CKJ REVIEW

A primer on metabolic memory: why existing diabesity treatments fail

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ABSTRACT

Despite massive government and private sector investments into prevention of cardiovascular disease, diabetes mellitus and obesity, efforts have largely failed, and the burden of cost remains in the treatment of downstream morbidity and mortality, with overall stagnating outcomes. A new paradigm shift in the approach to these patients may explain why existing treatment strategies fail, and offer new treatment targets. This review aims to provide a clinician-centred primer on metabolic memory, defined as the sum of irreversible genetic, epigenetic, cellular and tissue-level alterations that occur with long-time exposure to metabolic derangements.

Keywords: chronic kidney disease, diabetic kidney disease, epigenetics, metabolic memory, sirtuin 1

INTRODUCTION

Approximately 45% of the US population suffers from chronic disease, mainly pulmonary and cardiovascular diseases (CVD), diabetes mellitus (DM), obesity and cancer, while more than half of the annual deaths worldwide can be attributable to chronic conditions [1, 2]. We have moved past failed efforts at primary prevention to management of downstream morbidity and mortality, hopefully with detection of early biomarkers of progression [3].

Engerman and Kern [4], a relatively long time ago, described that diabetic retinopathy progressed despite good glycaemic control in dogs and coined the term metabolic memory. Despite almost 40 years of knowledge that tight glycaemic control does not completely prevent diabetic complications, we still follow glycated haemoglobin (HbA1c) to this day, with no better biomarkers in routine use.

Metabolic memory applies to chronic diseases [4] and describes microscopic to macroscopic irreversible changes that occur over time [5].

Primary alterations
- High plasma glucose
- Dyslipidemia
- High blood pressure
- Poor nutritional status

Early onset alterations
- ↑Polyol pathway
- ↑Protein kinase C pathway
- ↑Hexosamine pathway
- ↑AGE production
- ↑ROS production and oxidative stress
- ↑ER stress and mitochondrial damage

Intermediate onset alterations

Production of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, MCP-1 etc.)

- Proliferation of inflammatory cells
- Improved T-cell survival
- Monocyte adhesion
- Monocyte-to-macrophage differentiation

Late onset alterations
- ↑TGFβ1 pathway
- ↑mTOR pathway
- ↑Akt pathway

Genetic and epigenetic alterations
- miRNA and lncRNA (i.e. miR-192/200b/200c/216a/217 and lncRNA CJ241444)
- Histone acetylation and/or deacetylation (i.e. H3K9ac)
- DNA hypomethylation and/or hypermethylation (i.e. hypomethylation at 3' UTR of TXNIP)
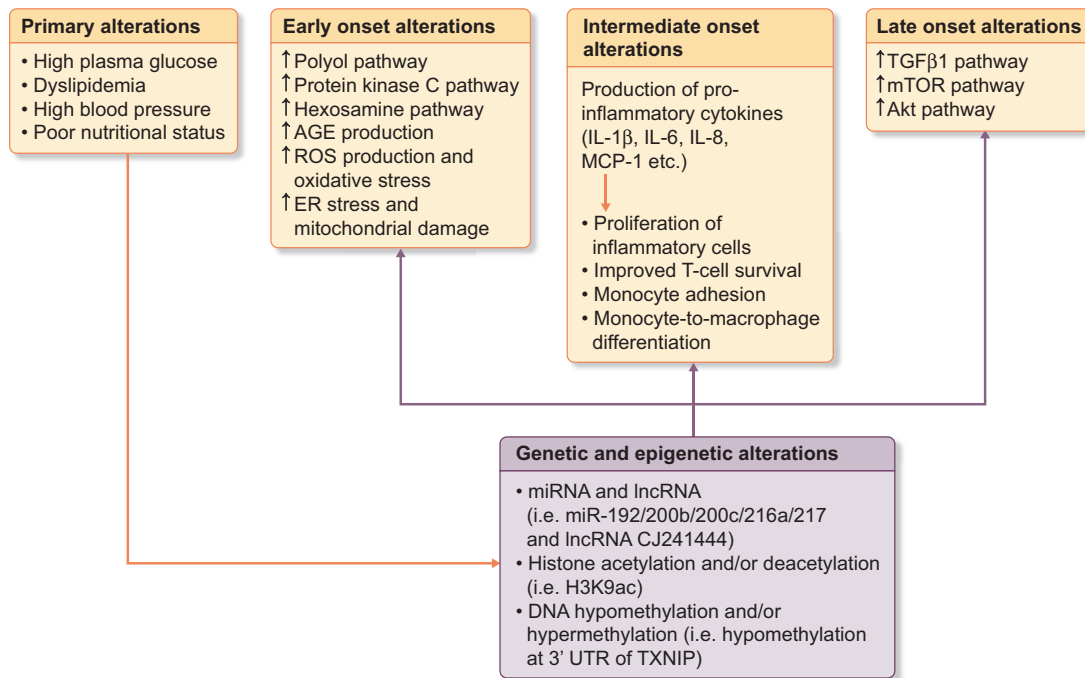
FIGURE 1: Triggering factors and mechanisms of development of metabolic memory. ER, endoplasmic reticulum.

Various primary, early, intermediate, late-onset alterations with genetic and epigenetic interactions play a role in the metabolic memory (Figure 1).

Lead time bias in the natural history of metabolic memory likely accounts for treatment failures in some patients while others have better outcomes. As this affects patients seen in the clinic daily, this report aims to provide a clinician centred review of metabolic memory.

THE METABOLIC MEMORY OF DM

Diabetes causes more blindness (microvascular), myocardial infarction (macrovascular), stroke (macrovascular) and renal failure (microvascular) worldwide than any other disease [6, 7], and the outcomes of the microvascular complications are not completely addressed by HbA1c, which is still the main biomarker used to follow 'successful' diabetes management. Diabetes does not cause these effects overnight; rather, microvascular damage leads to macrovascular consequences [8].

In a study involving 1441 Type I DM patients undergoing either intensive (≥3 daily insulin injections adjusted via frequent glucose monitoring) or conventional therapy (1–2 daily insulin injections) for a mean duration of 6.5 years, the Diabetes Complications and Control Trial (DCCT) demonstrated that delayed onset of microvascular complications, as well as slower progression, could be achieved via intensive therapy [9, 10] but the microvascular complications did not reach zero, even with these unrealistically strict treatment guidelines.

Furthermore, after the study, all patients were placed on intensive therapy, and the group that had received conventional therapy previously had higher rates of microvascular complications at 8-year follow-up, 17-year follow-up for CVD and even 22-year follow-up for low glomerular filtration rate. The metabolic memory of the conventional therapy could not be corrected even with the strictest glycaemic control, suggesting that other treatment strategies must be developed for true

microvascular prevention [11–15]. A separate study including 5102 newly diagnosed persons with Type II DM >10 years had similar findings [16, 17]. In patients with longstanding poor diabetic control, tight glycaemic control slightly improves cardiovascular outcomes compared with conventional management, a disappointing outcome for an intense intervention [18, 19]. Despite the best existing management, patients cannot escape the fate of their metabolic memory. In the below paragraphs, we give detailed description of mechanisms including immune mechanisms, oxidative stress, genetic and epigenetic changes in relation to tissue damage and pathological findings related to metabolic memory.

HISTOLOGICAL ALTERATIONS AND IMMUNE RESPONSE

The histopathological features of diabetic complications, most typically diabetic nephropathy, are mesangial expansion with increased extracellular matrix (ECM) production, formation of Periodic acid–Schiff (PAS) (+) diffuse thickening of glomerular basement membrane, effacement of podocyte food processes and hyaline arteriolosclerosis in both afferent and efferent arterioles of glomeruli and Kimmelstiel–Wilson nodules mostly as a post-mortem finding [20]. Similar patterns of histopathological changes are observed in both diabetic neuropathy and retinopathy with the addition of microaneurysm formation and punctate haemorrhages in retinopathy [21]. Expression of fibronectin and collagen mRNA, two predominant ECM proteins, has been shown to be significantly higher in human endothelial cells cultured in a hyperglycaemic environment [22, 23]. Elevated expression persisted after switch to normoglycaemic culture medium despite multiple cell divisions and replanting while cells with higher expression levels displayed proliferative advantage [22]. In addition to changes in ECM production, degradation of ECM is also impaired by hyperglycaemia leading to

glycation of mesangial proteins that impair matrix metalloproteinase-2 (MMP-2), the primary MMP secreted in mesangium responsible for the degradation of Type IV collagen [24].

Hyperglycaemia also promotes inflammatory changes in tissues, predominantly via activation of nuclear factor kappa B (NF-κB) and Toll-like receptor pathways [25]. Changes include release of inflammatory cytokines [interleukin (IL)-1β, IL-6, IL-8 and monocyte chemoattractant protein (MCP-1)], enhancement of monocyte-endothelial or vascular smooth muscle cell binding, monocyte-to-macrophage transition and increased vascular permeability [26, 27]. Furthermore, hyperglycaemia may cause premature senescence of various cell types and accelerated cellular ageing processes that may lead to senescence-associated secretory phenotype that secretes pro-inflammatory cytokines, leading to a destructive loop. Microbiotic alterations occur in people with pro-inflammatory cytokine levels and inducing senescence-associated secretory phenotype [28]. Furthermore, hyperglycaemia reduces CXC chemokine receptor type 4 (CXCR4) expression in CD34 (+) haematopoietic stem cells via altered DNA methylation patterns, improving T-cell survival via the activity of advanced glycation end-product receptors (RAGE) [29, 30].

Endothelial dysfunction and inflammation-induced insulin resistance also cause diabetic progression [31–34]. High-carb, high-fat diets inflame feeding control neurons in the hypothalamus [35, 36]. In addition, HbA1c positively correlates with IL-6 and tumour necrosis factor-α [37].

Hyperglycaemia leads to increased production and impaired degradation of ECM proteins, persistent pro-inflammatory state and altered immune response, causing net fibrosis and thickening of basement membranes causing diabetic microvascular and macrovascular complications. These are also the treatment targets.

OXIDATIVE STRESS AND BIOCHEMICAL CHANGES

Diabetic rats have more oxidative stress than those with immediately controlled diabetes, measured via reduced levels of nitric oxide (NO) and reduced glutathione in the urine [38]. Human studies revealed similar outcomes, though, the negative effects of oxidative stress on endothelial cell function are reversible in patients with initial HbA1c <7% with glycaemic control and use of anti-oxidants—while damage is irreversible if initial HbA1c is >7% [32, 33]. This is due to cytoplasmic reactive oxygen damage upon mitochondrial function and structure, leading to instability of electron transport chain (ETC), thus, cyclically higher levels of oxidative stress.

Long-standing high plasma glucose levels are associated with excess production of reactive oxygen species (ROS), namely superoxide radicals that may be converted into other species, by mitochondrial ETC. Hyperglycaemic status leads to activation of glycolysis, formation of higher concentration of pyruvate, activation of tricarboxylic acid cycle and in turn higher levels of electron donors (nicotinamide adenine dinucleotide and flavine adenine dinucleotide). High rates of electron flux between ETC and electron donors lead to increase in the electrochemical gradient across inner mitochondrial membrane until a critical threshold, which leads to inhibition of complex III of ETC when reached. Electrons are accumulated in coenzyme Q, which are then transferred to molecular oxygen, leading to generation of superoxide radicals [39]. These changes are reversible with the inhibition of ETC or by the use of uncoupler molecules to eliminate electrochemical gradient across inner membrane [39, 40]. In addition, high cellular glucose levels lead to the inhibition of a glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase, which leads to accumulation of upstream glycolytic intermediates. As a result, the protein kinase C (PKC) pathway, by formation of diacylglycerol, and the AGE products pathway, by the formation of main precursor called methylglyoxal, are activated.

In addition to increased ROS, many other pathways are used to describe biochemical changes occurring in hyperglycaemic states including the activation of the polyol pathway, AGEs, PKC pathway and hexosamine pathways. Activation of the polyol pathway, primarily the enzyme aldose reductase that converts glucose into sorbitol in the presence of nicotinamide adenine dinucleotide phosphate (NADPH), leads to depletion of NADPH stores of cells and may enhance the pre-existing status of oxidative stress observed in hyperglycaemia [41, 42]. However, in most cases of hyperglycaemia, the polyol pathway is not assumed to play major role since the requirement for cellular glucose concentration is too high according to the enzyme dynamics [43, 44].

Activation of the PKC pathway decreases NO production in vascular smooth muscle cells by inhibition of endothelial NO synthase, increase in the expression of transforming growth factor (TGF)-β1, NF-κB and plasminogen activator inhibitor-1, and increase in fatty acid oxidation in vascular endothelial cells, which may cause atherosclerosis [45–49]. Furthermore, increase in the expression of TGF-α, TGF-β1 and plasminogen activator inhibitor-1 is enhanced by the increase in O-GlcNAcylation of the transcription factor Sp1, as a result of activated hexosamine pathway [50, 51].

Increased levels of AGE in circulation leading to increased binding of AGE to its receptor referred to as RAGE result in increased expression of NF-κB, vascular cell adhesion molecule-1, trombomodulin, tissue factor, MCP-1 and vascular endothelial growth factor, and increased levels of ROS formation [52–58]. RAGE–NF-κB interaction has shown to be involved in the development of diabetic neuropathy and atherosclerosis as complications. Potential early biomarkers are summarized in Table 1 [59, 60].

GENETIC AND EPIGENETIC CHANGES

Genetics underpin maturity-onset diabetes of the young (MODY), and the development of Types I and II DM, with mutations at HNF4-alpha (MODY 1), glucokinase (MODY 2), HNF1-alpha (MODY 3) and HNF1-beta (MODY 4) [61]. On the other hand, epigenetic shifts (same DNA, different expression) likely play a more important role in metabolic memory. The main types of epigenetic modifications are DNA methylation, histone modifications, chromatin remodeling, non-coding ribonucleic acids (RNAs) as microRNA (miRNA), and lncRNA and RNA editing (Figure 1).

DNA methylation and histone acetylation, the two primary epigenetic mechanisms, are highly investigated in relation to their roles in metabolic memory in diabetic patients [62]. Monocyte and lymphocyte DNA analysis from patients in the DCCT and The Epidemiology of Diabetes Interventions and Complications (EDIC) trials showed elevated levels of H3k9ac, involved in the activation of affiliated genes, in many inflammatory genes in patients not receiving intensive therapy initially [63]. Another study demonstrated correlation between HbA1c levels and H3K9ac, thus, H3K9ac is one of the key contributors to

Table 1. Possible early biomarkers and alterations in patients with DM

Possible early biomarker categories	Examples
miRNA	Upregulation of expression of certain miRNA: • miR-192; miR-200b; miR-200c; miR-216a; miR-217; miR-21; miR-377 Downregulation of expression of certain miRNA: • miR-29; Let-7 family members
lncRNA	Upregulation of expression of certain lncRNA: • lncRNA Erbb4-IR; lncRNA Tug1 ; lncRNA RP23–298H6; lncRNA CJ241444 Downregulation of expression of certain lncRNA: • lncRNA Lin01619
Signaling pathways	Over-activation of certain pathways: • TGF-β1 signaling pathway; NF-κB signaling pathway
DNA methylation/demethylation	Hypomethylation at 3-UTR of TXNIP
Histone acetylation/deacetylation	Upregulation of certain regulators: • H3k9ac
Enzymatic control	Activation of certain enzymes: • Aldose reductase Inhibition of certain enzymes: • eNOS; glyceraldehyde 3-phosphate dehydrogenase; MMP-2
Upregulated molecules	Certain connective tissue regulators: • plasminogen activator inhibitor-1; TGF-α; vascular cell adhesion molecule-1; trombomodulin; tissue factor; MCP-1; vascular endothelial growth factor Biochemical outcomes: • ROS; AGE; Polyols Pro-inflammatory cytokines: • IL-1β; IL-6; IL-8; MCP-1; tumour necrosis factor-α
Downregulated molecules	• NO • NADPH • CXCR4 on T cells

NF-κB, nuclear factor-kappa light chain enhancer of activated B cells; CXCR4, C-X-C chemokine receptor Type 4; ncRNA, non-coding RNA; 3-UTR, three prime untranslated region.

metabolic memory. Another study utilizing both the initial and 17-year follow-up whole blood sample and monocytes of the participants of DCCT and EDIC trials demonstrated many hypo/hypermethylated DNA regions, for example, hypomethylation at the 3-untranslated region (UTR) of Thioredoxin-Interacting Protein (TXNIP) encoding thioredoxin-interacting protein, to be associated with diabetic complications [64]. Individualized analysis of the same data in accordance with the HbA1c levels during follow-ups revealed the persistent hypomethylation of 3′-UTR of the TXNIP gene, thus, epigenetic alterations of the TXNIP gene appear to be strongly related to metabolic memory [64]. In addition, a similar pattern has been observed in other studies in patients with high plasma glucose levels and hyperlipidaemia [65]. miRNAs, 20–25 nucleotides in length, are thought to regulate up to 60% of gene translation by binding to the 3′-UTR region of specific mRNAs. miRNAs are synthesized as a primary transcript by RNA polymerase II, the same enzyme involved in mRNA synthesis, and processed by Drosha-DGCR8 in the nucleus and Dicer in the cytoplasm into mature miRNA [66]. Despite being a highly investigated topic for many chronic diseases, the applicability to human diseases remains forthcoming with few actual human studies [67]. The main findings so far are in mice, where expression levels of five miRNAs (miR-192, miR-200b, miR-200c, miR-

216a and miR-217) are enhanced in renal glomeruli in the early stages of induced-diabetic states compared with control [68]. Among those miRNA, miR-192 appeared to be the key regulator since it upregulated expression of the others and was involved in the down-regulation of mRNAs involved in collagen and ECM protein synthesis [69]. In addition, mice treated with miR-192 inhibitors and miR-192 knockout mice progressed to diabetic nephropathy more slowly, while miR-192 gene amplification resulted in glomerular basement membrane hypertrophy and increased accumulation of ECM proteins including collagen [70]. Similar expression status can be achieved through treatment with TGF-β1, thus, effects of hyperglycaemia appear to be mediated through TGF-β1 expression at the genomic level [71–75]. Other highly investigated miRNA changes include upregulation of miR-21 and miR-377 and downregulation of miR-29, all of which lead to ECM hypertrophy and accumulation of ECM proteins through TGF-β1, Akt and mammalian target of rapamycin (mTOR) signalling [76–83]. Another crucial miRNA involved in the pathogenesis of hyperglycaemia is downregulation of Let-7 family members that inhibit collagen production and TGF-β1 expression

miRNAs may be the treatment biomarkers of choice, with high specificity for downstream morbidities. A study of 700 miRNAs in 40 patients with Type I DM with chronic kidney

disease (CKD) has been undertaken with this goal [84], and TGF-β1-regulated miRNAs and tissue-specific miRNA expression patterns have been proposed as biomarkers [68, 85–88].

Long non-coding RNA (lnc-RNA) are comprised of >100 nucleotides and regulate transcription and translation via histone modifications or regulating miRNAs [89]. Two miRNAs upregulated during hyperglycaemia and TGF-β1 signalling (miR-216a and miR-217) are regulated along with lncRNA RP23-298H6.1-001 [90, 91]. Similarly, coregulation of lncRNA CJ241444 and miR-192 has been reported. Diabetic nephropathy in mice is associated with downregulation of lncRNA Lin01619, and upregulation of both lncRNA Erbb4-IRand lncRNA Tug1 [92–97]. lncRNA NR_033515 upregulation is correlated with diabetic nephropathy and may be a treatment biomarker [98–100].

CLINICAL IMPLICATIONS

Obesity

Obesity is defined as a body mass index >30 kg/m^2, and affects more than one-third of men and women in the USA. It is accompanied by various comorbidities including Type II DM, metabolic syndrome, hypertension, dyslipidaemia, obstructive sleep apnoea, hyperuricaemia CVD and strokes [101]. The concept of genetic background in obesity was first hypothesized by Von Noorden in 1907, while mutations in leptin, leptin receptor, proopiomelanocortin, melanocortin 4 receptor, proconvertase-1/2, Neurotrophic Receptor Tyrosine Kinase 2 (NTRK2)/Brain-derived neurotrophic factor (BDNF) and many others have been identified since then as a genetic aetiology [102, 103]. Studies performed on a Dutch cohort comprised participants exposed to Dutch famine demonstrated DNA hypomethylation at the *IGF2* gene [104]. Similar outcomes have been observed in the Leningrad siege study, where participants were exposed to prenatal undernutrition and postnatal shortage of food supply [105]. Remarkable upregulation of genes involved in energy metabolism, inflammatory responses and cell growth/death via epigenetic mechanisms have been recorded on genome-wide association studies and epigenome studies. Human studies have demonstrated inverse correlation between the length of breast-feeding and DNA methylation levels of LEP promoter, a gene associated with low levels of plasma leptin and infant body mass index [106–108]. Histological, genetic, biochemical and epigenetic alterations have importance in the emergence and progression of obesity.

Parental nutritional status and hyperglycaemic exposure may be reflected *in utero* with an epigenetic signature, termed the Barker hypothesis. Association of high-fat maternal diet, low birthweight, short stature, hyperglycaemic state and undernutrition with epigenetic changes leading to obesity, insulin resistance and hypertension have been made. High-fat maternal diet leads to DNA hypermethylation at adipocytokine and leptin genes, leading to down regulation of those genes [109–117]. These changes persist over generations despite normal fat diet in the offspring and are not reversible with switches to normal fat diet prior to and during pregnancy [104, 118, 119]. Interestingly, paternal high fat diet also increases the risk of insulin resistance in the offspring. However, it is important to note that all studies are performed with animals in the absence of human studies, and there is uncertainty about their validity in humans. Similar effects are also indicated in cases of gestational DM and hyperglycaemia [120–123]. In addition to DNA hypermethylation of the adipocytokine and leptin genes, hypomethylation of mesoderm-specific transcript gene (MEST) and

hypermethylation of ATP-binding cassette transporter A1 (ABCA1) gene may occur in response to those stimuli [121, 122, 124–126]. Even though those epigenetic changes have not been proven in human subjects yet, significant correlation between those epigenetic markers, including hypomethylation of MEST and obesity, has been reported [127].

Hypothalamic control of obesity through various genes involved in appetite and energy metabolism have been proposed as potential epigenetic alteration sites, although no human studies have been conducted yet [128].

CKD

CKD most commonly results from diabetes and hypertension, affects more than a tenth of the world population and causes significant morbidity and mortality in adults [129]. End-stage renal disease (ESRD) is defined by histopathological findings including fibrosis, chronic inflammatory infiltrates, sclerosis of the glomeruli and hyaline obliteration. Certain prenatal conditions can predispose to ESRD by decreasing the number of functional nephrons, including prematurity, low birthweight, placental insufficiency and maternal diabetes, smoking, alcohol abuse, drugs and hypoproteinaemia, may affect the nephron number [130–135].

Transforming growth factor beta-1 (TGFβ-1)/SMAD signalling is crucial in the histopathological changes including ECM accumulation in CKD and renal fibrosis, as with diabetic nephropathy. In addition to the number of nephrons, epigenetic alterations play a crucial role in CKD development and progression.

Renal fibrosis is related to the activation of histone acetyltransferase p300/CBP-associated factor, involved in the activation of pro-inflammatory molecules such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, decreased histone acetylation at genes (HDAC1/2/5/6), involved in the downregulation of renal protective molecule BMP7, and upregulation of transcription factor ETS-1 (ETS-1 gene) via histone acetylation [136–139]. Furthermore, production of hypoxia-inducible factor 1 in cases with hypoxia results in the activation of histone demethylases mostly in genes involved in TGF-β1 pathway [140].

Increase in the production of Angiotensin Converting Enzyme-1 (ACE-1) in animal models in response to hypertension results in the enhancement of activatory epigenetic changes such as H3KAc and H3K4me and decline in repressive epigenetic modifications such as H3K9me2 at ACE promoter, thus inducing its over-production and a vicious cycle [141, 142]. Moreover, maternal low protein diet has been linked to ACE-1 overexpression.

As a model of RNA interference, regulation of miRNAs has been in the centre of epigenetics of CKD since animal studies involving downregulation of Dicer, the key enzyme in miRNA processing, were shown to cause to renal failure and death. miR-21, miR-29 and miR-200, downstream molecules in TGF-β signalling, have been shown to be important in renal fibrosis by pro-fibrotic effects of miR-21 via amplification of TGF-β signalling and anti-fibrotic effects of miR-29 and miR-200 via the inhibition of Epithelial to masenchymal transition (EMT). The latest studies identified other miRNAs with significant renal functions as miR-205, involved in apoptosis, and miR-146a, involved in ischaemia–reperfusion injury [80, 143–149].

CVD

CVD is the leading cause of morbidity and mortality in developed countries, developing as a consequence of either an embryological, anatomical, environmental and/or genetic conditions [150]. Despite the high prevalence and risk for mortality and morbidity in CVD, mostly secondary to ischaemic heart diseases, treatment modalities have limited influence on the progression of disease with significant efficiency and adverse reaction issues. Resistance to commonly prescribed coronary artery disease (CAD) medications such as nitrates, clopidogrel and aspirin have been reported [151–153]. The most common aetiologies of chronic heart diseases include atherosclerosis, dyslipidaemia and diabetes, all of which increases risk for vascular plaque formation and thrombosis. Similar histological and epigenetic alterations with diabetic patients have been reported in patients with chronic CVD.

DNA methylation levels vary significantly with gender, race and risk factors associated with CAD, while critical DNA methylation differences have been detected between atherosclerosis-prone and atherosclerosis-resistant arteries in the same CAD patients [154]. Alu and LINE-1, both retro-transposable elements involved in genomic rearrangements and control of genomic expression, are two commonly hypomethylated genes in patients with CAD. Also, certain CAD risk factors are proven to lead hypomethylation in those genes such as homocysteine, race, higher body mass index (for LINE-1) and height (for Alu), whereas no significant variation with gender or age has been determined [155–157]. Many other relevant genes have been identified, for example, ABCA1 methylation is highly correlated with atherosclerosis levels and is an important determinant of plasma high-density lipoprotein-cholesterol (HDL-C) levels [158]. The methylation status of ABCA1 has been considered a biomarker for early stages of atherosclerosis in patients with no other clinical or laboratory findings [159]. Additionally, methylation of forkhead box P3 (FOXP3) by mediating unstability in Treg cells and methylation at MMP-9, Collagen alpha-1(XIV) chain and INK4B (INK4B gene) have been proposed as candidates [160–163].

Nutritional intake as well as the medications used in the treatment of CAD influences the DNA methylation levels. Among these, homocysteine intake has crucial importance since it can be transformed into S-adenosyl methionine in the body, which is the primary methyl donor in biochemical pathways including DNA methylation [164]. Maternal nutritional status is an independent determinant of postnatal DNA methylation status of infants [165]. Age is another factor that leads to alterations in DNA methylation for which three age-related CpG islands have been identified, namely the genes ITGA2B, ASPA and PDE4C. Studies determining age by using the methylation status of those CpG islands have <5 years difference with the actual age [166]. To discriminate age and CAD-associated DNA methylation/hypomethylation another gene, ANGPTL2, has been proposed that has age-specific methylation status while elevated levels of methylation are observed in patients with CAD [167].

Although histone modifications have not been investigated in detail so far, few possible targets have been identified as downregulation of HDAC4, an enzyme that deacetylase histone molecules to alter gene expression levels. Over-expression of HDAC4 is known to induce mitochondrial dysfunction and apoptosis, while down-regulation has been shown to induce cardiac muscle and ECM hypertrophy and provides protection against oxidative stress and mitochondrial damage [168, 169].

On the other hand, miRNA in the context of CAD emerges as a possible site for earlier diagnosis and for intervention in order

to postpone disease onset and progression. miR-1 and miR-133, which are involved in the control of cell cycle and death, are significantly elevated in plasma and urine in patients with acute coronary syndrome [170, 171]. Elevations occur in the first 2 h of acute coronary syndrome, especially ST elevated myocardial infarction, and positively correlated with other commonly used cardiac markers such as CK-MB and troponin [170–172]. In addition, miR-208 is a cardiac-specific miRNA only expressed in cardiac tissue and only elevated in serum in cases of acute heart diseases [173, 174]. Elevated levels of miR-208 in plasma have high sensitivity and specificity for acute coronary syndrome [175]. miR-126, miR-132, miR-146 and miR-499 have been proposed as candidates in CVD to have importance diagnostically or prognostically [176–182]. The role of lncRNA is unclear in the development and progression of CVD due a scarcity of studies, though lncRNA necrosis-related factor, carcinoma-associated 1 (UCA1) and LIPCAR may be involved [96, 183, 184].

FUTURE DIRECTIONS

Multimodal diabetic complication management ranges from lifestyle modifications, to drugs, to surgeries such as photocoagulation/vitrectomy for diabetic retinopathy. Complications are usually a late finding, and intervention at this stage has been met with limited success overall. However, cognizance of metabolic memory, and intervention on the metabolic memory, may open up new treatment pathways [185].

Epigenetic alterations have been utilized in the treatment of oncological, autoimmune and neurological diseases with promising outcomes, such as four histone deacetylase inhibitors (vorinostat, romidepsin, panobinostat and belinostat) and two DNA methylation inhibitors (azacytidine and decitabine) that are approved by the Food and Drug Administration (FDA) [186, 187]. After detection of downregulation of the targets of transcription factor EP300-related genes via computed analysis of microarray data from many studies, vascular endothelial cells of patients with DM have been treated with EP300 inhibitors and HDAC inhibitors experimentally [188, 189]. In addition to the results of that cell culture study, animal studies investigating the efficiency of EP300 or HDAC inhibitors in DM are promising. A class III HDAC [Sirtuin-1 (SIRT1)] inhibitor and

Table 2. Possible novel therapeutic approaches proposed and/or investigating and targeting genetic and epigenetic modifications in DM

Possible target for therapeutic approaches	Examples
miRNA	• miR-192 inhibitor • miR-29c inhibitor • miR-21 inhibitor • miR-192 inhibitor
lncRNA	lnc-MGC
Histone acetylation/ deacetylation	• Histone deacetylase inhibitors: • Vorinostat; romidepsin; panobinostat; belinostat • EP300 inhibitors
DNA methylation/ demethylation	• DNA methylation inhibitors: • Azacytidine and decitabine
Genome editing	Not yet been investigated

EP300, Adenovirus early region 1 A-associated protein p300.

HMT EZH2 inhibitors demonstrated promising outcomes in animal models [190–193]. Additionally, a bromodomain-containing protein, JQ1, which blocks the expression of Angiotensin-II related genes, is successful in mice studies to reverse AngII-induced hypertension in animal models [194]. miRNAs are another possible site for intervention. Mice with induced DM that are treated with miR-192 inhibitor illustrated downregulation of many miRNAs such as miR-192 itself and others (miR-200, miR-216a and miR-217, all of which are involved in DM) and expression of p53, thus reducing the risk for diabetic complications, mostly diabetic nephropathy. Other studies investigating the inhibition of miR-29c, miR-21 and miR-192 also demonstrate success [76, 77]. lncRNA, namely lnc-MGC, has also been investigated as a target in DM, while studies regarding that topic are limited (Table 2).

As we develop a better understanding of genetic and epigenetic changes in chronic diseases including DM, CVD, CKD, metabolic syndrome and many others, genome editing may become a useful tool for treatment. Currently available techniques of genome editing include zinc-finger nucleases, first targeted nuclease developed by the fusion of FokI restriction endonuclease and custom-designed zinc finger domain, transcription activator-like effector nuclease, effector proteins derived from plant pathogenic bacteria genus *Xanthomonas*, homing endonuclease, referring to a number of endonucleases synthesized from the regions within introns and hydrolyzing genomic DNA within the same cell, and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9, the most commonly utilized method in gene editing [195–200]. In bacteria, the Type II CRISPR system is a protection mechanism against foreign DNA such as viruses and plasmids and provides some type of memory and adaptive immunity via RNA-guided DNA cleavage [201, 202].

CONCLUSION

Non-communicable cardio-metabolic chronic diseases are now a global health issue, spanning DM, CVD and CKD. Prevention strategies remain inadequate and the management of complications has not proven completely effective, so novel strategies are needed to approach these conditions. Metabolic memory, the sum of histological, biochemical, genetic, epigenetic and cellular alterations in response to specific stimuli including hyperglycaemia, dyslipidaemia or high blood pressure, have been identified and may provide new surrogate biomarkers for early detection and treatment response, as well as new targets for novel therapeutic actions.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflict of interest.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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