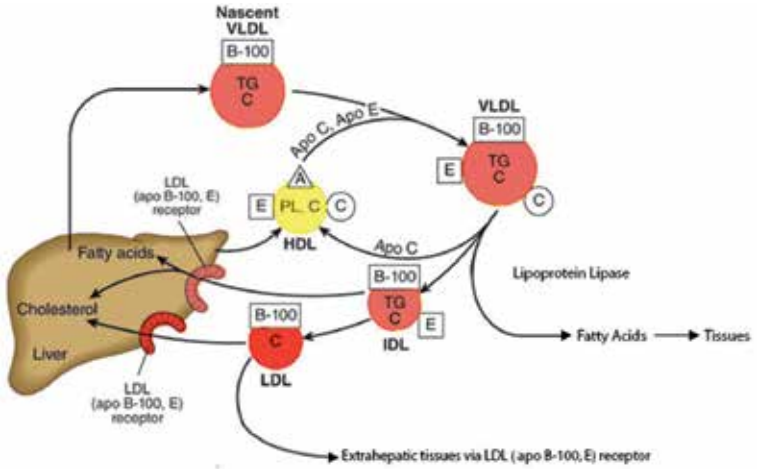

Figure 2.
Very-low-density lipoprotein metabolism. In circulation VLDL are transformed into intermediate-density lipoprotein after lipoprotein lipase activation by apolipoprotein C-II. IDL are removed by hepatic LDL receptors specific for apolipoprotein B and E. Apo E and Apo C-II are borrowed from high-density lipoprotein. A, Apolipoprotein a; B-100, Apolipoprotein B-100; C, Apolipoprotein C; E, Apolipoprotein E; LDL, low-density lipoproteins; TG, triglycerides; C, cholesterol; P, phospholipids.

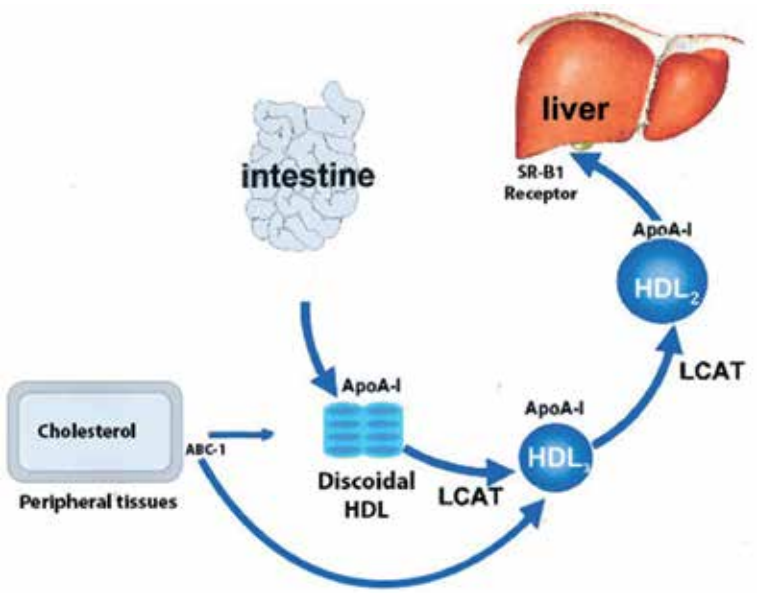

Figure 3.
High-density lipoprotein metabolism. As the main structural constituent of HDL, apolipoprotein A-I (Apo A-I), is the activator of enzyme lecithin-cholesterol acyltransferase. LCAT system is involved in HDL-mediated removal of excess unesterified cholesterol from tissues and its esterification. Scavenger receptor B1,and ATP-binding cassette transporter type I.

cholesterol from extrahepatic tissues requires attachment of nascent HDL to the ATP-binding cassette transporter type I (ABCA1). Binding to ABCA1 appears to trigger active transfer of phospholipids to nascent HDL, a step which is necessary for efficient translocation of free cholesterol from adjacent caveolae to the surface of HDL. Free cholesterol reaching the surface of HDL moves to the core of HDL. In this process nascent discoidal HDL is transformed into spherical HDL 3. After being accepted by HDL3, the free cholesterol is then esterified by LCAT to

cholesterol esters, increasing the size of the particles to form the less dense HDL2. In the next step, HDL2, released in circulation, participates in a series of elaborate exchanges of apoproteins and lipids with the Apo B-containing lipoproteins such as chylomicrons, VLDL, and IDL, before reaching the liver. Actually, HDL in circulation receives triglycerides from Apo B-containing lipoproteins in exchange for cholesterol esters, a process catalyzed by CETP. Finally, HDL-2, via Apo A-I, binds to the scavenger receptor B1, which has been identified as a HDL receptor in the liver. The cycle is completed by the reformation of HDL 3, either after selective delivery of cholesteryl esters to the liver via SR-B1 or by hydrolysis of HDL2 phospholipids and triglycerides by hepatic lipase. Released, free Apo A-I forms preβ-HDL with the minimum amount of phospholipid and cholesterol. Preβ-HDL is considered the most potent form of HDL in inducing cholesterol efflux from the tissues to form discoidal HDL (**Figure 3**).

3. Oxidative stress, lipid peroxidation, and lipoprotein modifications

CKD is associated with increased oxidative stress, which promotes covalent modifications of lipids and lipoproteins. Oxidative stress is an imbalance in the reactive oxygen species (ROS) production and their degradation ratio. ROS include various compounds such as superoxide anions, hydroperoxide, and hydroxyl radical. These compounds are produced under physiologic conditions, during energy production in mitochondria by reducing oxygen during aerobic respiration.

But excessive ROS levels may have a harmful effect on tissue function and structure, because of their interaction with different biomolecules in the human body, such as nucleic acids, proteins, and lipids. This interaction results with oxidative modifications of these biomolecules.

Under physiologic conditions, the production of ROS is balanced by antioxidant mechanisms that protect the cells from oxidative damages. The antioxidant mechanisms include enzymes; superoxide dismutase (SOD, EC 1.15.1.1) which catalyzes the dismutation of $O2^{\bullet -}$ into H_2O_2; and glutathione peroxidase (GPX, EC 1.11.1.9), which detoxifies H_2O_2 and other hydroperoxides. Reduced glutathione (GSH), as a non-enzymatic antioxidant, allows the scavenging of OH. The redox reactions are catalyzed by glutathione peroxidase. In antioxidant mechanisms also included several compounds such as HDL, albumin, tocopherols, ferritin, ceruloplasmin, transferrin, ubiquinol, flavonoids, and carotenoids.

HDL is well known for its protective antioxidant properties. Protein paraoxonase-1 (PON1, EC 3.1.8.1), bound to HDL, exhibited antioxidant effects, against lipid peroxidation. Selenium Glutathione-peroxidase 3, also known as glutathione peroxidase 3 (GPX3, EC 1.11.1.9), is another antioxidant enzyme, which is associated with HDL. Besides many functions in the human body, albumins are known for the antioxidant function too. In the first place concerning the lipid peroxidation, albumin can scavenge hypochlorous acid, responsible for chlorination of proteins mediated by myeloperoxidase, and through its reduced cysteine residue can scavenge hydroxyl radicals. One of the physiological functions of albumins is the transportation of insoluble components, through the blood plasma. In this way, albumins bind the long-chain fatty acids (LCFA), polyunsaturated fatty acids (PUFAs), and cholesterol and in the circulation, preventing them from oxidative modifications. Albumins bind also the ligands such as copper, iron, α-tocopherol, bilirubin, and homocysteine and prevent their antioxidant damages. Tocopherol is an important antioxidant in the human body, because of its ability to intercept intermediary radicals during the lipid peroxidation process. Most antioxidant mechanisms described above are decreased in patients with renal failure, leading to

a higher sensitivity to oxidative stress. These patients have low activity and concentration of Glutathione, low concentration of HDL, PON-1 and GPX3 enzymes, albumins and antioxidant vitamins such as vitamin E, D and C. This decreased antioxidant status, enhanced oxidative stress, and affected lipids and proteins leading to lipoproteins modifications and dysfunction. Lipids are one of the compounds mostly attached to oxidative stress. The peroxidation of lipids began with the reaction between a free radical with a polyunsaturated fatty acid containing more than two double bounds and formation of a lipid radical. In the next reaction, lipid radical can create lipid peroxyl radicals ($LOO^{\bullet}$) in reaction with oxygen, which can further react with other lipids forming new lipid radicals and lipid hydroperoxide (LOOH). Malondialdehyde (MDA) and 4-OH-2,3 alkenals are the end products of lipid hydroperoxide degradation. MDA covalently binds to proteins and nucleic acids, interfering with their normal biological functions. Binding to nucleic acids, MDA induce mutations and base-pair substitutions [6].

Binding to lysine amino group of protein part of lipoproteins, MDA created toxic adducts known as advanced lipoxidation end products (ALEs). In general, ALEs exhibit several pro-inflammatory effects and are involved in atherosclerosis [7]. These ALEs on Apo B result with oxidative modification of [8]. 4-OH-2,3 alkenals can also react with proteins, exactly with histidine, cysteine, and lysine residues and, create ALEs [9], which generate modified LDL. In this modified form, LDL can activate macrophages and increase the upregulation of class A scavenger receptors involved in the transformation of LDL into foam cells [10]. Another end product of lipid peroxidation is F2α-isoprostanes. Oxidation of arachidonic acid by a cyclooxygenase-independent pathway generates F2α-isoprostanes, known for atherogenic properties, because of their implication on platelet aggregation via Thromboxane A2 receptor, vasoconstrictive effects on smooth muscle cells, and endothelial cell proliferation and endothelin-1 secretion [11]. These three end products are routinely used for in vivo evaluation of lipid peroxidation level [12].

4. Lipid disorders in chronic uremia

Renal failure is characterized by specific metabolic abnormalities of plasma lipoproteins [13]. These abnormalities involve all lipoprotein classes and show variations depending on the degree of renal impairment. Uremic lipid profile includes increased VLDL, IDL, small dense LDL particles, lipoprotein (a), and decreased HDL. Besides the changes in their concentration and structure, as stated above, uremic environment can strongly modified circulating lipoproteins leading to profound alterations of their biological properties and toxic effects in different cells and tissues. This has led to the formulation of an accelerated atherogenesis hypothesis and has been commonly linked with the lipid metabolic alteration associated with uremia.

4.1 Chylomicrons and VLDL

Hypertriglyceridemia is common a disorder in uremic patients. Several studies have shown increased concentration of triglycerides even though serum creatinine levels are within normal range [14]. The predominant mechanism responsible for increased concentration of triglyceride-rich lipoproteins, including chylomicrons, VLDL, and their remains, is delayed catabolism and increased synthesis Apo B-48, the essential for chylomicrons metabolism. There are evidences that Apo B-48 levels are increased and inversely correlated with glomerular filtration and proteinuria [15]. In circulation, triglyceride-rich lipoproteins acquire Apo E and Apo C-II,

which are in the surface of HDL. In uremic patients, concentrations of Apo E and Apo C-II, which are necessary for activation of lipoprotein lipase and for uptakes of remnant chylomicrons and VLDL by a receptor specific for Apo E, are reduced. Such defect leads to a reduced release of triglycerides in peripheral tissues and to an accumulation of triglycerides. Delayed catabolism of triglyceride-rich lipoproteins occurs probably because of a decreased activity of hepatic triglyceride lipase and lipoprotein lipase. Moreover, significant evidence showed that enzyme lipoprotein lipase is lacking in renal failure [16]. There are evidences that diminished activity of enzyme is a consequence of the downregulation of the enzyme gene [17]. There is also downregulation of hepatic lipase expression [18].

The presence of lipoprotein lipase inhibitors also contributes to delayed triglyceride-rich lipoprotein catabolism. Apolipoprotein C-II is an activator, whereas apolipoprotein C-III is a direct lipoprotein lipase inhibitor. A decrease in apolipoprotein C-II/ apolipoprotein C-III ratio due to a disproportionate increase in plasma apolipoprotein C-III may be the cause of lipoprotein lipase inactivation, which further contributes to hypertriglyceridemia [19].

As it is mentioned above, triglyceride-rich lipoproteins, chylomicrons, and VLDL,need apolipoprotein C-II and apolipoprotein E for their maturation, which are delivered by HDL-2. In uremic patients HDL metabolism is impaired and HDL-3 are not maturated into HDL-2 due to a LCAT deficiency [20].

In healthy persons, VLDL and chylomicrons are transformed into IDL and chylomicron remnants after lipolysis in peripheral tissue. Chylomicron remnants are removed by the specific receptors of the liver, via LDL (Apo B-100 and Apo E) receptor and LDL receptor-related protein. It has been demonstrated that LDL receptor protein is downregulated in uremic patients [21] which leads to increasing levels of exogenous triglycerides. In physiological conditions, surplus IDL is transformed into LDL by the removal of their triglycerides by the hepatic lipase and enrichment in cholesteryl esters from HDL-2 by CETP. But the lack of HDL-2 impedes this process and leads to the accumulation of pro-atherogenic IDL [22]. There is a downregulation of hepatic lipase expression [18]; thus hepatic lipase deficiency which decreased conversion of IDL to LDL and lack of HDL work in concert to rise plasma concentration of IDL. A part of VLDL is removed by VLDL receptors, but in chronic uremia, the expression of VLDL receptors in tissues is also downregulated [23]. This makes impossible the VLDL binding with VLDL receptors in adipocytes and myocytes and their removal from the circulation (**Figure 4**). Insulin resistance is often associated with chronic uremia and seems to be responsible for a hepatic VLDL overproduction [24]. Secondary hyperparathyroidism, in renal failure, may play an additional role in triglyceride-rich lipoprotein catabolism impairment.

The predominant mechanism responsible for delayed metabolism of chylomicrons and very-low-density lipoproteins is increased synthesis apolipoprotein (Apo B-48) and low activity of lipoprotein lipase (LPL). Decrease concentration of high-density lipoproteins in renal failure results with decreased Apo E and Apo C-II, which are necessary for activation of LPL and for uptakes of remnant chylomicrons and intermediate-density lipoproteins by a receptor specific for Apo E. Such defect, together with the downregulation of LDL receptor protein and hepatic lipase (HL), leads to accumulation of chylomicron remnants and IDL, reducing the release of fatty acids into peripheral tissues. In physiological conditions, surplus IDL is transformed into LDL by the removal of their triglycerides and enrichment in cholesteryl esters from HDL-2 by CETP. But the lack of HDL-2 impedes this process and leads to the accumulation of pro-atherogenic IDL. Increased activity of CETP contributed in reducing HDL concentration. The presence of lipoprotein lipase inhibitor, Apo C-III, also contributes to delayed triglyceride-rich lipoprotein metabolism.

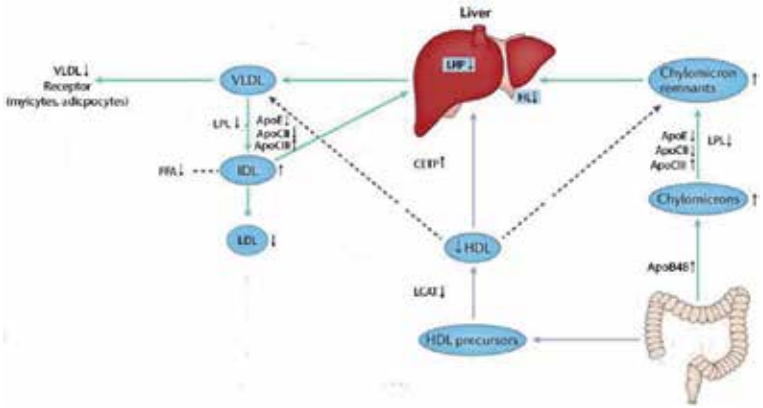

Figure 4.
Changes in chylomicrons and VLDL metabolism in renal failure.

4.2 HDL cholesterol

Uremic patients have decreased HDL in comparison with healthy population [25, 26]. Several mechanisms, working in concern, may underlie the reduction in HDL levels, which is usually indicative of impaired reverse cholesterol transport. Specifically, maturation of HDL is impaired and its composition is altered. Thus, uremic patients usually exhibit decreased levels of apolipoproteins A-I and A-II (the main protein constituents of HDL), diminished activity of LCAT, the enzyme responsible for the esterification of free cholesterol in HDL particles, as well as increased activity of CETP that facilitates the transfer of cholesterol esters from HDL to triglyceride-rich lipoproteins. One of the mechanisms for impaired HDL metabolism in uremia is the increased activity of enzyme ACAT which is responsible for intracellular cholesterol esterification. In physiological conditions, Apo A-I and Apo A-II, in the circulation, are loaded with cholesterol and phospholipids to form nascent HDL. Then, nascent HDL binds to the ABCA-1 receptor on circulating macrophages and activates cholesterol ester hydrolase allowing their loading with cholesterol. ACAT limits this reverse efflux of cholesterol from macrophages by catalyzing the esterification of intracellular cholesterol. Oxidative modification of Apo A-I can limit HDL binding on macrophages [27] and upregulation of hepatic ACAT [28] contributing in impaired cholesterol efflux. Therefore, an increase in ACAT activity can potentially limit HDL-mediated cholesterol uptake and contribute to the reduction in plasma HDL cholesterol and impaired maturation of HDL. Although the effect of chronic renal failure on ACAT expression and activity in the extrahepatic tissues is not known, chronic renal failure has been recently shown to markedly raise hepatic ACAT-2 mRNA and protein abundance, as well as total ACAT activity [29].

On the other hand, the activity of enzyme LCAT is decreased [30, 31]. Apo A-I is the activator of LCAT, the essential enzyme for the HDL-mediated cholesterol retrieval from extrahepatic tissues and as well as ligand for the SR-B1 and HDL-binding protein (ABCA1 transporter). Apo A-II serves as an activator of hepatic lipase, which plays a central role in the removal of HDL triglycerides by the liver. As mentioned above, in patients with impaired kidney function, Apo A-I and Apo A-II levels are decreased. This reduction contributes to diminished HDL concentration and impaired HDL maturation. Until recently, it was not clear whether the reported reduction in plasma LCAT activity is caused by the reduction in its hepatic production and plasma concentration or is a consequence of its inhibition by an

unknown uremic toxin [32]. Another enzyme with diminished activity is CETP. The enzyme mediates transfer of cholesterol ester from HDL to IDL in exchange for triglycerides. Increased activity of CETP in uremic patients facilitates the transfer of cholesterol esters from HDL to triglyceride-rich lipoproteins, reducing the HDL cholesterol ester and elevation of HDL triglycerides. The mechanism responsible for the elevation of CETP is unknown, but some investigation connected its increased synthesis with proteinuria. Probably the same mechanism is responsible for the dysregulation of hepatic SR-B1. Hepatic SR-B1 is the primary pathway for the disposal of HDL-borne cholesterol ester and triglycerides, and dysregulation of this protein can impact HDL metabolism. Heavy glomerular proteinuria has been shown to significantly reduce hepatic SR-B1 protein expression in experimental animals [29]. HDL has a protective effect against inflammation, platelet adhesion, and LDL oxidation. Those protective functions of HDL can be attributed to HDL-associated enzymes on its surface. Paraoxonase-1 is considered as the main antioxidant enzyme bound to HDL. Mainly expressed in the liver and the kidney, this enzyme exhibited antioxidant properties against lipid peroxidation as it binds to HDL and in a minor part to VLDL [33]. Glutathione seleno-peroxidase 3, also known as glutathione peroxidase 3, is another antioxidant enzyme associated with HDL [34].

One of main anti-atherogenic properties of HDL is a reverse cholesterol transport from circulating macrophages. HDL also increases the production of nitric oxide (NO), through the activation of the endothelial NO synthase in endothelial cells resulting in a vasorelaxant phenotype. In CKD the production of NO by endothelial cells is significantly reduced with HDL [28]. HDL also inhibits the expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1), which prevent the attachment of circulating monocytes to endothelial cells. In uremic patients, HDL promotes an enhanced expression of VCAM-1 and ICAM-1 on endothelial cells [35, 36]. Moreover, CKD-HDL upregulates the expression of pro-inflammatory mediators such as monocyte chemoattractant protein-1 (MCP-1), interleukin-1ß (IL-1ß), and tumor necrosis factor α (TNF-α) [36, 37]. And finally normal HDL exhibit anti-apoptotic effects on endothelial cells through the downregulation of caspase-3 (a member of the cysteine-aspartic acid protease) activity [38]. All these diminished protective functions of HDL can contribute to accelerated atherogenesis [39]. HDL is very sensitive in oxidative stress and posttranslational modifications. Renal failure is associated with an enhanced activity of enzyme myeloperoxidase (MPO, EC 1.11.2.2) that plays a crucial role in the generation of posttranslational modification derived products (PTMDPs). MPO catalyzed the oxidative reactions and formation of a variety of chlorinated protein and lipid adducts. MPO-modified ApoA-1 results in decreased reverse cholesterol efflux and a reduced binding with ABCA-1 receptor, which disturbed cholesterol homeostasis (**Figure 5**). 3-chlorotyrosine, an oxidation product of MPO, impairs the activity of enzymes, LCAT, and PON-1, resulting with decreased anti-inflammatory effects of HDL. And through the activation of SR-B1 in macrophages, MPO-modified HDL directly contributes in atherosclerosis (**Figure 5**).

In renal failure, decreased activity of lecithin-cholesterol acyltransferase impaired the transformation of nascent cholesterol into HDL3 and then into HDL2. Increased activity of cholesteryl ester transfer protein facilitates the transfer of cholesterol esters from HDL to triglyceride-rich lipoproteins, reducing HDL concentration. Removal of free cholesterol from macrophages proceeds by scavenger receptor 1. Nascent HDL is generated when Apo A-I interacts with ATP-binding cassette transporter type 1 (ABCA1). Than nascent HDL activates cholesterol ester hydrolase allowing their loading with cholesterol. ACAT limits this reverse efflux of cholesterol from macrophages by catalyzing the esterification of intracellular

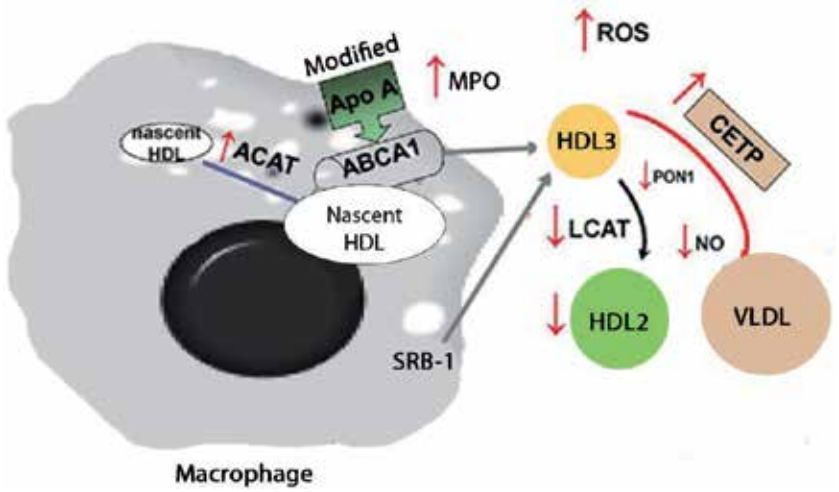

Figure 5.
HDL metabolism in renal failure.

cholesterol. Increased activity of ACAT, in renal failure, participates in impaired cholesterol efflux. Antioxidative and anti-inflammatory functions of HDL are impaired due to reduced activity of HDL enzyme PON1. HDL from patients with renal failure loses its vasoprotective properties, inhibiting nitric oxide production. Oxidative modification of Apo A-I decreases HDLs binding to macrophages. Myeloperoxidase-modified Apo A-I decrease reverse cholesterol efflux, reduce binding with ABCA1, and impair HDLs anti-apoptotic properties.

4.3 LDL cholesterol

Beyond atherogenic risk of LDL level itself, renal failure leads to various structural modifications of LDL particles. The lipoproteins found in uremic patients are disproportionately modified, with LDL that is enriched in triglycerides. These modified LDL particles tend to be smaller and denser in their form. Small dense LDL is believed to be markedly pro-atherogenic, and this is attributed to its ability to infiltrate the vessel wall and its increased susceptibility to oxidative modification. Because of the significantly modified lipid subfraction turnover, residence time of lipoproteins in the circulation is prolonged. Thus, lipoproteins are at risk of posttranslational modification. LDL receptor-mediated cholesterol uptake plays an important role in cholesterol homeostasis. Modified LDL have reduced affinity for the classic LDL receptors and are taken up by the scavenger receptors on the surface of the macrophages. These receptors are increased in chronic uremia. High affinity for macrophages results in the accumulation of cholesterol and the formation of foam cells in the vascular walls, resulting in the development of atherosclerotic plaques [40, 41]. Heavy proteinuria alone or in combination with chronic uremic state results in acquired LDL receptor deficiency and plays a central role in the genesis of the atherosclerosis and cardiovascular diseases. Several levels of LDL oxidation can coexist in the bloodstream and lead to the activation of several pathways involved in atherosclerosis through their binding to scavenger receptors [42] and smooth muscle cell proliferation. There is an evidence that OxLDL are accumulated in uremic patients and are correlated with the intensity of peripheral arterial disease [43]. Oxidized epitopes of LDL can activate immunity and then lead to the formation of antibodies directed against OxLDL. OxLDL/antibodies against OxLDL ratio were also correlated with carotid atherosclerosis

and cardiovascular events [44]. Formation of OxLDL is a consequence of oxidative stress. As mentioned above, the breakdown of polyunsaturated fatty acids produces highly reactive molecules, such as MDA and 4-OH-2,3 alkenals. MDA and 4-OH-2,3 alkenals can form Schiff bases and covalent Michael-type adducts, with lysine residues of Apo B-100, in LDL (**Figure 6**). The oxidized fatty acid fragments which can remain attached via ester bridges, may also contain terminal reactive phospholipids which may form adducts with Schiff base lysine residues of Apo B-100. Similarly with HDL modifications, increased levels of MPO are involved in LDL modifications. MPO can modify LDL through several mechanisms. MPO initiated the reaction between hypochlorous acid and tyrosine residues of Apo B-100, protein part of LDL, resulting with 3-chlorotyrosine formation, which is known for pro-atherogenic properties through its binding with lectin-like oxidized LDL receptor 1. MPO also generated reactive nitrogen species, converting LDL into a nitrosilated-LDL form. This reaction resulted in nitration of Apo B-100 tyrosyl residues of LDL. Carbamylated LDL (cLDL) is another modified form of LDL, initiated by MPO. In this reaction MPO catalyzed the addition of thiocyanate, derived from the decomposition of urea to the lysine residues of LDL, and leads to the formation of carbamylated LDL [45, 46]. The carbamylation occurs by spontaneous, nonenzymatic chemical modification of Apo B-100, by thiocyanate. It is a irreversibly reaction of thiocyanate with free amino groups and ε-NH2 of lysine residues in protein part of LDL (**Figure 7**). cLDL have pro-atherogenic effects such as the transformation of macrophages into foam cells [47] through their binding to the pro-atherogenic CD36 receptor [48, 49]. cLDL are associated with endothelial toxicity [50, 51] through lectin-like oxidized LDL receptor 1 [52] (**Figure 8**). cLDL levels are raised by chronic uremia [53, 54].

Modified forms of LDL; carbamylated LDL and oxidized LDL; activated lectin-like oxidized LDL receptor 1, on endothelial cells; and initiated formation of macrophages and smooth muscle cell proliferation.

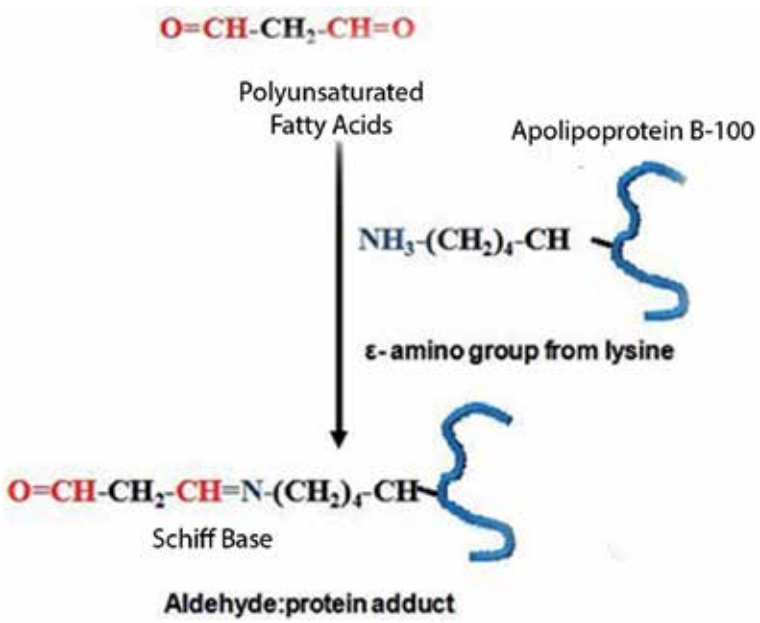

Figure 6.
Formation of oxidized LDL.

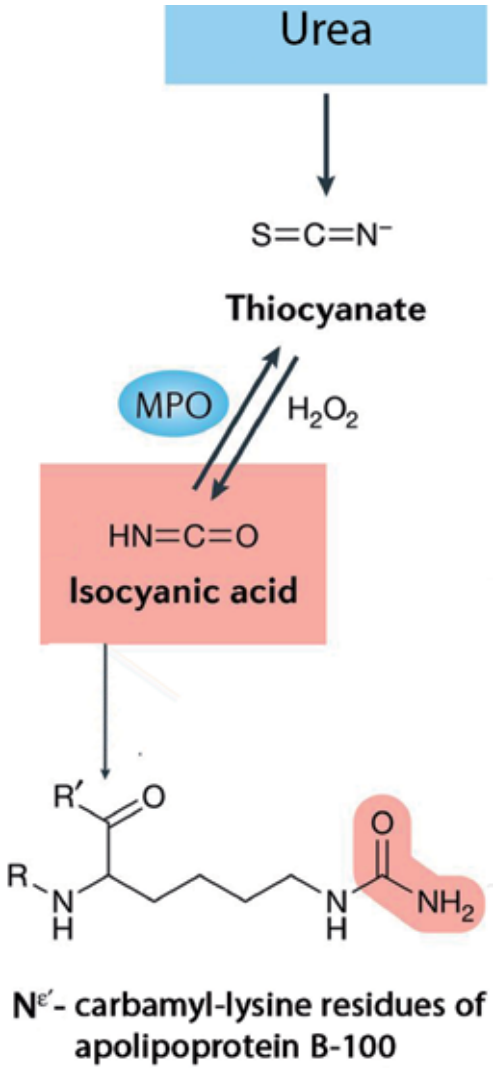

Figure 7.
Formation of carbamylated LDL.

4.4 Lipoprotein (a)

The contribution of cardiovascular events to the extraordinary high mortality in CKD has generated some interest in nontraditional atherosclerotic cardiovascular disease risk factors, which are prevalent in this population, such as Lipoprotein (a) [Lp (a)]. Lp (a) is an LDL-like lipoprotein containing a unique apolipoprotein called Apo(a). Serum levels of Lp(a) are determined largely by genetic variation in the gene encoding for Apo(a). Apo(a) is very homologous to plasminogen [55] and exhibits an extreme size polymorphism with the Apo(a) isoproteins, ranging in size from 420 to 840 kDa. Inherited in an autosomal codominant fashion, the Apo(a) isoprotein is closely correlated with serum Lp(a) concentrations, with an inverse correlation between the size of the Apo(a) isoprotein and the serum Lp(a) concentrations. Lp(a) has been implicated in the regulation of plasminogen activator inhibitor-1 expression in endothelial cells and shown to inhibit endothelial cell surface fibrinolysis to

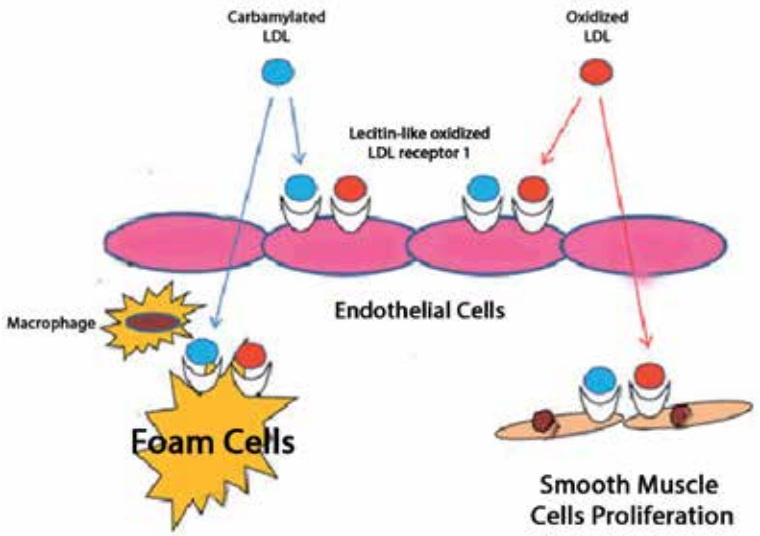

Figure 8.
Oxidized LDL and carbamylated LDL effects.

attenuate plasminogen binding to platelets and to bind to plaque matrix components. Autopsy studies in humans have documented the presence of Lp(a) in aortic and coronary atherosclerotic plaques and an apparent colocalization with fibrinogen [56]. Lp(a) levels are frequently elevated in uremic patients with CKD [57] and have been associated with a frequency distribution of apolipoprotein (a)-Lp(a) isoforms, similar to those found in general population. This indicates that elevated Lp(a) levels in these patients are not due to the genetic origin [58]. It has been suggested that kidneys have an important role in Lp(a) metabolism [59]. In CKD, Lp(a) occurs at high concentrations, largely because of reduced clearance or as a result of increased hepatic synthesis, induced by an acute-phase reaction or by protein losses from proteinuria [60]. Uremia can be considered to be a state of activated acute-phase response, and in the micro-inflammatory milieu, a number of atherogenic proteins like Lp(a) are acting as an acute-phase reactant. Based in all these properties, Lp(a) is a prototype candidate to be classified as a uremic toxin.

5. Conclusion

Chronic uremia causes profound alteration in lipoprotein metabolism, promoting the development of atherosclerosis and cardiovascular disease. Besides the changes in their concentration, enhanced oxidative stress and uremic environment can strongly modify circulating lipoproteins leading to profound alterations of their biological properties and can be considered as uremic toxins. Uremic lipoprotein profile is directly involve in glomerular capillary endothelial damage and in the progression of renal disease. This "reverse epidemiology" shows the importance of lipid control to prevent the progression of renal failure.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

ABCA1	ATP-binding cassette transporter type I
ACAT	acyl-CoA cholesterol acyltransferase
α-TNF	alpha tumor necrosis factor
ALEs	advanced lipoxidation end products
Apo A,B,C,E	apolipoprotein A,B,C,E
Apo (a)	apolipoprotein
CETP	cholesteryl ester transfer protein
CKD	chronic kidney disease
cLDL	carbamylated low-density lipoprotein
DGAT	acyl-CoA diacylglycerol acyltransferase
GPX	glutathione peroxidase
GPX3	glutathione peroxidase 3
HDL	high-density lipoproteins
IDL	intermediate-density lipoproteins
ICAM-1	intercellular adhesion molecule-1
IL-1ß	interleukin-1ß
LCAT	lecithin-cholesterol acyltransferase
LDL	low-density lipoproteins
Lp(a)	lipoprotein (a)
LRP	LDL related protein
MCP-1	monocyte chemoattractant protein-1
mRNA	messenger ribonucleic acid
MDA	malondialdehyde
MPO	myeloperoxidase
NO	nitric oxide
OxLDL	oxidized-LDL
LCFA	long chains fatty acids
$LOO^{\bullet}$	peroxyl radicals
LOOH	lipid hydroperoxide
PON1	paraoxonase 1
PTMDPs	posttranslational modification derived products
PUFAs	polyunsaturated fatty acids
ROS	reactive oxygen species
SOD	superoxide dismutase
SR-B1	scavenger receptor B1
VCAM	vascular adhesion molecule-1
VLDL	very-low-density lipoprotein

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Section 7

Glycogen Storage Disease

Chapter 10

Sports and McArdle Disease (Glycogen Storage Disease Type V): Danger or Therapy?

Georg Bollig

Abstract

McArdle disease (glycogen storage disease type V) is an inborn error of energy metabolism in the muscle. The effects of McArdle disease on physical performance have similarities with the metabolic state of marathon runners after glycogen depletion and can therefore be seen as a nature's experiment in the field of sports medicine. Many patients with McArdle disease avoid sports in general because physical activity usually leads to muscle pain and muscle cramps. Often patients therefore regard physical activity as both painful and possibly dangerous. This chapter is about the advantages and possible risks of sports for patients with McArdle disease. The scientific literature will be discussed highlighting both endurance and muscle strength exercise. It will discuss the differences of aerobic and anaerobic exercise in individuals suffering from McArdle disease. Complications as rhabdomyolysis, myoglobinuria, kidney failure, and malignant hyperthermia will be discussed. The chapter will summarize the current knowledge about the possible dangers versus possible benefits of sports for patients with McArdle disease. A summary of recommendations for physical exercise and training for McArdle patients will be provided.

Keywords: McArdle disease, glycogen storage disease type V, rhabdomyolysis, endurance exercise, muscle strength exercise, aerobic exercise, anaerobic exercise, sports medicine

1. Introduction and background

1.1 No sports?

Many people with McArdle disease do not like sports or physical activity and say therefore no to participation in sports activities or regular physical exercise. The aim of this chapter is to address patients and health care providers' queries about vMcArdle disease and sports as well as to provide guidance on physical activity for those who have to live with McArdle disease.

McArdle disease (glycogen storage disease type V) is an inborn error of energy metabolism in the muscle [1–5]. It hampers physical exercise in affected patients due to the restriction of the availability of glucose as energy source for muscular work. It can be seen as a nature's experiment in the field of sports medicine as the underlying defect of the myophosphorylase enzyme leads to metabolic effects

that are similar to the effects of glycogen depletion in marathon runners [2]. Many patients with McArdle disease (McAd) avoid sports because physical activity usually leads to muscle pain and muscle cramps. Often patients with McAd therefore regard physical activity as both painful and dangerous. On the other hand, physical activity is of great importance to manage daily life. Living is based on regular motion, and muscles have to be used in order to be healthy. Not using our muscles will in the long run lead to weakness, immobility, and frailty. Therefore people affected by McArdle disease do benefit of keeping a certain degree of fitness. Regular physical exercise might play a key role in delaying progressive muscle wasting, weakness, and frailty in later life of people affected by McAd.

- But how much physical activity is beneficial and what might be dangerous?
- Are certain types of physical exercise better than others?

These and many other questions arise when talking about physical activity and sports with patients with McAd. People with McAd do therefore need guidance on physical activity based on scientific and evidence-based facts. Due to unpleasant experience with sports in the form of pain, cramps, weakness, or myoglobinuria, many patients with McAd show a tendency to avoid sports and physical activity because they are afraid that sports may be not only painful but also harmful. Unfortunately this behavior can decrease their physical activity and physical capacity further. This chapter will shed light on McArdle disease and sports in general and try to answer the above mentioned questions.

2. Method

This chapter is based on a review of the existing publications on McArdle disease (glycogenosis type V) and sports and the authors' personal experience with the topic based on his German PhD thesis on the subject [2] and practical experience with patients with McArdle disease from sports medicine and anesthesiology. A literature search with the Medical Subject Heading (MeSH) words "McArdle disease" and "sports" was performed using the search engines PubMed and Medline. Publications that had McArdle disease and sports as a topic were included. In addition reference lists of books and other sources were assessed by hand search. An overview of the existing literature on this topic is provided.

3. McArdle disease: a nature's experiment

McArdle disease was first described in 1951 by Brian McArdle, a British neurologist. It is known by different synonyms as myophosphorylase insufficiency, glycogen storage disease type V, or myophosphorylase deficiency [1–5]. **Table 1** provides an overview of the different types of glycogen storage diseases [6]. McArdle disease is caused by a lack of myophosphorylase (alpha-1,4-glucan orthophosphate glycosyl transferase) that normally initiates muscle glycogen breakdown during exercise by removing 1,4-glycosyl groups from the glycogen molecule leading to the release of glucose-1-phosphate [1–5] and thus providing fuel for muscular work. Patients with McAd are unable to use the glycogen storage in the muscles as an energy source to enable physical activity.

In about 50% of patients with McAd, a positive family history can be found. For most of the patients, the diagnosis is first established between the age of 10 and 30 [3]. The disease is described to be autosomal recessive, although transmission

Type of glycogen storage disease	Enzyme defect	Inheritance	Organs involved	Clinical symptoms
Type 0	Glycogen synthase deficiency		Liver	Fasting hypoglycemia, tiredness, pallor, vomiting, muscle cramps
Type I **Von Gierke disease**	Glucose-6-phosphatase deficiency	Autosomal recessive	Liver, kidney	Growth retardation, hypoglycemia
Type II **Pompe disease**	Acid maltase deficiency	Autosomal recessive	Muscle, heart, liver	Hypotonia, muscle weakness (progressive), affected: proximal and respiratory muscle, cardiac enlargement and failure
Type III **Cori disease**	Debrancher enzyme deficiency	Autosomal recessive	Liver, muscle, heart	Growth retardation, muscle weakness (liver cirrhosis can occur)
Type IV **Andersen disease**	Branching enzyme deficiency	Autosomal recessive	Liver, kidney, heart, muscle	Mild hypoglycemia
Type V **McArdle disease**	Myophosphorylase deficiency	Autosomal recessive	Skeletal muscle	Exercise intolerance, muscle cramps and pain, myoglobinuria on strenuous exercise
Type VI **Hers disease**	Liver phosphorylase deficiency	Autosomal recessive	Liver	Mild hypoglycemia
Type VII **Tarui disease**	Phosphofructokinase deficiency	Autosomal recessive	Skeletal muscle	Muscle pain and fatigue on exercise. Muscle cramps and tenderness
Type VIII	Phosphorylase b kinase deficiency	X-linked recessive	Liver, brain	Ataxia, spasms, brain degeneration
Type IX	Phosphoglycerate kinase deficiency	X-linked recessive	Liver	Mild hypoglycemia
Type X	Phosphoglycerate mutase deficiency	Autosomal recessive	Liver, muscle	Exercise intolerance, muscle pain

Table 1.
Overview of glycogen storage diseases (modified from [6]).

clinically appears to be autosomal dominant in some affected families [2–4, 7]. The gene for myophosphorylase lies on chromosome 11q13. There are a number of different mutations described in the scientific literature; the most frequent mutation is named R50X [5].

The prevalence of McArdle disease is not known exactly due to the relative benign course of the disease and the often mild and frequently misinterpreted clinical symptoms. The clinical symptoms are summarized in **Table 2**. Haller has estimated the prevalence of McArdle disease in the Dallas-Fort Worth region as 1 in 100,000 [8].

As the pathophysiological effects of McAd are similar to the state of glycogen depletion in marathon runners, it is of special interest from the view of sports medicine and has been called a nature experiment [2].

- Muscle pain (myalgia)
- Fatigue
- Cramps
- Exercise intolerance
- Intermittent claudication
  - (Muscle pain on mild exertion in the calf muscle, usually attributed to peripheral artery disease)
- Second wind phenomenon
- (Exercise becomes easier after a period of moderate and tolerable exercise)
- Stiffness
- Muscle swelling after exercise
- Myoglobinuria
- Muscular atrophy
- (Mostly proximal muscles affected and in elderly patients)

Table 2.
Clinical symptoms and signs of McArdle disease [1–4].

4. Typical clinical picture and diagnosis of McArdle disease

4.1 Typical clinical picture

Typical clinical symptoms of McArdle disease are muscle pain and fatigue during exercise. Pain is often located in the knee, calf, and legs. Normally pain vanishes after a few minutes rest. Clinical symptoms and signs are shown in **Table 2** [1–4].

4.2 Diagnosis of McArdle disease

Diagnosis is based on the clinical picture, muscle biopsy, biochemical tests, exercise testing, and genetic testing [2–4, 7, 9] as listed in **Table 3**.

The absence of rising lactate is a diagnostic criterion in McAd. Usually this has been tested using the forearm ischemic exercise test [2–4]. Both Bollig [2] and Vissing and Haller [9] have suggested to use cycle ergometry testing instead of the forearm ischemic exercise test. The ischemic forearm exercise is more painful for the patients and might put them at risk for severe rhabdomyolysis and myoglobinuria. The cycle ergometry as diagnostic tool from sports medicine is useful in the diagnosis of McAd and may be used to assess the actual state of cardiopulmonary fitness and may help to give patients guidance for further training. **Figure 1** shows the results of cycle ergometry testing in a typical patient with McArdle disease [2].

- Clinical picture
- Elevated creatine kinase (CK) levels in the blood
- Absence of increased venous lactate during forearm ischemic exercise test or cycle ergometry
- Low or absent myophosphorylase activity on histochemical or biochemical examination of muscle biopsy
- Genetic testing (the muscle phosphorylase gene is located on chromosome 11q13, and several mutations have been described—the most common mutation is called R50X)

Table 3.
Diagnosis of McArdle disease [2–4, 7, 9, 10].

Workload (watt)	Heartrate / min.	Bloodpressure (mmHg)	Lactate (mmol/l)
0	76	120/85	0,8
30	116	130/-	0,8
70	164	170/-	1
110	204	180/-	0,8

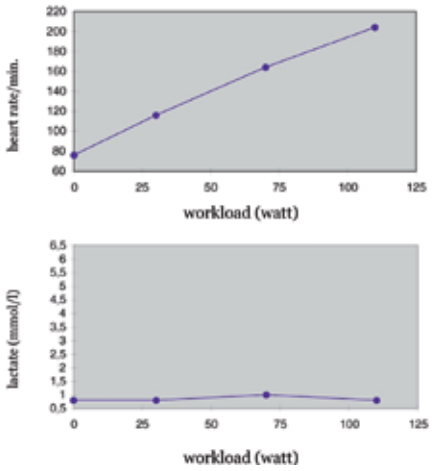

Figure 1.
Cycle ergometry of patient F.M., born 1967, 183 cm, 80 kg; modified from [2].

The prognosis of McArdle disease is usually good, and life expectancy is normal although severe cases with muscle wasting and extreme weakness and death in childhood have been described [1–4, 10].

5. Treatment options

At present there are no causal treatment options available. Some symptomatic treatment options may reduce symptoms or enhance the amount physical activity that can be tolerated. These treatment options include oral sucrose before exercise [11], a low dose of oral creatine [12], vitamin B6 [2, 10], and coenzyme Q10 [2, 10]. Our study about the use of clenbuterol over a 12-month period leads to a subjective improvement of exercise tolerance in three patients. Some relatives of the patients noted an improved exercise tolerance after clenbuterol intake over some weeks [2]. One patient from our study has used a low-dose clenbuterol (0.005–0.02 mg once daily) to enhance exercise tolerance for more than 10 years. This patient used clenbuterol for some months with regular breaks of weeks up to months between the therapy cycles. A systematic Cochrane review on pharmacological and nutritional treatment options has been published by Quinlivian et al. [12]. There do exist animal models for McArdle disease in sheep, cows, mice, and rats that may be used to test potential therapies in future studies [10]. Gene therapy of McArdle disease might be a future option, but its dangers outweigh the possible advantages at present [10].

6. Health problems and possible risks associated with McArdle disease

Patients with McArdle disease are at risk of developing myoglobinuria and even kidney failure due to rhabdomyolysis after exercise or anesthesia [2, 3, 12, 13]. Therefore, patients affected by McAd should learn how to accomplish daily activity

with McAd and how to avoid major muscle damage and the risk for massive rhabdomyolysis and acute kidney failure.

Some cases with insulin resistance and a diabetes type II-like clinical picture in patients with McArdle have been described, but there is no known connection between type I diabetes and McArdle disease [10]. Increased glycogen storage in the muscle of McArdle patients has been suggested as a probable cause of insulin resistance in McArdle patients [10]. Overweight has been observed in many patients with McAd [14]. This might be a potential risk factor for developing type II diabetes as it is for other people without McArdle disease.

Another potential problem is the possible risk of malignant hyperthermia which is a complication during general anesthesia associated with different muscular diseases. Although no case of malignant hyperthermia during anesthesia has been described in McAd so far, it is a potential risk when patients with McAd have to undergo operations with the need for general anesthesia. Therefore, precautions have to be taken by the anesthesiologist, and local or regional anesthesia may be preferred whenever feasible [6, 13].

7. Effects of physical activity and sports in patients with McArdle disease

As described above patients with McAd might suffer from exercise intolerance and pain, fatigue, and cramps during exercise, typical clinical symptoms of McArdle disease. Up to 50% of the patients with McAd show myoglobinuria, and unfortunately acute renal failure has been described in 27% following rhabdomyolysis as a result of vigorous or strenuous exercise [3]. Cases with extreme rhabdomyolysis and myoglobinuria have, e.g., been reported after a swimming competition, an asthma attack, after carrying a TV, and after the diagnostic use of the ischemic work test using a tourniquet [15–19]. The variation of creatine kinase (CK) levels in the blood has been investigated in a male with McAd over a period of several months by the author [2]. **Figure 2** shows the results from this German doctor thesis from the year 2000. The results indicated that anaerobic exercise and physical activity demanding great strength or strenuous exercise lead to huge increases in creatine kinase activity, whereas aerobic exercise did not increase blood creatine kinase levels to a great extent. Aerobic exercise was shown to be associated with lower creatine kinase levels after physical activity in a number of instances during the study period [2, 20]. This finding was later proofed by other researchers [10]. The oxygen uptake and physical ability of patients affected by McAd is usually limited to about 50% of comparable healthy individuals [2].

7.1 The second wind phenomenon

The second wind phenomenon is defined as "a period of less painful and more effective exercise associated with a decrease in heart rate after the initial period of cramping and/or weakness." [21]. Many patients with McArdle disease do experience this phenomenon that was first described by Pearson et al. [22]. During exercise this phenomenon can lead to better endurance because patients are able to exercise for a longer period and experience physical activity as less painful in the long run.

7.2 Aerobic exercise (endurance exercise)

Aerobic exercise is endurance exercise where oxygen is needed in energy production. Aerobic energy production takes some minutes to start but can help to

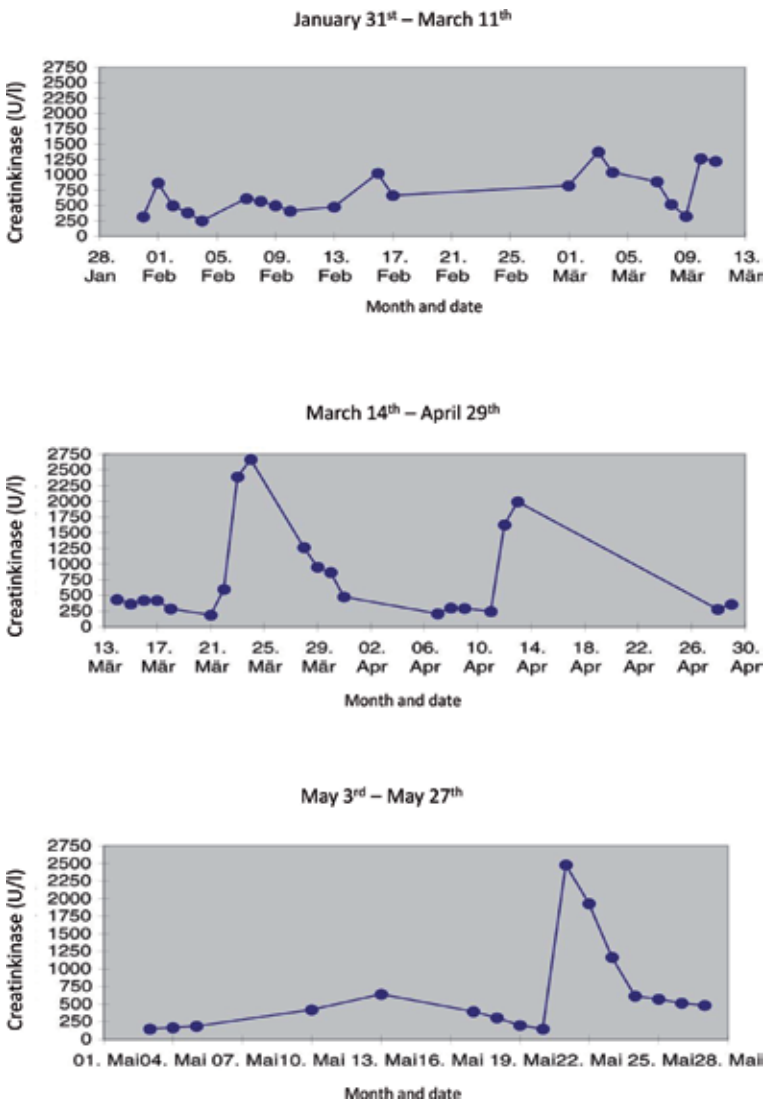

Figure 2.
Creatine kinase levels from a long-term follow-up over 5 months [modified from [2]].

supply energy for muscular activity over a longer period of time (several minutes up to several hours). Due to the lack of glucose that cannot be released from the glycogen deposits in the muscle, patients with McArdle disease rely on fatty acids, amino acids, and glucose from the liver as energy source during exercise [2, 10]. These mechanisms are based on aerobic metabolism. Patients with McAd can therefore tolerate longer periods of physical activity well if it is aerobic exercise of mild-to-moderate intensity. The work intensity that patients with McAd do tolerate can show big variations between different patients.

During the study period, different types of physical activity were recorded in a diary by the patient. Aerobic exercise as cycling and walking/hiking did not lead to CK elevation, whereas anaerobic exercise leads to CK elevation.

Different researchers have recommended aerobic training and aerobic conditioning in order to improve physical activity, oxygen uptake, cardiovascular fitness, and energy supply via the blood in McAd [2, 10, 23–26]. Especially walking and cycling with mild or moderate intensity can be recommended for all McAd

patients to improve their physical capacity [2, 10, 25–27]. Aerobic metabolism usually starts after 7–10 min of exercising. Therefore, patients with McAd should warm up with low intensity and may increase the intensity of physical work after 7–10 min. Some of the patients experience the above described second wind phenomenon.

7.3 Anaerobic exercise

During anaerobic exercise (within the first seconds and minutes or using great strength), energy is supplied by anaerobic mechanisms as anaerobic glycogenolysis without oxygen. Short periods of activity with high intensity such as running, walking upstairs, and carrying or lifting heavy weights require anaerobic metabolism. Due to the deficiency of the myophosphorylase enzyme in the muscle of McAd patients, this is hampered. Anaerobic physical activity can thus lead to muscular damage in patients with McAd and should be avoided as far as possible by patients with McAd [2, 10, 25]. Nevertheless supervised resistance training has been shown to improve muscle strength in patients with McAd [28]. Pietrusz et al. state that strength training for McArdle patients is safe when it is tailored to the patient as "short bursts of resistance activity lasting no longer than 10 seconds preceded and followed by 30 seconds to 3 minutes rest." In a case report of two McAd patients, an improvement of both muscular strength and quality of life was observed after a period with resistance training [29].

8. Discussion and conclusions

Sport is the most important therapeutical option for patients with McArdle disease. Aerobic conditioning can be recommended to all McAd patients, but anaerobic exercise may lead to muscular damage. It has been shown by different researchers that regular physical activity may lead to improved exercise capacity [2, 10, 23–26]. As we have learned from practical experience and the scientific literature, extensive physical and strenuous exercise may lead to muscle damage, myoglobinuria, and even acute kidney failure [15–19]. Nevertheless Santalla et al. and Pietrusz et al. have shown that resistance training under expert supervision is feasible and improves muscle strength in McArdle patients. But it is important that this type of training is performed under supervision in order to avoid muscle damage [28, 29]. On the other hand, a case study with a long-term follow-up of one patient with McAd has shown that mostly aerobic activity did not lead to an increase in the creatine kinase level. Instead, moderate cycling or hiking led to a decrease in the creatine kinase [2]. In the same patient, anaerobic exercise lead to increased CK levels suggesting muscle damage after carrying heavy weights [2]. In order to avoid muscle damage by vigorous exercise or in a risky way, all patients with McAd should receive sport medical advice on an individualized training plan that meets their individual training needs. In order to enhance patient compliance, common aims and routines for physical activity and sports should be established.

In conclusion, regular activity and sport are paramount for patients with McArdle disease. Patients benefit from regular physical activity. Sport should be based on aerobic conditioning such as walking and cycling, whereas anaerobic exercise of high intensity over short periods should be avoided in general. Physical activity must be individualized to the patients' capacity and needs. Some case

- Do not be afraid of physical activity.
- Individualize your personal training goals.
- Compete with yourself and not with others.
- Aerobic conditioning (walking or cycling) is the preferable training method for patients with McAd.
- Keep on doing physical activity on a regular basis three to five times a week using aerobic exercise, such as walking or cycling for about 30–40 min on each occasion.
- Regular training of mild-to-moderate intensity will improve physical capacity and may postpone weakness and muscle wasting in elderly patients.
- Preexercise nutrition may enhance physical performance.
- Resistance training should only be used under competent supervision of physicians and/or physiotherapists with experience in treating McArdle patients in order to avoid muscle damage.

Table 4.
Recommendations for physical activity for patients with McArdle disease.

reports suggest that even resistance training might be feasible, effective, and safe for patients with McAd. Obviously there is individual variation of the intensity that is appropriate for different patients. Therefore, a cooperation with a doctor experienced on sports medicine, trainer, and physiotherapist can help to establish an individualized training plan in order to maintain and possibly to improve physical capacity without increasing the danger for undesirable effects of too much physical activity.

Probably a self-monitoring of the CK blood level (like measuring blood-glucose in diabetes patients) could help to guide training and individual response to exercise in the future. More research on specially designed training programs for McAd is needed.

The following general recommendations shown in **Table 4** are based on personal experience of the author and the current state of the scientific literature [2, 10, 20–30].

As shown above, sport has therapeutic potential for people with McArdle disease. Sport is used with reason and is therefore not a danger but a powerful medicine.

Conflict of interest

The author declares no conflict of interest.

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Section 8

Imaging Studies

Chapter 11

Emerging Knowledge From Noninvasive Imaging Studies: Is Ammonia Control Enough?

Andrea L. Gropman

Abstract

Multiple lines of research suggest that ammonia is harmful to the brain if the levels remain elevated for extended periods of time. Several decades ago, there was no testing or standard of care to monitor the effect of hyperammonemia (HA) on neurological function in urea cycle disorders (UCD), and the timing of HA encephalopathy is still not clear. Magnetic resonance imaging (MRI) was not done routinely, if at all, so it was not known what changes were occurring in the brain, during and after recovery from HA. Decades ago, a diagnosis of a UCD meant severe disability and early death. Earlier diagnosis, improved management, and nitrogen scavenger therapy have improved the lives and life span of patients with UCD. However, many patients suffer from learning difficulties under the umbrella "executive function" which comprises neurologically based skills involving mental control and self-regulation. The general agreement of the core elements of executive functions includes inhibition, working memory, and cognitive flexibility and is necessary in development of skills in reasoning, fluid intelligence, problem-solving, and planning. Our research focuses on the use of noninvasive neuroimaging coupled with neuropsychological testing to understand the complex relationship between ammonia, glutamine, cognitive function, seizures, and specifically impact on development of working memory.

Keywords: ammonia, EEG, glutamine, MRI, neuroimaging, urea cycle disorder

1. Introduction

The urea cycle disorders (UCD) represent one of the most common groups of inborn errors of metabolism, with an overall incidence of 1 in 30,000 [1, 2], and involve deficiency of one of six urea cycle enzymes or of a related cofactor or transporter [3, 4]. The most common of these, ornithine transcarbamylase deficiency (OTCD), is the only disorder of ureagenesis inherited in an X-linked manner, with an estimated incidence of 1 in 70,000 [5]. Over 240 missense mutations have been identified in the OTCD gene but overall 400 including nonsense, frameshift, in-frame indels, splice site errors, and one in a regulatory domain [6, 7]. About 60% of hemizygous males harbor a mutation around the enzyme active site and present with hyperammonemic (HA) coma in the newborn period [8, 9]. The remaining 40% of patients demonstrate more peripheral mutations in other parts of the gene, associated with less severe phenotypes and later onset presentation [10].

A majority of children with OTCD have cognitive and motor deficits due to hyperammonemic episodes [8, 11–13]. Neonatal onset disease mortality rate is high. Prior to advances in recognition and treatment, it was not uncommon for survivors of neonatal onset disease to have intellectual disability, cerebral palsy, and seizures [14]. Neonatal survivors have a decreased IQ which may be as low as 43. In males with partial deficiencies, disease onset is later, and outcome is better, although still associated with high mortality and morbidity with many individuals manifesting cognitive, motor, and psychiatric sequelae [15–17], in particular impaired working memory and other measures of executive function which are essential for performing well in school, vocations, and relationships. Treatment of OTCD involves a combination of protein restriction (which is also a restriction in nitrogen, leading to ammonia accumulation) and medications that invoke an alternative pathway of waste nitrogen excretion [18, 19]. Females heterozygous for OTCD have a variable phenotype and display a broad range of symptoms from apparently asymptomatic to fully affected, owing to both allelic heterogeneity and differential X-inactivation patterns.

A common presumption for years has been that approximately 85% of heterozygous females are asymptomatic based on history, whereas the remainder show symptoms ranging from behavioral and learning disabilities and protein intolerance to cyclical vomiting, stroke-like episodes, and hyperammonemic coma [20–23]. Symptomatic women who harbor mutations seen in the neonatal onset disorder in hemizygous males [10] may develop HA due to skewed X-inactivation. There is therefore a range of residual enzyme capacities and urea synthetic capacities that result in this variation [24]. However, advances in neuroimaging and the work of the Urea Cycle Disorders Consortium (UCDC) have demonstrated that many of these previously presumed asymptomatic females have similar brain structural, biochemical, and cognitive biomarkers seen in those who are clinically impacted, yet they may be mild under conditions of low demand. More obvious symptoms were uncovered when cognitive demand increases or there is superimposed illness or stressor [25].

2. Pathophysiology of the UCDs

Ammonia is a product of the metabolism of proteins and other compounds, and it is required for the synthesis of essential cellular compounds. However, a five- to tenfold increase in ammonia in the blood induces toxic effects in most animal species, with alterations in the function of the central nervous system. Ammonia is a normal constituent of all body fluids. At physiologic pH, it exists mainly as ammonium ion. Reference serum levels are less than 35 mmol/L (outside the newborn period, where higher levels are seen). Excess ammonia is excreted as urea, which is synthesized in the liver through the urea cycle. Sources of ammonia include bacterial hydrolysis of urea and other nitrogenous compounds in the intestine, the purine-nucleotide cycle and amino acid transamination in skeletal muscle, and other metabolic processes in the kidneys and liver. Increased entry of ammonia to the brain is a primary cause of neurological disorders associated with HA, such as congenital deficiencies of urea cycle enzymes, hepatic encephalopathies, Reye syndrome, several other metabolic disorders, and some toxic encephalopathies [26–28].

On the basis of studies in animal models and other preclinical model systems, several mechanisms of ammonia neurotoxicity at the molecular level have been proposed.

While the exact pathophysiology remains unclear, current theories include (1) glutamine accumulation, with associated impaired cerebral osmoregulation, and (2) glutamate/*N*-methyl D-aspartate (NMDA) receptor activation, with resultant excitotoxic injury and energy deficit [26–30]. The WM is preferentially affected in proximal UCD, and the extent of injury has been shown to depend upon the duration of HA coma and the interval between coma and death [28].

Acute ammonia intoxication in an animal model leads to increased extracellular concentration of glutamate in the brain and results in activation of the NMDA receptor. Activation of this receptor mediates ATP depletion and ammonia toxicity; blocking the NMDA receptor with dizocilpine (MK-801) prevents both phenomena. The ATP depletion is due to activation of Na^+/K^+-ATPase, which, in turn, is a consequence of decreased phosphorylation by protein kinase C. Activation of the NMDA receptor may account for seizures seen in some patients during acute HA [29].

High levels of ammonia in the brain also induce other metabolic changes that are not mediated by activation of the NMDA receptor and thus are not involved directly in ammonia-induced ATP depletion or neurotoxicity. These include increases in brain levels of lactate, pyruvate, glutamine, and glucose, with concomitant decreases in brain glycogen, ketone bodies, and glutamate [30].

Chronic HA is associated with an increase in inhibitory neurotransmission as a consequence of two factors. The first involves the downregulation of glutamate receptors secondary to excessive extrasynaptic accumulation of glutamate. The second mechanism implies increased GABAergic tone resulting from benzodiazepine receptor overstimulation by endogenous benzodiazepines and neurosteroids. These changes likely play a role in central nervous system features of intellectual function, decreased consciousness, and coma [26, 30].

In the brain, glutamine represents a storage depot for nitrogen, binding excess ammonia and offering a short-term buffering of excess ammonia in patients with HA, likely as a protective mechanism. It is these high levels of glutamine in the brain that are also hypothesized to be neurotoxic and one of the factors leading to injury (UCDC unpublished). Brain astrocytes are key players in the interactions of glutamine and ammonia via the Gln/Glu cycle (**Figure 1**).

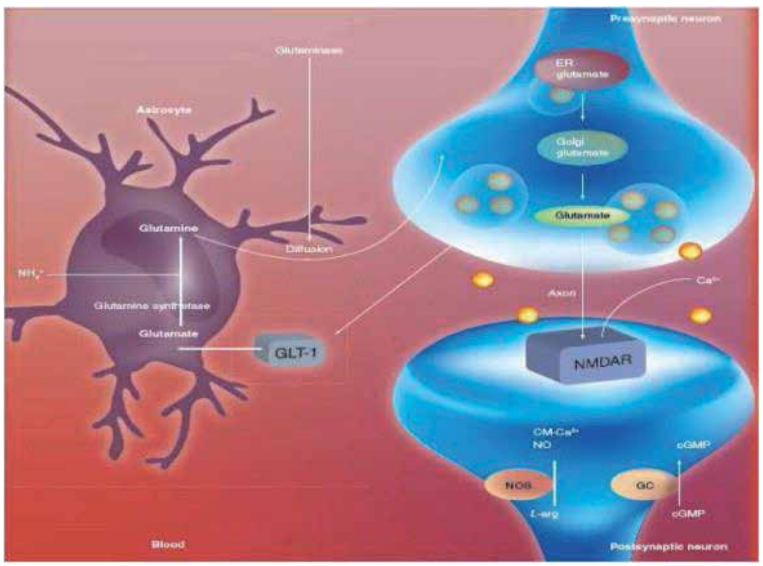

Figure 1.
The glutamine-glutamate cycle. Reprinted with permission from Pediatric Health, 2008, **2***(6):701–713.*

When ammonia is not adequately detoxified by the hepatic urea cycle, there is an increase in scavenger amino acids, including glutamine. Ammonia entering the brain is rapidly incorporated into the formation of glutamine by glutamine synthetase, present in the astrocyte. Glutamine concentrations increase in hyperammonemic states. While not measured directly, indirect measures with ^{1}H magnetic resonance spectroscopy (MRS) studies of patients with urea cycle disorders have demonstrated elevations of the glutamine/glutamate complex [30]. Glutamine has been implicated in hyperammonemic encephalopathy. It has been shown that a rise in plasma glutamine levels precedes HA. There is a sustained positive correlation between plasma glutamine and ammonia levels.

Inhibition of glutamine synthetase in hyperammonemic rats by treatment with enzyme inhibitors prevents the rise in cortical glutamine levels and cortical water content [31]. Clearance of synaptic glutamate by glial cells is required for the normal function of excitatory synapses and to prevent neurotoxicity. This process occurs in the atrocity, which takes up synaptic glutamate and returns glutamate to the neurons in the form of glutamine, a non-neuroactive amino acid that the neurons subsequently reconvert to glutamate via the action of mitochondrial phosphate-dependent glutaminase.

2.1 Short-term clinical effects of HA

Clinical signs of HA may occur at concentrations >60 micromol/L and are very individual as some patients may tolerate higher levels before symptoms are noticed. The short-term changes may include initially anorexia, irritability, lethargy, somnolence, disorientation, vomiting, and asterixis (flapping tremor). As symptoms progress and ammonia is not lowered, cerebral edema, coma, herniation and death [30]. In the acute stages, there is increased blood brain barrier permeability, leading to depletion of intermediates of cell energy metabolism. On an anatomic level, there is disaggregation of microtubules [29].

2.2 Chronic effects of HA

Chronic effects of HA include alterations in axonal development as well as alterations in brain amino acid and neurotransmitter levels. Electrophysiologic effects of HA include direct effects on inhibitory postsynaptic potentiation (IPSP). Neurotransmission is impacted due to increased extracellular glutamate levels and downregulation of AMPA-kainate receptors, enhanced tryptophan uptake, elevated quinolinic acid levels, and enhanced NMDA activity. Activation of NMDA receptors increases calcium in postsynaptic neurons which binds to calmodulin and activates neuronal nitric oxide (NO) synthase, increasing NO, which activates guanylate cyclase, increasing cyclic guanine monophosphate (cGMP), part of which is released to the extracellular space [31, 32]. Activation of this glutamate- NO-cGMP pathway may be involved in some forms of learning.

Recent reports indicate that guanylate cyclase and cGMP are important in learning and memory; induction of LTP is the molecular basis of some forms of learning and memory [33]. Because glial cells also have these receptors, the excessive glutamate leads to glial cell swelling, which seems to protect the neurons from excitotoxic injury. Studies in spf mouse models of OTC and other animal models of HE show neuropathological evidence of excitotoxic neuronal cell death which suggests that overactivation of NMDAR is a feature of urea cycle disorders [34] and may be age dependent [35].

3. What are the cognitive implications of HA on the brain?

A proportion of individuals with OTCD have a wide spectrum of neuropsychological complications including developmental delay, intellectual disability, and executive function deficits [36]. Most adult-onset patients remain asymptomatic, until they present with rapid decline in mental status and subsequently chronic encephalopathy [36, 37].

Fluctuating HA may cause delirium, confusion, and incoherent speech. In addition to subsequent regression, lack of attention leads to unemployment and introverted behavior [37]. Waisbren et al. demonstrated that nearly all asymptomatic 156 women with OTCD attained a full-scale intelligence quotient (IQ) of 102 ± 16. Among 25 men, the full-scale IQ measured was 101 ± 21. No differences were noted between the verbal and performance scores. In addition, in 27% of females and 33% of males, working memory deficiency was observed as a constant finding. The ammonia concentration and its duration appear to be key determinants of the long-term outcome [37].

3.1 Long-term sequelae of HA: Executive function

Executive function (EF) is the ability to control and regulate actions and thoughts [38]. It includes processes such as working memory, self-regulation, and inhibitory control.

Executive functions are a set of cognitive processes and competencies that control behavior and learning. It is an umbrella term which comprises neurologically based skills involving mental control and self-regulation. The general agreement of the core elements of executive functions includes inhibition, working memory, and cognitive flexibility [38, 39]. These elements are highly interrelated, and the interplay of these processes is vital in flexible, goal-directed behaviors. From the core elements of basic executive functions, higher-order executive functions such as reasoning, fluid intelligence, problem-solving, and planning are built [40].

Historically, executive functioning has been thought to be regulated by the prefrontal cortex of the frontal lobes; however reviews found indications for the sensitivity, but not for the specificity, of executive function measures to the frontal lobe [41]. Both the frontal brain regions and other structures of the brain are involved and necessary for successful application of these skills.

Individuals are not born with executive function skills, but rather are born with the potential to develop them. With any genetic or environmental insult to the brain, the executive functions and prefrontal cortex are one of the first to suffer and suffer disproportionately. The disruption of the brain architecture can seriously delay or impair the development of executive functioning [38].

To date, most evaluations of EF rely on parents' reports such as the Behavior Rating Inventory of Executive Function Preschool (BRIEF-P) form, which may not capture the development of executive skills to its full expression [42].

EF has been previously studied using task-based functional MRI (fMRI) scans, which can be difficult to adapt for children [43]. Instead, multiple studies have relied on resting state functional MRI [44]. Reineberg et al. investigated differences in brain connectivity in relation to individual performances during different EF behavioral tasks [45]. However, to our best knowledge, resting state fMRI has not been applied to characterize EF-related brain connectivity differences in children, especially at a young age (2–5 years old).

4. How can neuroimaging help us probe markers of neurological dysfunction in IEMs?

Multiple studies using multimodal MRI suggest its value in the recognition of microscopic anatomic damage that *precedes clinical symptoms* in many inborn errors of metabolism and neurodegenerative disorders. Depending upon the type of imaging study, it may answer a different question regarding the pathology, biochemistry, or physiology. Neuroimaging may detect subtle abnormalities that can be correlated with neurocognitive abnormalities even in asymptomatic OTCD heterozygotes. The neuroimaging/neurocognitive studies we performed as part of the UCDC focused on adolescents and adults with OTCD. Our collective studies demonstrated that OTCD heterozygous females have changes in function of the prefrontal cortex (PFC) in association with an altered neurocognitive profile in working memory, executive functioning, and attention [46].

fMRI can allow us to understand how the brain constructs neural networks to perform cognitive tasks, probe how these networks are altered in brain disorders, and allow us to follow recovery. Magnetic resonance spectroscopy using hydrogen (^{1}H) or carbon (^{13}C) allows us to probe both static and dynamic changes in brain metabolism [47]. The benefits of neuroimaging using MRI are the ability to view the brain in the three orthogonal views, the lack of radiation exposure, and the ability to target the organ or pathology being studied. In addition, high-performance MR hardware is available resulting in faster scans and higher resolution with higher field.

4.1 Use of neuroimaging to assess brain injury in UCDs

Neuroimaging in recent years has come to encompass many different modalities that can be combined in a single imaging session to gain complementary information regarding the brain's structural, functional, and metabolic dimensions.

A typical routine structural MRI protocol includes not only T1- and T2-weighted sequences but also, in most academic and teaching hospitals, fluid attenuation inversion recovery (FLAIR) and voxel-based morphometry (VBM) or other ability to measure tissue volume from acquired structural images on a clinical scanner. Diffusion weighted and diffusion tensor imaging (DWI and DTI) are used to study microstructural variance in WM fiber tracts [48], and proton magnetic resonance spectroscopy is used to measure brain metabolism in static and dynamic models [47]. Multimodal assessment batteries and data fusion give investigators a complex and varied perspective into the structural, functional, and biochemical parameters of the central nervous system in IEMs [49].

4.2 What MRI modalities are available and what do they measure?

4.2.1 Magnetic resonance imaging (MRI)

MRI interrogates tissue water protons via differential populations of proton spins that result when a biological sample is placed in a strong magnetic field. Using MRI, one can define brain anatomy and characterize gray matter and WM microstructural and macro-structural changes. These are read as signal abnormalities on T1- and T2-weighted images which correspond with the specific tissue pathologies. With MRI one can detect damage at a macroscopic level. One must remember that MRI findings can lag behind clinical changes and stages of disease as well as recovery processes.

4.2.2 Fluid-attenuated inversion recovery

Fluid-attenuated inversion recovery imaging is usually a routine part of most radiology clinical imaging sequences. Diffusion tensor imaging (DTI) is used due to its sensitivity in detecting increases in interstitial water content. Such applications include imaging brain tumors, demyelinating diseases (i.e., multiple sclerosis), metabolic WM disease cerebral infarcts, and gliotic scars.

4.2.3 Diffusion weighted imaging and diffusion tensor imaging

Diffusion MRI is another MRI method which allows fine mapping of the diffusion process of molecules (i.e., water) in biological tissues. DWI and DTI can be used in vivo noninvasively. DWI and DTI techniques are focused on the fact that molecular diffusion in tissues is not free but, rather, is impacted by obstacles, such as macromolecules and cell membranes (myelin). By using DTI, water molecule diffusion patterns can inform about microscopic details regarding myelin integrity and architecture.

DTI relates image intensities to the relative mobility of water molecules in the tissue. It can also imply direction of the motion [48]. In general, areas that have a relatively high mean diffusion appear dark on the diffusion weighted MRI images. Diffusion MRI can also be used to make inferences about WM architecture since the diffusion of water corresponds to cell geometry in axons [48].

Cytotoxic edema in contrast follows sodium/potassium pump failure, often due to energy metabolism failure due to ischemic insult. It is quick and can occur within minutes of the onset of ischemia and can produce increased brain tissue water of up to 3–5%. In HA, cytotoxic edema also results. Therefore, DTI can be used in patients with UCD at baseline to assess whether patients differ with respect to WM integrity. DTI has a role in measuring functional connectivity differences between control groups and patients and to follow up patients over time to monitor disease progression, recovery, or impact of therapies [50]. It is a very good technique to also look at rapid fluxes in water content such as during a HA episode in a patient with UCD and allows follow-up noninvasively during recovery and/or with introduction of a therapeutic agent.

The most commonly used indices for the measurement of anisotropic diffusions by DTI include the relative anisotropy measure, fractional anisotropy (FA), and the volume ratio indices. These indices provide quantitative measurements of the changes of WM integrity in brain regions that are affected by diseases.

We have used DTI techniques together with advanced fiber tracking algorithms, to evaluate the 3D trajectories of neural tracts. This has allowed us to model WM neural connectivity in UCDs. DTI was used to determine whether there are WM microstructural abnormalities in partial OTCD that could underlie the cognitive phenotype. Our focus on WM alterations was based on prior neuropathology studies in HA. These studies have shown WM is almost exclusively affected. There is also a relationship between Gln toxicity and WM damage [51]. Anisotropy was calculated by standard methods, from the eigenvalues of the diffusion tensor by using the FA metric. After comparison between UCD patients and age-matched control groups, we established that FA of the frontal WM was significantly decreased in patients with UCD compared to the age-matched controls. This, in turn, is indicative of changes in WM microstructure (**Figure 2**). Additionally, we found an inverse relationship between FA and disease severity that was not age dependent. Based on this, we could conclude that MR imaging in OTCD may be normal in patients with late-onset disease, heterozygotes, or those not in hyperammonemic crisis at the time of the study.

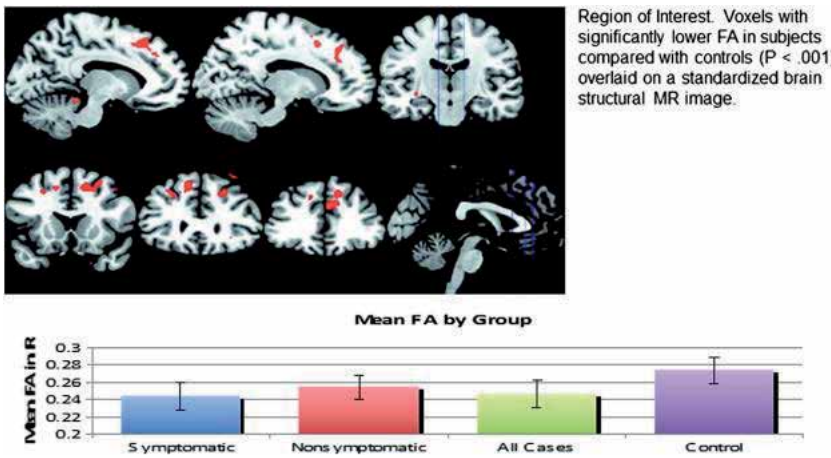

Figure 2.
Decreased FA in the anterior cingulate. The decrease in FA is most significant in patients with OTCD who are symptomatic, but note also FA decreased in those "asymptomatic" patients as compared to controls without any urea cycle disorder.

DTI was much more sensitive to changes in WM microstructural differences than fast spin echo (FSE) T2-weighted imaging for detecting abnormalities in normal-appearing WM. We also found that the degree of the abnormality correlated with degree of cognitive deficits. The location of the deficits in the frontal WM is highly significant as this area is important in the connectivity of fibers vital to executive function, working memory, and attention.

4.3 Progressive WM injury predicts cognitive decline with the most pronounced effects on processing speed and executive function

With our research we have shown DTI evidence of WM injury in motor tracts that subserve executive attention and working memory and can correlate measures of FA with specific working memory tasks. These changes result from WM tract disruption and related cortical disconnection. Quantitative data on the WM microstructure provide a more direct measurement of brain tissue integrity than standard MRI sequences.

4.3.1 WM damage in OTCD

Neuropathological findings have been extensively examined in patients who have died due to urea cycle disorders. These findings shared pathology with other more common conditions such as hepatic encephalopathy as well as hypoxic ischemic encephalopathy. Previous autopsy and, more recently, neuroimaging studies suggest that OTCD results in a predilection for WM injury. And in patients several months prior to death after surviving a neonatal presentation, neuropathological findings consisting of cortical atrophy, ventriculomegaly, gliosis with Alzheimer type II astrocytes, spongiform changes at the gray/white junction, ulegyria, and spongiform changes in the deep gray nuclei-basal ganglia and thalamus have been reported in the literature [52–55].

Neuroimaging studies which have been performed several months after a neonatal hyperammonemic event, months later in neonatal coma survivors, are consistent with these pathological findings, correlating with hypomyelination of WM, myelination delay, cystic changes of the WM, and gliosis of the deep gray matter nuclei. The original reports were small case series using clinical CT initially and then, only

later, MRI. Survivors of prolonged hyperammonemic coma had severe anatomic abnormalities including ventriculomegaly and cortical atrophy. Today, these severe findings are rarely encountered if patients are diagnosed promptly, and duration of hyperammonemia is shortened.

4.4 Functional MRI and UCD research (fMRI)

The basic premise behind fMRI is the increase in blood flow to the local vasculature that accompanies neural activity in the brain. This leads to a local reduction in deoxyhemoglobin. An increase blood flow occurs without an increase of similar magnitude in oxygen extraction.

Deoxyhemoglobin is paramagnetic; it alters the T2*-weighted magnetic resonance image signal and serves as the source of the signal for fMRI [56]. Coupling between neural activity and changes in blood flow was first reported in 1890 (Roy and Sherrington) [56]. By using fMRI, one can observe how the brain is functioning and what areas of brain are activated while a person is performing a specific task. It can allow unmasking of regional vulnerability, circuitry, and recovery of function after damage or intervention.

4.5 Magnetic resonance spectroscopy

MRS is another clinical sequence that provides noninvasive analytic method of identifying and measuring the individual brain chemicals present in various brain regions. ^{1}H–MR spectroscopy is widely used in clinical practice to provide information on brain metabolites. The major metabolites that can be seen include choline (Cho), creatine (Cr), N-acetyl aspartate (NAA), glutamine (Gln), and the osmolytes: myoinositol (mI) and taurine (Taur). Metabolites that can be detected have a unique frequency resonance that is termed the chemical shift. The chemical shift is reported as parts per million (ppm). The advantage of this measure in ppm is that it is the same at any magnetic field strength. The basis of the signal derives from Larmor frequency and coupling. The frequency of individual nuclei is compared to a reference compound called tetramethylsilane (TMS). The MRS is read from right to left. The metabolites that are disrupted in OTCD include Gln, Cho, and mI [57, 58]. One can quantitate the metabolites by either using in house software or using a commercially available program such as linear combination modeling or LCModel [59].

The magnetic resonance (MR) signal detectable is directly proportional to the concentration of the nuclei in the prescribed voxel. Because the brain is mainly composed of water which has a concentration of 55.5 mmol per gram, this must be subtracted in the analysis as the concentration of other chemicals such as NAA or PCr is on the order of 0.015 and 0.5 mmol per gram of tissue. Common voxel sizes used in MRS are from 1 to 5 mm^3.

Both the size and shape of a peak seen on a spectrum are due to the contribution of five attributes:

1. The concentration of nuclei.

2. The T1 and T2 relaxation times of the metabolite. These are also affected by the TR (relaxation time) and TE (echo time) of the MRS sequence as certain metabolites at low concentration may only be seen best at low TE.

3. Magnetic inhomogeneity across the sample. This can be corrected to some extent by a process called shimming. This implies the process by which the main magnetic field (Bo) is made more homogenous by applying small electrical

currents. This can be done passively, as many vendors have automated shimming packages on the scanners, or manually.

4. Another consideration, especially in the case of ^{1}H MRS in the UCD, is the presence of overlapping peaks since several metabolites in whole or in part may have overlapping peaks at a certain ppm.

5. Whether the line is expected to be single or a multiplet. This is determined by the chemical structure J coupling effects.

Some of these principles are explained below.

MR proton spectroscopy has great utility in evaluation of brain metabolic disturbances. Although a nonspecific pattern (elevated Cho, depressed NAA) is common in many types of brain disease, short echo time (TE) MRS (i.e., TE < 30 msec) can reveal more specific metabolic signatures. It is also useful to focus on the temporal changes of chemicals rather than only what is abnormal. Furthermore, temporal changes on subsequent exams can help support or refute the benefit of ongoing therapeutic measures. A simple single voxel technique boasts better signal-to-noise ratios (SNR) and allows shorter TE options than multivoxel technique.

Voxel size is always a consideration, since there is a balance between signal-to-noise ratio and tissue specificity; ideally, it should be as large as possible to achieve satisfactory SNR but small enough to target the area of interest. Generally, a 2 × 2 × 2 cm (2 cm^3) voxel is sufficient; voxels smaller than 1 cm^3 are unlikely to be worthy of the acquisition time it would require to achieve reasonable SNR. Voxel location and echo times should be selected based on the suspected and/or discovered disease patterns. We typically perform ultrashort (TE 14, TR 1500; STEAM technique), short (TE 35, TR 1500-2000; PRESS technique), and intermediate (TE 144, TR 1500-2000; PRESS) or long (TE 288, TR 1500-2000; PRESS) echo time sequences. Examples of metabolites that are best seen at short echo include glutamine and glutamate.

In the case of glycine at 3.55 ppm, it is necessary to obtain at least one MRS data point using an intermediate (i.e., 144 msec) or long (i.e., 288 msec) echo time to remove the spectral contamination of mI that is also around 3.5 ppm.

When are longer echo times preferred? Longer echo times improve diagnostic specificity in disorders such as maple syrup urine disease (MSUD) by eliminating the normal background macromolecular signal that can hide branched-chain amino and ketoacid peaks.

The noninvasive detection of elevated brain glutamine by ^{1}H MRS has also been shown to be a useful biomarker in chronic hepatic encephalopathy [60–62]. We have observed clinically, and it has been shown that glutamine has been implicated in hyperammonemic encephalopathy. A rise in plasma glutamine levels precedes HA [60–62]. The importance of glutamine in this process is further strengthened by the relationship between HA, neurologic dysfunction, and cerebral spinal fluid glutamine concentrations observed in patients with hepatic encephalopathy.

The UCDC presented the largest series of adult patients with OTCD who were imaged using ^{1}H MRS at 3 T and discuss the utility of advanced imaging in understanding the underlying mechanisms of dysfunction [57]. ^{1}H MRS studies have demonstrated elevations in Gln and decreases in mI and Cho in patients who are clinically symptomatic [51]. We showed with ^{1}H MRS decreased mI is also an important biomarker and also seen in females who describe themselves as asymptomatic [57]. We have hypothesized that the decrement of mI might constitute a useful biochemical marker with which to discriminate females with a partial deficiency.

4.5.1 ^{13}C MRS

Although ^{1}H MR spectroscopy is a sensitive tool to detect biochemical abnormalities in individual patients, in vivo ^{13}C MR spectroscopy can reliably be used to quantitate distinct signals from glutamate and glutamine. Unambiguous assignment of these metabolites can contribute to a better understanding of the pathogenesis and treatment of brain dysfunction in UCDs. With the use of carbon 13 (^{13}C) MR spectroscopy, abnormalities in cerebral glutamate metabolism have been noted in patients with chronic hepatic encephalopathy. Therefore, the next step was to use this technique to investigate cerebral glutamate turnover rate in patients with partial OTCD.

This method allows study of glutamate neurotransmission which is carried out by a glial neuronal process that includes the oxidation of glucose and the Gln/Glu cycle [63]. The metabolic model predicts that under conditions of elevated plasma ammonia, the increase in the rate of Gln synthesis is stoichiometrically coupled to increase in the uptake of the anaplerotic substrates CO_2 and ammonia with concurrent efflux of Gln from the brain. Furthermore, studies in hyperammonemic rats suggest that only a fraction of Gln is used to synthesize GABA via Glu. The remainder passes through the neuronal TCA cycle. Bluml et al. have previously shown that there is disturbed neurotransmitter Glu/Gln cycling in chronic hepatic encephalopathy [63, 64]. In their studies, Glu enrichment was decreased and Gln enrichment was increased.

5. Beyond ammonia: relationship of dysregulation of glutamatergic and GABAergic neurons in patients with HA and occurrence of seizures

Despite decades of research, the mechanisms leading to neural injury in HA are still not well understood. Ammonia toxicity is not necessarily an adequate explanation for the degree of cognitive dysfunction seen in patients with argininosuccinate synthase (ASS), argininosuccinate lyase (ASL), and arginase deficiency (ARG). ASS is also referred to as citrullinemia.

Recent studies by our group and others, however, have shown that HA exposure alters several amino acid pathways and neurotransmitter systems, cerebral energy metabolism, nitric oxide synthesis, oxidative stress, and signal transduction pathways which all increase the risk for seizures [30, 65].

Epilepsy had previously been considered an infrequent manifestation in urea cycle disorders, but our longitudinal study (LS) of infants with UCD at a single site (Children's National Health System) found subclinical electrographic seizures (ES) (detected on EEG without clinical manifestations) to be surprisingly common during acute hyperammonemic episodes [66].

This unanticipated finding was particularly identified in neonates with HA. This new finding raises the question of whether seizures play an important role in the etiology of neurocognitive deficits in UCD and whether seizures could afford a biomarker that correlates with brain damage in these disorders.

We have observed ES developing in patients in whom HA rebounded following discontinuation of ammonia scavengers. During HA, the brain is vulnerable to injury as a result of increased permeability and alterations in energy metabolism. In this small cohort, we observed that children with evidence of ES had abnormal MRI scans and/or adverse neurodevelopmental outcomes. This is consistent with what is seen in animal models. The animals develop change in behavior and ataxia and ultimately seizures [67].

HA episodes are critical periods in which to intervene in order to prevent long-term cognitive disability. In neonates and children, HA episodes occur in the context of a developing brain that already has vulnerabilities as a result of normal remodeling, synaptogenesis, and ion channel development. It is also the period in life recognized to be at highest risk for seizures. It is possible that seizures in HA infants and children with UCD are an early biomarker of perturbed metabolism and, left untreated, may contribute to brain injury and subsequent intellectual and other developmental disabilities.

Considerable evidence shows that HA compromises brain energy metabolism which predisposes the patient to seizures due to neuronal depolarization in association with lower energy. The seizures then in turn further lower brain energy, setting in motion a physiologic cycle: HA → lower ATP → depolarization/increased vulnerability to seizures → frank seizures (or, at least, ES) → further lowering of ATP → more seizures (or ES) [68].

5.1 Seizures in UCD

While there is a paucity of investigation of the incidence/prevalence of seizures in UCD, one early study evaluated 11 EEG tracings of 4 infants, irrespective of clinical seizure status [69]. This small study identified epileptiform EEG alterations and hypothesized that they may be a characteristic manifestation of UCD. Later, a retrospective analysis of EEG tracings and head CT scans in 49 UCD patients revealed EEG abnormalities during a clinically stable period that were predominantly observed in patients with abnormal CT scans [70].

In agreement with these reports, our group has identified that patients with distal UCD (where ammonia levels are not as elevated as in proximal disorders) have a high frequency of epilepsy and cognitive dysfunction, raising the possibility that seizures may be associated with other biochemical abnormalities in distal UCD [36, 71]. Additionally, we have shown that disrupted neural networks underlying working memory are a consistent finding in UCD patients with mild as well as severe symptoms [25, 46]. These studies strengthen the importance of understanding the incidence/prevalence of epilepsy in patients with UCDs and if and how this may affect later cognitive function. It further suggests that patients may be undertreated if we focus solely on ammonia-lowering agents and fail to recognize and treat concurrent seizures, as there are clearly other factors, such as disturbed mitochondrial function and oxidative stress, implicated in ammonia-induced neurotoxicity [68, 72–74].

5.1.1 Animal models of UCD and seizures

Both myoclonic and tonic–clonic seizures have been reported in the OTCD spf-ash mouse, an animal model of late-onset UCD with variable phenotype and severity. One study showed that the seizures were linked to the neurotoxic effect of HA on astrocytes, which increased and desynchronized astrocytic Ca^{2+} signaling and compromised the ability to buffer extracellular potassium. Using an animal model of inducible HA, the NAGS knockout (NAGSko) mouse, develops HA within a few hours of withdrawal of effective treatment with N-carbamylglutamate and L-citrulline (NCG + Cit) [75]. Studies in these mice demonstrated that they manifested seizures during HA but unexpectedly also had seizures during baseline recording when blood ammonia levels were normal. In this study, EEG seemed to be a sensitive measure of detecting neuronal dysfunction from HA and suggested its use as an early biomarker of its damaging effects.

The common hypothesis as to how ammonia may lead to seizures is based on the idea that reducing ammonia in the blood will reduce the influx into astrocytes, thereby inhibiting the glutamine synthetase enzyme. This is the basis of ammonia lowering agents in clinical use. However, the work of Rangroo-Thrane et al. challenged that idea by showing that this only may worsen the neurological condition by increasing neuronal exposure to both [NH_4^+] and [K^+]. They have shown that failure of buffering potassium in astrocytes actually is the critical mechanism that contributes to ammonia neurotoxicity. Using awake, intact mice that were induced to have HA, this research group reported that when ammonia was blocked from entering the astrocyte via the potassium transporter Na^+-K^+-$2Cl^-$ cotransporter isoform 1 (NKCC1), seizures developed. They also conclude that a therapeutic intervention should work by blocking this pathway by inhibiting NKCC1 [75].

To determine the incidence of seizures in our UCD cohorts, we conducted a data mining study of six children who were enrolled in the UCDC since 2003 and who had continuous video EEG (cVEEG) during a HA episode and sufficient data to abstract. We accessed their data including clinical information, MRI scans, and metabolic laboratory results including ammonia and glutamine levels. We found that seizures occurred in neonates with UCD even when ammonia and glutamine levels had returned to normal. We further found that interburst interval duration (the time between brain activity and silences) correlated with ammonia levels. During periods of HA, the duration of electrical silence was prolonged, and the EEG pattern could be used to predict elevated ammonia levels (**Figure 3**). A prolonged interval was also correlated with cerebral dysfunction and an abnormal follow-up MRI showing injury (**Figure 4**) [76]. cVEEG therefore can be a useful tool for managing infants with HA and may be essential for seizure management, especially for infants in deep metabolic coma. Features of the EEG appeared predictive of short-term cognitive outcome and structural injury on MRI in this cohort.

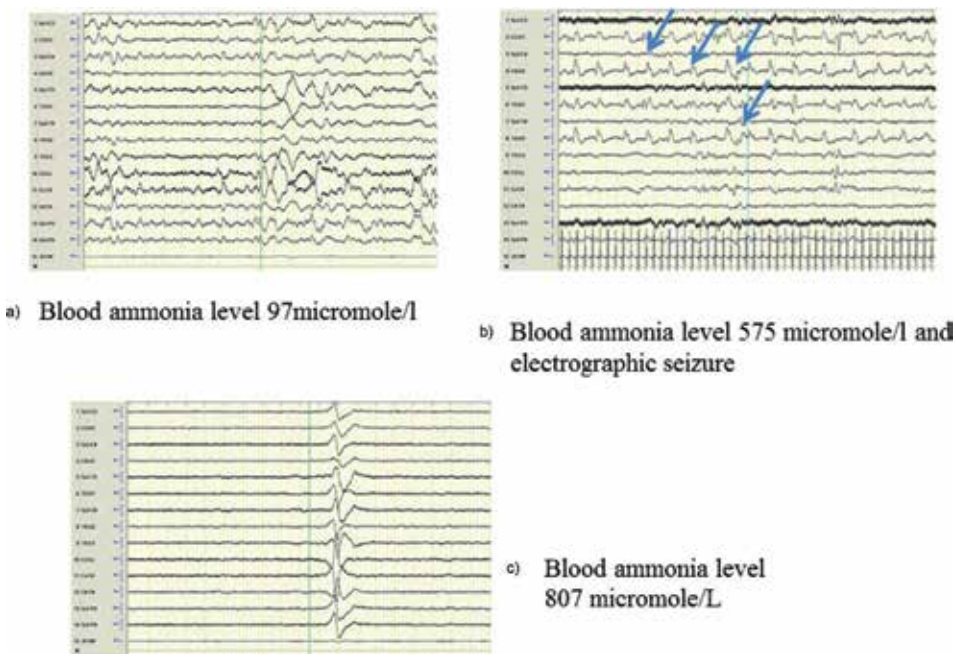

Figure 3.
EEG patterns change with concentration of ammonia from normal neonate (a) to increased interburst intervals, in this case also showing focal seizure (arrows) (b) to more significant suppression of brain activity as ammonia concentration increases further (c).

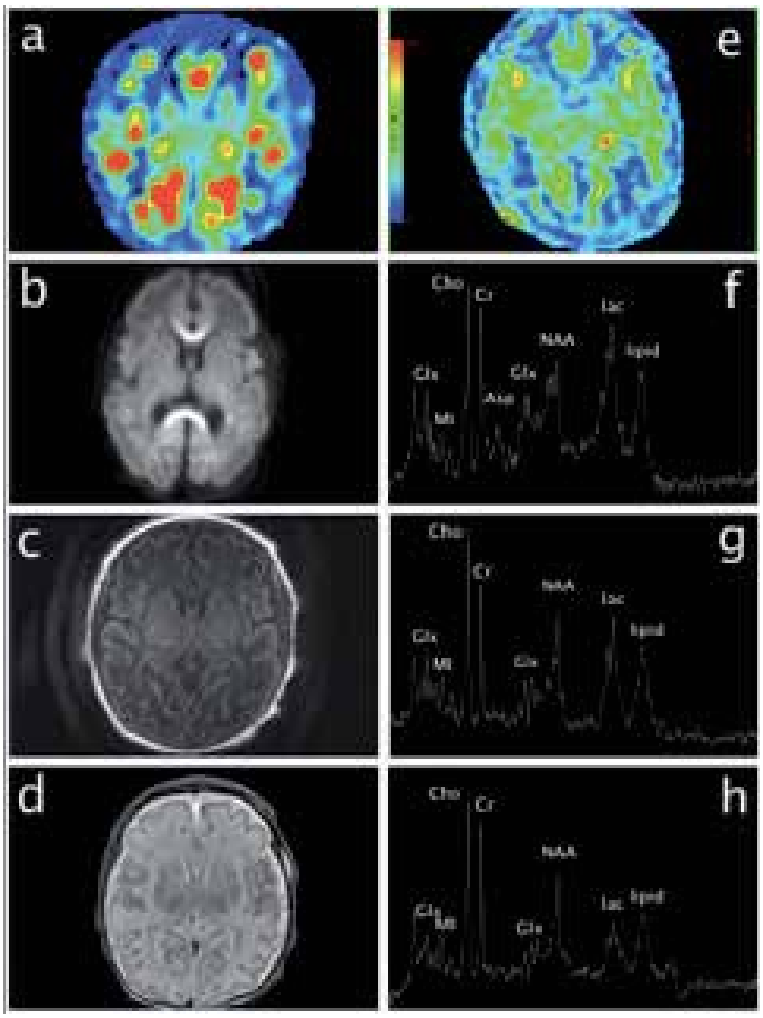

Figure 4.
Selected axial brain MR images at the level of the basal ganglia at day of life 14 (a–d) and 18 (e). Heterogeneous cerebral hyperperfusion improves over time between exams. (a and e) reduced diffusion is present showing hyperintense signal in the callosal splenium and genu, sagittal stratum, internal capsules, frontal WM, and, to a lesser extent (with partial pseudonormalization), the cerebral cortex and deep gray nuclei in correlation with the ADC map (not shown). (b) Hyperintensity on T1WI and (c) hypointensity onT2WI (d) are present extensively throughout most of the cerebral cortex, and mild signal changes are present affecting the cerebral deep gray nuclei. The cortical signal changes on the T1 and T1WI represent laminar necrosis. The unmyelinated cerebral WM demonstrates excessive T1 and T2 prolongation. There is mild diffuse cerebral volume loss with prominent sulci and ventricles.

6. Conclusions

Our understanding of the neurocognitive challenges of OTCD has been improved with the study of advanced MR imaging techniques; however, many issues remain unresolved. We are beginning to understand the neural networks impacted and have been able to correlate imaging findings with specific cognitive outcomes. We now need to scale it back to determine the earliest markers of brain injury. New findings of electrographic seizures in neonates with UCD raise questions of whether seizures play an important role in the etiology of neurocognitive deficits in UCD and whether changes on the EEG could afford a biomarker that correlates with brain changes in these disorders. Future studies are directed towards

studying a more diverse group of UCD patients during baseline as well as during HA events. Pattern recognition to inform the structural and biochemical changes on MRI will allow us to move towards precision management where neuromonitoring may inform moment to moment changes in clinical management.

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Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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Section 9

Toxicity of Associated Drug

Chapter 12

Dipeptidyl Peptidase-4 Inhibitor-Associated Bullous Pemphigoid

Ágnes Kinyó

Abstract

Bullous pemphigoid (BP) is the most common type of autoimmune bullous diseases; drug-induced bullous pemphigoid is a rare variant of it. In the last decade, there is an increasing prevalence of BP, especially dipeptidyl peptidase-4 inhibitor-associated BP (DPP-4i-BP), with the higher prevalence of BP in diabetic patients. Recently, several clinical phenotypes of BP were detected, but there is a tendency in BP cases related to DPP-4 inhibitors to show an atypical noninflammatory form with less distributed skin symptoms, mild erythema, decreased eosinophilic infiltration in the periblister area, and normal or slightly elevated peripheral eosinophil count. Anti-NC16A BP180 autoantibodies are less frequently detected by ELISA, but they response to other epitopes of BP180. Clinical outcome is similar such as in classical non-DPP-4 BP patients, regardless of withdrawal of DPP-4 inhibitors.

Keywords: bullous pemphigoid, noninflammatory, dipeptidyl peptidase-4 inhibitor, gliptins, eosinophil

1. Introduction

Bullous pemphigoid (BP) is a rare autoimmune blistering disease of elderly patients, but in the last decades, it shows increasing incidence [1–8]. Higher prevalence of BP may be according to the increasing global life expectancy of the population, increasing incidence of predisposing neurological diseases, growing numbers of provoking drugs, and improving awareness of newly recognized atypical clinical phenotypes and better diagnostic methods [1]. The role of culprit drugs such as neuroleptics, diuretics, and antidiabetics is reported in several studies [1, 2, 9]. BP is typically present in elderly with a higher predominance in female patients [4, 7]. The classical clinical features of BP are generalized bullous skin eruptions with surrounding erythema and itching; peripheral eosinophilia is also common. Mucosal involvement was observed in 10–30% of patients [2]. Atypical clinical variants may be present up to 20% in BP, including the more common prurigo-like or urticarial type, eczema-like type, dyshidrosiform type, erosive type, and erythema annulare centrifugum-like phenotype [1, 7]. The diagnosis is based on the histological findings, including direct and indirect immunofluorescence microscopy and anti-BP180/BP230 enzyme-linked immunosorbent assays (ELISA) [2]. The gold standard for the treatment of the disease according to guidelines is corticosteroid, in topical or systemic administration and in severe cases with adjuvant immunosuppressive medications, such as azathioprine, methotrexate, or mycophenolate mofetil [2]. In the case of drug-induced BP, the most important therapeutic step is the withdrawal of the culprit drug [9].

2. Bullous pemphigoid and diabetes

BP is a chronic, relapsing disease in patients with several comorbidities and significant morbidity. Investigating the prevalence of diabetes mellitus (DM) in BP, a higher frequency of DM has been found in the last decade [10, 11]. In accordance to several case reports [12–18], case series, case-control studies, pharmacovigilance reports, and retrospective investigations [19–37], the growing number of DM in BP patients is in association with the increasing use of an antidiabetic drug, the dipeptidyl peptidase-4 inhibitor (DPP-4i). DPP-4i, also called gliptins, was approved in 2006 to treat type 2 DM. Sitagliptin, vildagliptin, linagliptin, saxagliptin, and alogliptin have been approved by FDA or EMA; anagliptin, trelagliptin, omarigliptin, and teneligliptin have approval only in Japan. Gliptins are used in monotherapy or in combination with metformin. However, there is a clear evidence of provoking role of DPP-4 inhibitors in BP; the pathomechanism of it is still not understood.

3. Demographics of DPP-4 inhibitor-related BP

DPP-4 inhibitor intake was associated with a threefold increased risk for BP [27, 31, 38]. According to the former investigations [14, 23, 26, 27, 29, 31, 38, 39], vildagliptin has the strongest association with BP; the risk was tenfold (ranged between 7.23 and 11.8) in a systemic review and meta-analysis by Kridin et al. [38]. A higher, sixfold risk was also observed with linagliptin [27, 38]. Higher risk of DPP-4i-BP was also found with sitagliptin by Lee et al. [40], and they also found in a larger sample size (patients with DPP-4i-BP n = 260) that the risks associated with specific DPP-4 inhibitors were lower than the previous studies [27, 38], 1.81 for vildagliptin, 1.64 for linagliptin, and 1.7 for sitagliptin. However, a significant association was detected with vildagliptin, linagliptin, and sitagliptin in age- and sex-matched controlled population; the association with saxagliptin in BP was not significant [27, 31]. Saxagliptin, anagliptin- and alogliptin-induced BP were presented only in a few sporadic cases [29, 31, 32, 40, 41]. DPP-4 inhibitors are often given in combination with metformin; the two recent publications reported that the association of BP and gliptins is independent of metformin exposure [27, 30]. Despite the BP is more common in females, a multicenter investigation and EudraVigilance data showed that DPP-4 inhibitor-associated BP tends to be more common in men [14, 23], similarly to Kridin et al. [27] and Lee et al. [40] and in contrast to Varpuluoma et al., who found a higher risk for BP in women taking DPP-4 inhibitors [30]. The mean age of DPP-4i-related BP patients ranged between 76.6 and 79.1 years [23, 27, 39]. Kridin et al. presented the strongest association in patients younger than 70 years [27], while Benzaquen et al. found stronger association in patients older than 80 years [23], while Lee et al. observed 1.76-fold risk in patients younger than 75 years and 1.5-fold risk in patients older than 75 years [40]. The mean latency period between the initiation of gliptin and the appearance of BP is ranging from 6 to 26.4 months [23, 27, 29, 30, 33]. That means DPP-4 inhibitors can be suspected in the pathogenesis of BP if the drug has been initiated at least 6 months, but it also has to be considered if the drug intake last for more than 2 years prior to the onset of BP.

4. Clinical features of DPP-4 inhibitors-related noninflammatory BP

The classical clinical picture of BP is generalized as bullous skin lesions, tense blisters with severe urticarial erythema. Recent publications characterized a noninflammatory form of BP with limited distribution, smaller blisters, and scant erythema

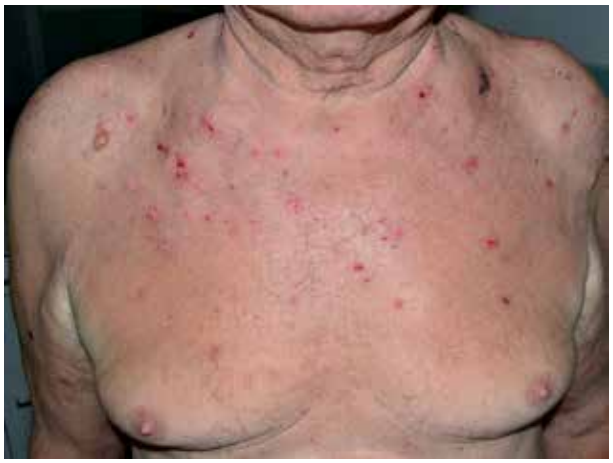

Figure 1.
DPP-4i-related bullous pemphigoid. Mild skin involvement localized to the upper part of the trunk with small blisters and erosions without erythema in a male patient.

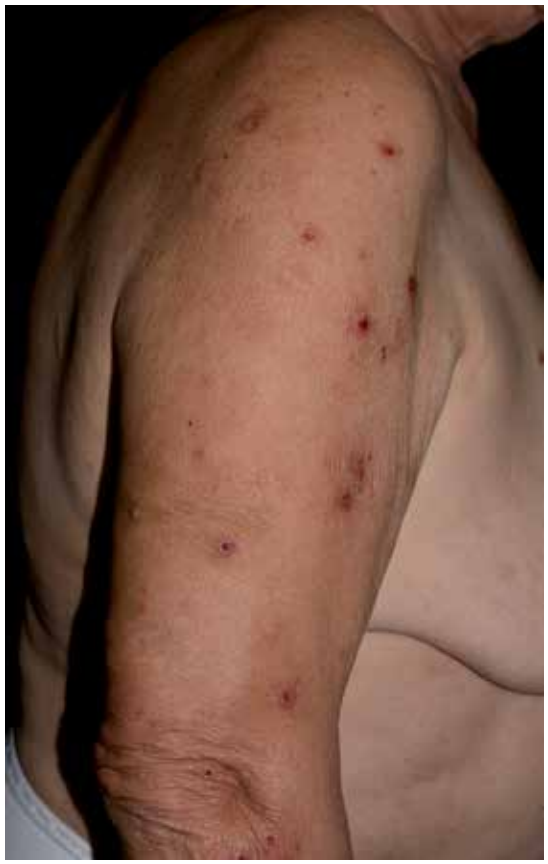

Figure 2.
DPP-4i-related bullous pemphigoid in a female patient with small, round erosions without erythema on the upper extremities.

(**Figures 1** and **2**) [37, 42, 43]. These noninflammatory phenotypes do not react with the NC16A domain of BP, show better clinical outcome, and has a higher prevalence in DPP-4 inhibitor taking patients [22, 32, 34–37, 42, 43]. Noninflammatory BP can

also be found in non-DPP-4-related cases but in a significantly lower manner. Higher frequency of mucosal involvement was reported in gliptin-associated BP in two studies (Kridin et al., 22.2%, n = 36, and Chijiwa et al., 78%, n = 9) [27, 33], but this observation was not supported by Plaquevent (n = 108) [31]. Interestingly, in gliptin-associated mucous membrane pemphigoid (MMP) there is a significantly lower buccal and more common cutaneous involvement [44]. Previous studies demonstrated that eosinophil count is in correlation with the severity of BP [45–47] and with BPDAI score [46]. Comparing the Bullous Pemphigoid Disease Area Index (BPDAI) [48], BPDAI scores for urticaria/erythema (U/E) were significantly lower in noninflammatory phenotypes [33, 36, 42, 43], and lower BPDAI U/E was in correlation with decreased eosinophil count in the perilesional skin [33, 42]. Significantly decreased peripheral eosinophil count was detected in patients of Kridin et al. [27].

5. Immunological characterization of DPP-4 inhibitor-related BP

In BP, there are two targets for autoantibodies, the hemidesmosomal BP180 and BP230 and the juxtamembranous extracellular non-collagenous 16A (NC16A), both of them can be easily detected by commercially available ELISA tests [2, 49, 50]. The domain of BP180 (also called COL17) is a major target epitope in 80–90% of cases [49]. In several investigations, noninflammatory BP patients did not show reactivity against the NC16A domain of BP180, but they were positive for full-length BP180 and its ectodomain midportion with ELISA [34, 36, 42]. The midportion of BP180 is more likely to be recognized than the NC16A domain in DPP-4i-associated noninflammatory BP patients; they are presented with localized symptoms and mild erythema [32, 35, 36, 42, 51]. Although there was a positive reaction to anti-NC16A in DPP-4i-BP cases, but they were mainly presented with prominent erythema and inflammation, concurring with classical phenotype of BP [31, 35, 42]. Kawaguchi et al. showed that the rate of ELISA positivity and antibody titers for anti-BP180 NC16A was significantly lower in DPP-4i-BP than the non-DPP-4 group [22], and this lower titer was also observed by García-Díez et al. [52]. Kawaguchi has also emphasized that patients with DPP-4i-BP tended to have noninflammatory phenotype of BP and presented with negative ELISA for BP180 NC16A domain [22]. Some studies reported noninflammatory DPP-4-induced BP patients who were negative for anti-NC16A domain initially but responded to the full-length BP180 and became positive for NC16A during the course of the disease. This epitope spreading was observed in several cases, after the prolonged use of DPP-4 inhibitors after the onset of BP [17, 37, 52]. Other investigations also demonstrated that multiple epitopes of BP180 are targeted in DPP-4i-BP (midportion, C terminus, and LAD1) [35, 52], and it may suggest that epitope spreading is more common in DPP-4i-BP than in classical BP cases [35]. García-Díez et al. suggested the major role of the midportion of BP180 in DPP-4i-BP, while other BP180 regions are involved later by epitope spreading [52]. Indirect IF positivity was also detected in DPP-4i-BP patients [13, 27, 34], and anti-BP230 autoantibodies were present [34, 35], but the sensitivity of the test was only 38%, which is lower than usually reported [51].

6. Clinical outcome

Based on several investigations, withdrawal of the DPP-4 inhibitors was the first therapeutic step in most cases in the treatment of the disease [23, 34]. Regarding to these data, discontinuation of DPP-4i treatment seems to have a favorable

impact on the clinical outcome of BP [23, 27, 34], but Plaquevent et al. have found that there is no difference in outcome in patients who have further got the gliptin treatment after the diagnosis of BP [31]. Regardless of DPP-4 inhibitor withdrawal, standard treatment protocol was applied in most cases, topical potent corticosteroid treatment in localized form and gold standard systemic corticosteroid treatment with adjuvant immunosuppressive therapy, such as azathioprine, mycophenolate mofetil, or methotrexate in severe cases [27, 31]. Relapse rates were similar in DPP-4i-BP patients such it was previously reported [31].

7. Conclusions

Dipeptidyl peptidase-4 inhibitors (also called gliptins) are widely used drug in the treatment of type 2 diabetes mellitus. There is an increased risk of BP in patients during DPP-4 inhibitor treatment. The exact pathomechanism of DPP-4i-associated BP is still unclear. Dipeptidyl peptidase 4 (also called CD26) is a 110 kDa transmembrane glycoprotein, which is expressed on the surface of several cells, such as T cells [53, 54]. DPP-4 has antihyperglycemic effect and enzymatic activity; it plays a major role in glucose metabolism by blocking incretin [54]. DPP-4 is a plasminogen receptor that activates plasminogen resulted in plasmin formation [55, 56]. Plasmin, a serine protease, which has a high level in lesional skin and in blister fluid in BP [57], cleaves BP180 within the NC16A domain [58]. Cleavage of BP180 in the NC16A region can induce neoepitopes with altered antigenicities [42, 59]. The antifibrotic effect of DPP-4 inhibitors in the skin also supports the role of DPP-4 in collagen metabolism [56]. DPP-4 is involved in immune cell activation, and its inhibition can modify the immune response, which may increase the activation of eosinophil recruitment into the dermis, which is considered to be essential in blister formation in BP [60]. In contrary to these findings, in patients with gliptin-associated noninflammatory BP, both peripheral and perilesional skin eosinophil counts are significantly lower than in classical BP [27, 33], so the exact pathognostic role of eosinophils in DPP-4-related BP needs further investigations. It is also not elucidated why vildagliptin has the strongest association with BP, but it is known that vildagliptin has the lowest selectivity among gliptins with strong inhibition of DDP8 and DPP9 isozymes [22, 61]. Some results suggest that DPP-4 inhibitor has immunomodifier effect mainly in

	DPP4i-related BP	Classical type BP
Distribution	Limited	Generalized
Bullous eruption	Smaller blisters	Tense bullas
Erythema	Absent or sparse	Pronounced
Perilesional eosinophilic infiltration	Absent or mild	Pronounced
Serum eosinophil count	Normal or slightly elevated	Elevated

Table 1.
Comparison of clinical features in DPP-4i-related and classical type bullous pemphigoid.

genetically susceptible individuals, and they have detected that HLA-DQB1*03:01 allele has higher prevalence in DPP-4i-BP patients [22, 62].

In conclusion, DPP-4 inhibitor-related BP tends to be presented with noninflammatory phenotype of BP, with limited extension, smaller blisters, scant perilesional erythema and eosinophilic infiltration, and normal or slightly elevated peripheral eosinophil count (**Table 1**). Anti-NC16A BP180 positivity is less common, and ELISA titers are slightly elevated, similarly to anti-BP230, but positivity for full-length BP180 or other epitopes of BP180 may be detected. Response to therapy is similar such as in classical non-DPP-4i-BP patients, regardless of withdrawal of DPP-4 inhibitors.

Conflict of interest

There is no conflict of interest.

Acronyms and abbreviations

BP	bullous pemphigoid
BPDAI	bullous pemphigoid disease area index
COL17	collagen XVII
DM	diabetes mellitus
DPP-4	dipeptidyl peptidase-4
DPP-4i-BP	dipeptidyl peptidase-4 inhibitor-associated bullous pemphigoid
ELISA	enzyme-linked immunosorbent assays
EMA	European Medicines Agency
FDA	Food and Drug Administration
NC16A	non-collagenous 16A
MMP	mucous membrane pemphigoid
BPDAI	BP disease area index

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This book deals with a vital topic: metabolism in the cells of the body and various disorders due to its imbalance and/or diseases that disrupt the metabolism of the body. The objective of this book was to collect and compile up-to-date information from reputed researchers in their respective fields to disseminate the latest information about topics that have profound effects on the metabolic processes in the body including insulin resistance, diabetes mellitus, hypothyroidism, metabolic syndrome, glycogen storage disease, and the urea cycle disorder. In total, there are 12 chapters in this book in which the authors have shared their research findings and real-life experiences in managing their patients.

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