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RESEARCH ARTICLE

CELLULAR FIBRONECTIN AS A BIOMARKER FOR ASSESSING ENDOTHELIAL FUNCTION IN DIABETIC PATIENTS

***Saidu Kasimu and Mainasara, A.S.**

Department of Chemical Pathology and Immunology, Faculty of Basic Clinical Sciences, College of Health Sciences, Usmanu Danfodiyo University, Sokoto

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ABSTRACT

Background*:** Retinopathy and neuropathy are major chronic complications of diabetes mellitus as result of poor glycaemic control. Cellular fibronectin is derived from an endothelium of the blood vessel, it plays role in sub endothelial matrix assembly. Increased in cellular fibronectin in the plasma is associated with injury to the endothelium of blood vessels. ***Aim of the study: Is to evaluate the plasma levels of cellular fibronectin in diabetic patients. ***Materials and methods***: Cellular fibronectin was determined in one hundred (100) diabetic subjects and one hundred (100) non-diabetic subjects using sandwich Enzyme-Linked immunosorbent Assay. While (45%) of the diabetic subjects were males, (55%) were females, (48%) of the non-diabetic subjects were males and (52%) were females. However, out of (45) of the diabetic male subjects, 20(44%) present with erectile dysfunction and 25% of the diabetic subjects have blurred vision. Approval of the study was obtained from the Ethics and Research Committee of the Specialist Hospital, Sokoto. ***Results*:** Cellular fibronectin was elevated in diabetic subjects (31.21±1.62mg/L) compared with non-diabetic subjects (13.19±1.20mg/L). Cellular fibronectin was elevated in diabetic subjects with erectile dysfunction (49.00±3.76mg/L) compared to diabetic with no erectile dysfunction (32.20±2.31mg/L).Cellular fibronectin was also elevated in diabetic subjects with blurred vision (45.52±3.32mg/L) compared to diabetic subjects with no blurred vision (34.00±1.91mg/L). ***Conclusion*:** Determination of plasma level of cellular fibronectin in diabetic subjects could be useful as an adjunct in predicting early vascular complication in diabetic patients.

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INTRODUCTION

The endothelium is made up of a single layer of cells, that covered the vascular lumen. (Moreira *et al.*, 2018). It is now identified, however, that endothelial cells are metabolically active with important paracrine, endocrine and autocrine functions which are indispensable for the maintenance of vascular homeostasis under physiological conditions (Sena *et al.*, 2013; Tabit *et al.*, 2010). The functions of vascular endothelium include; regulation of vessel integrity, vascular growth and reconstruction, tissue growth and metabolism, immune responses, cell adhesion, angiogenesis, haemostasis and vascular permeability (Cheok *et al.*, 2018). The endothelium plays an initial role in the regulation of vascular tone, controlling tissue blood flow and inflammatory responses and also, maintaining blood fluidity (Li *et al.*, 2017b; Cheok *et al.*, 2018). It does so, by producing components of the extracellular matrix such as cellular fibronectin (cFn) and variety of regulatory mediators like; NO (nitric oxide), ET-1 (endothelin-1), Ang II (angiotensin II), t-PA (tissue-type plasminogen activator), PAI-1 (plasminogen activator inhibitor-1), VWF (von Willebrand factor) and tumour necrosis factor alpha (TNFα) (Klemis *et al.*, 2017). Failure of the endothelium to perform its functions from any means is referred to endothelial dysfunction (Yada *et al.*, 2018). The hallmark of diabetes mellitus, chronic hyperglycaemia, has been implicated in the development of endothelial dysfunction (Habib and Ali, 2018). The Principal mechanisms of endothelial dysfunction by hyperglycaemia involve Protein Kinase C (PK C) activation, Activation of the hexosamine and polyol pathways, Formation of advanced glycation end-products (AGEs). These pathways are said to mediate vascular dysfunction through Reactive Oxygen Species (ROS) overproduction, most importantly is the increases in O_2^- (Marzona *et al.*, 2017). Activation to the endothelium could result to increased synthesis of cellular fibronectin in response to the endothelial injury (Kassas *et al.*, 2016). Fibronectins (FN) are large glycoproteins found in plasma, in extracellular matrix, and on cell surfaces (Doddapattar *et al.*, 2018). They promote cell-cell and cell-matrix interactions and thus play a

***Corresponding author:* Saidu Kasimu**
Department of Chemical Pathology and Immunology, Faculty of Basic Clinical Sciences, College of Health Sciences, Usmanu Danfodiyo University, Sokoto

role in tissue construction and reconstruction (Kanters *et al.*, 2001). The abundant (FN) in plasma is secreted by hepatocytes and lacks extra-domain III (ED) segments, refers to plasma fibronectin (pFn) (Kanters *et al.*, 2001). However, fibronectin found in sub-endothelial and connective tissue matrices are produced locally by endothelial cells and fibroblasts and mostly contain an extra type III structural domain ED-A and is called cellular fibronectin (cFn). It's Usually made up of <2% of the total FN in plasma (Kanters *et al.*, 2001).*Fibronectin:* Fibronectins (FNs) are a family of large molecular weight (≈ 440 KDa) dimeric glycoproteins involved in a variety of biological processes (Osawa *et al.*, 2017). The protein is composed of similar but not identical polypeptides, which share a common molecular organization (Cabello *et al.*, 2018). These glycoproteins are found in plasma, in extracellular matrix and cell surfaces (Hamidi and Ivaska, 2017). Fibronectin is one of the most important extracellular matrix proteins that mediate a number of functions in basement membranes (Klemis *et al.*, 2017). It is a major extracellular matrix component closely associated with trabecular cells and capable of interacting with integrins and syndecans (Filla *et al.*, 2017). Fibronectin has been found in the juxtacanalicular region and along the inner wall of Schlemm's canal (Vranka and Acott, 2017). It is also present as a soluble protein in aqueous humor (Yuksel *et al.*, 2017). Granulosa cells in culture secrete high amounts of Fibronectin, and it is one of the major glycoproteins in follicular fluid as well as being a major component of extracellular matrix (Zeebaree *et al.*, 2018). It is an important matrix constituent in cartilage, and its content is markedly elevated in articular cartilage lesions within osteoarthritic joints (Mehta *et al.*, 2018). Fibronectin is a major secretory product of lung fibroblasts which represents 35 - 40% of the parenchymal cell population of normal lung (Jun and Lau, 2018). Fibronectin is also produced by alveolar macrophages, a cell type representing 90% of the inflammatory and immune cells of the lower respiratory tract (Jun and Lau, 2018). Fibronectin is found in plasma, biological fluids, lose connective tissues and some basal lamina (Cappellari *et al.*, 2016). It is synthesized by a variety of cells including liver hepatocytes, lung fibroblasts, endothelial cells, macrophages and Type 2 alveolar epithelial cells (Klemis *et al.*, 2017). Fibronectin has numerous biological functions relating to specific binding domains for cells and for extra cellular macromolecules including collagens (Cappellari *et al.*, 2016).

As a major protein inthe extracellular matrix, Fibronectin provides positional assistance to help direct a wide variety of biological processes including cell migration, cell differentiation and apoptosis (Alghadir *et al.*, 2016). These biological activities of Fibronectin are mediated through interactions with various members of integrin family and cell surface proteoglycans (Kanters *et al.*, 2001). Also, these activities are contained within discrete structural domains and that neighbouring domains within Fibronectin can either suppress or enhance a particular activity (Klemis *et al.*, 2017).

Fibronectin modules: The amino acid sequence of fibronectin reveals three types of internally homologous repeats or modules separated by (usually) short connecting sequences (Sankiewicz *et al.*, 2018). There are 12 type I, 2 type II and 15 type IIImodules, also referred to as fibronectin I, II and III.Each module constitutes an independently folded unit, often referred to as a domain but not to be confused with "functional domains" that frequently contain more than one module (Sankiewicz *et al.*, 2018). Modules homologous to those in fibronectin are also found in other proteins. The type II which is the most ubiquitous of all modules, is found in about 2 % of animal proteins and occasionally in plants (Eimont *et al.*, 2018). Amino acid sequences of fibronectin modules are highly conserved, differing from their counterparts in other species by only a few percent (Alghadir *et al.*, 2016). The similarity between different modules of the same type within a given protein is much less. All three modules contain several conserved core residues. Type I and II modules each contain four conserved cysteines constituting two disulfide bonds that are crucial for stability and function (Becke *et al.*, 2018). Type IIIis devoid of disulfides bond but III- 7 and III-15 each contain an unpaired buried cysteine (Zollinger and Smith, 2017). Fibronectin modules fold independently and this can exist in isolation from their neighbors. This was demonstrated in a few cases with proteolytic fragments that were small enough to contain only a single module and which, upon exposure to heat or chemical denaturants, underwent reversible unfolding (Becke *et al.*, 2018).

Forms of fibronectin: Fibronectins exist in two forms; circulating plasma fibronectin (soluble protomeric form) and locally synthesized cellular fibronectin (insoluble multimeric form).

Plasma fibronectin: Plasma fibronectin is synthesized by hepatocytes and circulates in the blood (Ismail *et al.*, 2018). Plasma Fibronectin has been shown to be a good indicator of pathological conditions associated with injury of the reticulo - endothelial system, radiation - induced lung injury, and in patients with Broncho-pulmonary carcinoma (CHOY *et al.*, 2017). Recent studies indicates that a significant increase in plasma Fibronectin occurs following exposure to ozone (O_3) and also that, the measurement of total plasma Fibronectin levels offers a potentially sensitive tool for detecting O_3 effects (Néri *et al.*, 2017).

Cellular fibronectin: Cellular fibronectin is synthesized by several cells including epithelial and connective tissue cells and is deposited into the extracellular matrix (Raitman *et al.*, 2017). Cellular fibronectin is a protein derived from an endothelium, it plays an important role in the assembling of sub endothelial matrix (Osawa *et al.*, 2017). Increased plasma levels of cellular fibronectin therefore, indicate loss of endothelial cell polarization or injury to blood vessels (Osawa *et al.*, 2017). Furthermore, elevated plasma levels of circulating cellular fibronectin have been described in clinical syndrome with vascular damage, although not in diabetes or atherosclerosis (Takii *et al.*, 2017). Both forms of fibronectin are encoded by a single gene, whose transcript can be alternatively spliced at three intron-exon boundaries, yielding fibronectin protein subunits containing, in the rat, the extra type IIIA (EIIIA) repeat, the extra type IIIB (EIIB) repeat, and/or variable region (V) region. Equivalent regions are designated extra domain - A (EDA), extra domain B (EDB) and connecting segments (III CS) in humans. During splicing of fibronectin mRNA, these exons are included or excluded depending on the tissue and cell type (Huang *et al.*, 2018).

Alternative splicing of fibronectin: There are five sites of alternate splicing of fibronectin mRNA (Murphy *et al.*, 2018). The first two results in the insertion of extra type III domains, EDA and EDB after modules III- II and III - 7 respectively. These modules are virtually absent from adult tissue but are

differentially expressed during embryonic development, in malignant or injured tissue, and during angiogenesis. The inclusion of EDA renders fibronectin a better substrate for cell spreading and migration and has been used as a marker for certain types of cancer (Zhang *et al.*, 2018a). EDA - containing fibronectin is up-regulated as part of response to wound healing (Yan *et al.*, 2017). Both EDA and EDB are increased in human plasma following trauma (Bogoslovsky *et al.*, 2015). This module is reported to serve as a ligand for integrins $\alpha_9\beta_1$and $\alpha_4\beta_1$. No specific receptors or other ligands have been identified for EDB, although it also is reported to support cell adhesion. EDB is also of interest as a marker for angiogenesis and as a potential target for tumor therapy (Sackey-Aboagye *et al.*, 2016). In plasma fibronectin mRNA, exons encoding the EDA and EDB segments are excluded, whereas the exon encoding the IIICS is either partially or completely included depending on the species. In cellular fibronectin mRNA, EDA and / or EDB exons are wholly retained and the IIICS appears to be at least partially included (Kaspar *et al.*, 2017). The third site of splicing is in the V (variable) (ITICS) region, which can be included either in its entirety or only partially, depending on the tissue. In plasma fibronectin the V region is fully incorporated into one chain but entirely absent from the other. Since the V region has at least two different integrin binding sites, its presence or absence will affect the adhesion of some types of cells. The multiple splice sites within this region produce five IIICS variants in humans namely IIICS-0, IIICS - 120, IIICS - 95, IIICS - 89, and IIICS - 64 (Scanzello *et al.*, 2015). A fourth site of splicing occurs primarily in cartilage where the dominant form of fibronectin lacks not only the entire V region but modules IIICS -15and 1 - 10 as well. This site is located at the carboxyl termini of fibronectin (Scanzello *et al.*, 2015). A truncated single chain form of fibronectin first identified in zebra fish, has been reported in mammals, including humans (Qiao *et al.*, 2014).

Glycosylation of fibronectin: In addition to the primary mRNA transcript processing, the fibronectin molecule can also undergo post-translational modifications notably O-linked glycosylation (King *et al.*, 2017). Fibronectin is a glycoprotein sites of N - linked glycosylation include the 2nd type II module, the 8th type I module, the 3rd, 5th and 7th type IIImodules and the V region, which also contains **O –** linked carbohydrates (King *et al.*, 2017). The extent and type of glycosylation varies depending on the tissue source. Amniotic fluid fibronectin contains about twice as much carbohydrate as the plasma form and this includes the presence of polylactosamine in the gelatin - binding domain (Ayodele *et al.*, 2017). Fibronectin isolated from malignant human tissues, fetal tissues or placenta also contains a unique "oncofetal " epitope, involving N-Acetylgalactosaminylation of a threonine in the V region, that" is recognized by the monoclonal antibody FDC- 6 and serves as the basis for a widely used gynecological test to predict pre-term delivery (Kumra and Reinhardt, 2016). The actual function of the various carbohydrate groups on fibronectin, beyond protecting certain domains from proteolysis or stabilizing them toward heat remains unclear. Thus, differential pre-mRNA processing and post-translational modification can lead to 20 different fibronectin isoforms, Usually less than 2% of total fibronectin in plasma is derived of cellular fibronectin (Stewart and O'connor, 2015).

Functions of fibronectin: Fibronectin has numerous biological functions relating to specific binding domains for cells and for extracellular macromolecules including collagens (Prakash *et al.*, 2015). It plays key roles in various cellular events that include cell migration and proliferation of fibroblasts and endothelial cells (Grigoriou *et al.*, 2017). Physiologically, fibronectin plays an important role in cell adhesion, motility and tissue repair among others (Lenselink, 2015). However, its overproduction may decrease motility and replication of many cells including endothelial cells (Viola *et al.*, 2017). This dimeric glycoprotein has been implicated in a variety of biological processes, including tissue remodeling during wound healing and embryonic development (Grigoriou *et al.*, 2017). Fibronectins influence these processes by affecting cell adhesion and extracellular matrix assembly (Eimont *et al.*, 2018). Fibronectins promotes cell-cell and cell matrix interactions and thus plays a role in tissue construction and reconstruction (Ayodele *et al.*, 2017). This glycoprotein is also important in such diverse activities as cell adhesion, cell migration, cellular differentiation, blood clotting, opsonization, wound healing and neoplastic transformation (Grigoriou *et al.*, 2017). It is also involved in many complex functions, some of which include the enhancement of the binding of a lymphokine to macrophage and the expression of iron receptors on macrophages. It also increases adherence and chemotaxis of phagocytes and helps maintain the oxidative bactericidal capacity of macrophages (Akenami *et al.*, 1997).

Functional domains of fibronectin: The diverse recognition functions of fibronectin are located on distinct fragments or domains, many of which have been expressed on recombinant form or isolated from proteolytic digest with retention of specific binding properties (Piprek *et al.*, 2018). Digestion of fibronectin with any of a variety of proteases generates a collection of fragments that are sometimes referred to as functional domains because they retained the ability to interact with other macromolecules (Piprek *et al.*, 2018). When thermolysin is used, one obtained an N-terminal 29 KDa Fib-l/Hep-l fragment (1_{1-5}) that binds to fibrin, heparin and some bacteria, and is important for fibronectin matrix assembly. This is followed by a 42 KDa gelatin binding fragment (GBF, $I_6II_{1-2}I_{7-9}$) that binds to denatured collagen (gelatin) and also to tissue transglutaminase. Then a small ≈9 KDa fragment (III-I) that binds weakly to heparin and is thought to be important for self-association and fibril formation. Then the large 110kDa central cell- binding fragment (III_{2-10}) contains the famous tripeptide RGD that was the first integrin recognition sequence to be identified. This is followed by either a 30 or 40 kDa Hep-2 fragment (III_{-14} or III_{12-15}) contains the highest affinity heparin- bindings site. Finally, from the C-terminus one obtains a second fibrin-binding fragment, 19-kDa Fib-2 (1_{10-12}). The variably spliced V region, also referred to as the type IIIconnecting strand contains two sites for recognition by integrin 4β1; however, it is not recovered intact from proteolytic digests. Its presence in only one chain of plasma fibronectin and its vulnerability to proteolysis digest accounts for the occurrence of 30 and 40kDa Hep-2 fragments as well as a small 8kDa fragment consisting of module Ill-15 whose function is unknown (Hecker *et al.*, 2018). The biological activities of fibronectin are mediated via interactions with various members of the integrin family (Park *et al.*, 2018). Integrins are transmembrane receptors composed of an α- and β - subunits covalently associated into a heterodimer (Yang *et al.*, 2018). There are more than 20 different integrin receptors each having their own ligand specificity. In general, however, they bind various extracellular matrix proteins usually through a specific ARG sequence and cell surface proteoglycans.

It has been shown for some time that these biological activities of fibronectin are contained within discrete, structural domains and that neighbouring domains within fibronectin can either suppress or enhance a particular activity (Yang *et al.*, 2018)

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Fibronectin and pathological states: Fibronectin has been implicated in many pathological states (Kumar *et al.*, 2014). These include cutaneous wounds, tumour metastasis, rheumatoid arthritis; cardiac allograft rejection and liver fibrosis (Piperigkou *et al.*, 2018). Fibronectin is also markedly elevated in articular cartilage lesions within osteoarthritic joints (Tanoue *et al.*, 2018). Fetal tissues and tumours express a higher percentage of fibronectins with ED-A and ED-B repeats, but in adult life, they are particularly absent (Kumra and Reinhardt, 2016). The extra type IIIdomain fibronectin is prominent during the injury response of adult tissues and mediates important early events in the response (Wang *et al.*, 2016). This form is particularly apparent in acute liver injury, where it has been shown that sinusoidal endothelial cells produce ED-A fibronectin (Kornblihtt *et al.*, 1996). Expression of the ED-A and ED-B isoforms are also increased during wound healing (Maione *et al.*, 2016). Fibronectin synthesis is increased in macrophages and polymorphonuclear leukocytes isolated from inflammatory foci (Akenami *et al.*, 1997). One of the most important extra cellular matrix proteins that are over expressed in retinas, glomeruli and the hearts in diabetes is fibronectin (Hu *et al.*, 2017). As reported by Doddapattar *et al.* (2018), high glucose concentrations increase a number of extra cellular matrix related genes, including fibronectin.

Vascular smooth muscle cells, cultured under hyperglycaemic conditions proliferate at a significantly faster rate than those cultured under normal glucose conditions (Xi *et al.*, 2019). High glucose increases the de novo synthesis of the protein Kinase-C activator, diacylglycerol (Xi *et al.*, 2019). Thus, one hypothesized mechanism by which High glucose induces VSMC proliferation is through the chronic activation of one or more isoforms of protein kinase – C (Xi *et al.*, 2019) . Also, Chen and colleagues in 2003 used human macrovascular and microvascular endothelial cell lines to evaluate the key molecular signaling events involved in high glucose -induced fibronectin over-expression. This expression was shown to be dependent on endogenous endothelin receptor - mediated signaling. They also examined the roles played by protein kinase-C and the transcription factors, nuclear factor $_{K}$Band activating protein-I with respect to such changes (Chen *et al.*, 2003). Protein Kinase C (PKC), a family of phospholipids dependent serine/ threonine protein kinases, plays a central role in the growth factor signal transduction pathway and regulates a wide variety of cellular functions, including cell proliferation, differentiation and cell growth (Fu *et al.*, 2017). PKC represents a family of II isoenzymes that have been categorized into three groups: group A or cPKCs (α, βI, βII and γ), group B or nPKCs (α, ε, η, θ) and group C or aPKCs (ζ and λ_l). In addition, PKC resembles nPKCs structurally but aPKCs functionally (Isakov, 2017). While cPKCs require Ca^{2+} - and diacylglycerol and phorboesters for their activities, PKCs are Ca^{2+} insensitive, but they are still activated by DAG or phorbol esters, aPKCs are independent of both $Ca^{2+}$and DAG/phorbol esters. The PKC isoenzymes differ in biochemical properties, tissue specific distribution and intracellular localization(Frank *et al.*, 2016). Increased activity of PKC in high glucose could be explained by the de novo synthesis of DAG via the polyol pathway - dependent increase in cytosolic *NADH/NAD*$^{+}$ratio (Frank *et al.*, 2016). Transforming Growth factor B (TGF - B) is a key cytokine for matrix protein production(Roy *et al.*, 2015). TGF- β a multifunctional cytokine that operates in either a paracrine or autocrine manner stimulates fibronectin synthesis and regulates production of the EllA - fibronectin isoform by acting on fibronectin pre - mRNA(Duran-Salgado and Rubio-Guerra, 2014). TGF - β is derived from a number of cell types including platelets wound macro phages and fibroblasts myofibroblasts, as well as injured proximal tubular epithelial cells(Moses *et al.*, 2016). High glucose has been shown to induce coordinated increases in TGF - β protein, the TGF Type 2 receptor, and the extracellular matrix protein fibronectin). High glucose has also been shown to trigger a series of events including protein kinase - C and mitogen - activated protein (MAP) kinase activation, elevation of reactive oxygen species, and increased expression of c - fos and c-jun proto-oncogenes which form a heterodimer, AP - I, that activates the TGF - β gene promoter (Yang *et al.*, 2014). Also, fibronectin splicing is known to be regulated in part by TGF - β. Inthe setting of injury in vivo, TGF - β overrides the promoter - dependence of fibronectin splicing in normal cells. This suggest that TGF - β, modifies the spliceosome if not through its known signaling intermediates, then it's through the products of genes regulated by this cytokine(Wang *et al.*, 2017).

DIABETES MELLITUS: Diabetes mellitus is one of the most common metabolic diseases in which either the hormone insulin is lacking or the body's cells are insensitive to insulin effects. The multi - system effects of diabetes such as retinopathy, nephropathy neuropathy and cardiovascular diseases are considered important impinging on the public health (Gingras *et al.*, 2017). A persistently increased blood glucose level characterizes diabetes. The diagnosis of diabetes mellitus is established by a raised blood glucose level: the venous whole blood glucose must be over 10mmol/l in samples taken at random or 2 hours after a 75g oral glucose load *(de Oliveira et al., 2018)*. Diabetes mellitus is generally categorized into two types of disease. Type I diabetes mellitus (also known as insulin - dependent diabetes mellitus (IDDM) is characterized by an absolute deficiency of insulin. In some clinical studies, type I diabetes mellitus is assumed when diagnosis occurs at or before the age of 30 in patients who require continuous insulin use. Type 2 diabetes mellitus (also known as non-insulin -dependent diabetes mellitus (NIDDM) refers to patients with diabetes mellitus characterized by insulin resistance or a state of relative insulin deficiency. Clinical studies will often use diabetes onset after age of 30 years as an operational criterion for type 2 diabetes mellitus (Gospin *et al.*, 2017). These two differ in their clinical characteristics, etiological and pathophysiologic basis for diseases. The main clinical difference is their propensity to develop diabetic ketoacidosis in the basal metabolic state; insulin is required in type I to prevent ketoacidosis, whereas in Type 2, ketoacidosis is unlikely even' when the glycaemic control is poor. Typically, type 1 presents acutely and manifests the typical symptoms of polyphagia, polydipsia and polyuria (Gospin *et al.*, 2017). In contrast, Type 2 is insidious and may be present for years before being diagnosed. Approximately, a good percentage of all diagnosed cases of diabetes mellitus is Type 2 and may be as many undiagnosed cases of Type 2 as diagnosed cases (Matsugasumi *et al.*, 2018).

Prevalence of diabetes mellitus: **The** number of people with diabetes mellitus is increasing due to population growth,

aging, urbanization, and increasing prevalence of obesity and physical inactivity (Gjesing *et al.*, 2017). The adoption of western lifestyles is known to lead to increasing prevalence of type 2 diabetes mellitus m Africa, although epidemiological studies using standardized methods are rare (Uloko *et al.*, 2018). The prevalence of diabetes for adult worldwide was estimated to be 8.8% in 2015 (Uloko *et al.*, 2018) and 6% in Sokoto (Makusidi *et al.*, 2013). The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2003 (Alexander *et al.*, 2003). The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men (Rathmann and Giani, 2004). This common endocrine disease affects more than 15 million Americans and contributed to ≈47.9 billion dollars in health care expenditure in 1995 (Bouguerra *et al.*, 2007). Both type 2 diabetes mellitus and type I diabetes mellitus show a wide variation in incidence and prevalence in different populations (Bouguerra *et al.*, 2007). The prevalence of type 2 varies from close to zero in some populations to 40 - 50% in the adults of Nauru (Pacific) and in the Pima Indians (North American) (Misra *et al.*, 2010). The incidence of type I in children under 16 years range from around 301,100,000 children per year in Finland and other Northern European countries, to less than 111,00,000 per year in Japanese children (Dabelea *et al.*, 2014). Many genetic and environmental factors combine to produce this variation. Among the suggestions made for Type 2 are the thrifty genotype and more recently the thrifty phenotype hypotheses of Nee! and of Hales and Barker respectively (Hales and Barker, 2001). Genetic and environmental factors in type I diabetes mellitus in children have combined to create an apparent gradient of decreasing incidence from northern to southern European countries but with at least one local 'hot spot': The Island of Sardinia (Cucca *et al.*, 1995). The factors responsible for this pattern and the increasing incidence overtime require further studies (Cucca *et al.*, 1995)

Type 1: Insulin Dependent Diabetes Mellitus (IDDM): The aetiology and pathogenesis of Type 1 diabetes mellitus are not fully understood, but Mandrup-Poulson grouped patients with Type 1 diabetes mellitus according to their presenting characteristics as well as immune and genetic markers into early onset, older children and adolescent and late onset (Mandrup-Poulsen, 2003). He reported that Type 1 diabetes mellitus in early childhood is characterized by abrupt onset and by high frequencies of ketoacidosis and of insulin and IA2 autoantibodies (Pociot and Lernmark, 2016). It is associated with the major histocompatibility complex heterozygosity, HLA DR ¾ (Pociot and Lernmark, 2016). Type 1 diabetes mellitus in older children and adolescents is associated with a high frequency of islet cell cytoplasmic autoantibodies, glutamic acid decarboxylase (GAD) autoantibodies and HLA DR 3. Typical immune markers often accompany autoimmune diabetes mellitus of late onset (Leslie *et al.*, 2016). Whether these groupings represent separate diseases or simply reflect the rapidity with which β- cell destruction takes place is unclear (Asmat *et al.*, 2016). Some learn people with type 1 diabetes mellitus lack the classic manifestations of autoimmune disease, and their diabetes may therefore have a different aetiology and pathogenesis (Leslie *et al.*, 2016). β-cell destruction in immune mediated type 1 diabetes mellitus is caused by an inflammatory reaction in the islets of Langerhans, triggered by environmental factors in genetically susceptible individuals (Mandrup-Poulsen, 2017).

Aetiology: Although the incidence is highest in school children and adolescents, studies have shown that in its classic form, Type 1 diabetes mellitus may develop at any age (Newton *et al.*, 2016). Between 1960 - 1989, steady temporary increases were reported in North America, Northern Europe, Japan and New Zealand (A.D.A., 2015). Data from Finland and United Kingdom show that the growing incidence is mainly explained by an increase in the frequency of onset in children under 5 years (A.D.A., 2016). This finding points to changes in major environmental aetiological factors early in life, such as viral infections and nutritional factors (Enander *et al.*, 2018).

Viral infections: Studies had shown that mothers whose children became diabetic before age 13 had increased titers of low avidity antibody to enteroviruses such as the Coxsackie β group (indicating recent viral infection) compared with mothers whose children were not diabetic by age 15 (Enander *et al.*, 2018). Immune cross reactivity to similar sequences in the Coxsackie β virus and the putative β-cell autoantigen, glutamic acid decarboxylase, has been found, suggesting that molecular mimicry may be the underlying mechanism (Enander *et al.*, 2018).

Nutritional factors : Early exposure to cow's milk proteins is associated with an increased risk of childhood diabetes (Lamb *et al.*, 2015). Molecular mimicry, between bovine serum albumin and lCA 69, another putative β-cell autoantigen, has been suggested (Dubridge *et al.*, 2019). However, use of bovine serum albumin to induce tolerance in animal models of spontaneous type 1 diabetes mellitus failed to show protection against diabetes despite tolerance to the bovine serum albumin (Li *et al.*, 2017a). Also, of interest is formula milks based on cows' milk and on hydrolysed casein contain bovine insulin. Children fed with cows' milk formula had highertiters or IgG antibodies to bovine insulin that cross reacted with human insulin, and the presence of these autoantibodies correlated with the presence of autoantibodies to human insulin (Gil *et al.*, 2017). These findings may explain the high incidence of insulin autoantibodies in young children and raise the possibility that sensitization to bovine insulin could affect immune system auto aggression towards β-cells in people who are genetically predisposed (Gil *et al.*, 2017). Finally, excess intrauterine growth has been reported as an independent risk factor for type 1 diabetes mellitus, but the mechanism remains to be explain (Sauder *et al.*, 2017).

In some genetically susceptible individuals, this immune process can persist In association with chronic progressive beta cell destruction over many months, even years, and lead to IDDM, but in others it may remit spontaneously without diabetes developing (Gil *et al.*, 2017). At diagnosis, about 80% of islets contain no beta cells and the islets may be heavily infiltrated with lymphocytes. There is no evidence that the exocrine pancreatic cells or the other islet cells are involved in this destructive process. The limited secretion of insulin by patients with IDDM results in them being prone to ketoneogenesis (Gormsen *et al.*, 2017). In the absence of insulin treatment such patients will die in diabetic ketoacidosis (Gormsen *et al.*, 2017).

Type 2: Non - Insulin Dependent Diabetes (NIDDM): Type 2 diabetes affects at least 2% of the population and no race is immune from the disease; among the Pima Indians of Arizona and Nauruan from Polynesia, half the adult population is diabetic. The prevalence of type 2 diabetes increases with

increasing age: in the elderly the prevalence can be striking, reaching 45% in men aged 75 - 79 years in east Finland (Rewers and Ludvigsson, 2016). In part this increase may be due to an age- related deterioration in glucose tolerance. Thus, while fasting blood glucose levels remain fairly constant with age the glucose level 2 hours after oral glucose rises steadily. In the absence of prospective studies this age- related effect is ignored for the purposes of defining the disease (Rewers and Ludvigsson, 2016).

Pathogenesis: The islets of Langerhans in-patient with type 2 appear normal except for amyloid deposits and a reduction in the beta cell mass to about 60% of normal (Chiang *et al.*, 2017). In general, the secreted insulin has a normal structure though there is a tendency to secrete a relative excess of pro-insulin. The disease is characterized by a decreased beta cell, secretory capacity, insulin resistance and hepatic glucose overproduction (A.D.A., 2010). The onset of the diabetes is insidious, probably occurring several years before the clinical diagnosis (Alberti and Zimmet, 1998). Since hyperglycaemia itself can induce beta cell dysfunction and insulin insensitivity, it has proved impossible to distinguish primary changes leading to diabetes from those, which are secondary to the disease (Tilg *et al.*, 2017). To address this problem, studies have been performed on non - diabetic children of diabetic patients. These children show fasting hyperglycaemia, impaired glucose tolerance, decreased glucose clearance, fasting hyperinsulinaemia, either decreased or increased insulin response to glucose, and impaired insulin - mediated glucose disposal due to reduced non - oxidative glucose metabolism (DeFronzo *et al.*, 2015). It remains to be determined whether these changes presage diabetes. The one study which followed patients prospectively found that offspring who developed diabetes, as compared with those who do not, were initially more obese and had decreased glucose tolerance and glucose clearance, fasting hyperinsulinaemia and increased second phase insulin responses (Koivusalo *et al.*, 2016). The development of type 2 was both preceded and predicted by defects in insulin - dependent and insulin, independent glucose uptake changes, which could precede the onset of hyperglycaemia and diabetes by more than a decade (Zheng *et al.*, 2018). These observations are consistent with the finding that initial lession in type 2 is due to peripheral insulin resistance not beta cell dysfunction(Zheng *et al.*, 2018). However, it is widely believed that neither abnormal insulin secretion nor abnormal sensitivity to insulin alone can explain the glucose intolerance of type 2 diabetes (Zheng *et al.*, 2018).

Aetiology: The most powerful evidence that type 2 is predominantly inherited comes from the study of identical twins of diabetic patients concordant for type 2 diabetes (Willemsen *et al.*, 2015). Evidence suggest that some individuals with maturity onset diabetes in the young (MODY) have a defect (both missense and nonsense mutations have been reported) in their glucokinase gene promoter region which might account for up to 40% of these cases. Rare genetic defects in the insulin gene and in the insulin, receptor have also been described which may cause diabetes (Willemsen *et al.*, 2015). The estimated heritability of type 2 is about 82%. In assessing the role of the environment in causing NIDDM, it is important to understand that heritability is not an invariant index of a genetic influence. It describes the genetic effect under particular environmental conditions (DeFronzo *et al.*, 2015).

Different estimates of heritability might be obtained if twins were studied in different environments. A number of non-genetic factors have been implicated in the aetiology of this disease, including nutrition, obesity, and aging and reduced exercise (van Dongen *et al.*, 2015).

Nutrition: The incidence of type 2 decreases during food shortage (Ragnarson Tennvall and Apelqvist, 2004). However, high carbohydrate diets improve insulin sensitivity in both normal, and Type 2 subjects through physiological adaptation to an altered fuel supply and not reversal of a pathological process (Wilding, 2014). The change from hunter - gatherer to a modern diet may be responsible, in part, for the virtual epidemic of diabetes in migrant populations and previously isolated communities. At present, however, there is no direct evidence that dietary factors cause type 2 diabetes although they may influence rate of progression to clinical symptoms. Studies do indicate a relationship between low birth weight and impaired glucose tolerance in later life (Crusell *et al.*, 2017). Fetal malnutrition may contribute to the later development of type 2 diabetes mellitus by impairing early fetal growth and reducing the β-cell mass. This might limit β-cell function in adult life and hamper the ability of the β-cell to compensate for insulin resistance caused by obesity, pregnancy, or drugs such as glucocorticoids (Crusell *et al.*, 2017). Support for this view shows that, low birth weight in adult first degree relatives of patient, with type 2 diabetes mellitus is associated with impaired β-cell function, and monozygotic twins who became concordant for type 2 diabetes mellitus after 60 years of follow up were growth retarded at birth compared with discordant twins (Beaumont *et al.*, 2017). Furthermore, low birth weight has been found to predispose to obesity and hypertension, suggesting that intrauterine growth retardation is a risk factor for several features of metabolic syndrome (van Dijk *et al.*, 2015). These data show that increased focus on early fetal growth and nutrition may prevent type 2 diabetes mellitus in the coming generation (van Dijk *et al.*, 2015).

Obesity: Obesity is a major factor and can potentiate type 2 diabetes mellitus in genetically susceptible individuals (Costanzo *et al.*, 2015). Offspring of type 2 diabetes mellitus themselves are more likely to be obese when young. In one study, those subjects with low birth weight who became obese were particularly prone to diabetes mellitus (Duque-Guimarães and Ozanne, 2017). Individual with upper body obesity are also particularly high risk of type 2 diabetes mellitus (Kratochvilova *et al.*, 2019). Nevertheless, there are no differences in the relative contributions of decreased insulin secretion and decreased insulin sensitivity between obese and lean type 2 diabetes mellitus subjects (Sattar and Gill, 2014; Kratochvilova *et al.*, 2019).

Aging and Exercise: Ageing is not associated with decreased insulin secretion nor with decreased insulin sensitivity in physically active subjects (Lalia *et al.*, 2016). However, lack of exercise in the elderly may hasten the appearance of hyperglycaemia. Prospective studies of populations at risk of diabetes suggest that physical activity protects against the development of type 2 diabetes mellitus (Shih *et al.*, 2018).

Genetics of diabetes: Two different methodological approaches have been taken to clarify the genetic bases of insulin dependent and non-insulin dependent diabetes (Burgio *et al.*, 2015).

The random marker approach uses information from the human genome project on the location of so called chromosomal "tags" spread all over the human genome, and the segregation of these tags with diabetes in genetically informative families. This method identifies chromosomal regions that may contain genes predisposing to disease; subsequent analysis is necessary to identify the structural genes of pathogenetic relevance. A complementary method is the candidate' gene approach (Mandrup-Poulsen, 2017). Here genes encoding for proteins believed to have a role in the pathogenesis of a disease (based on formulated pathogenetic model) are searched for variations associated with the disease in case-control studies or linked to 'the disease in family studies, or both (Mandrup-Poulsen, 2017).

Type 1 diabetes mellitus: In insulin dependent diabetes mellitus, the random marker approach has identified 18 chromosomal regions that show evidence of linkage to insulin dependent diabetes mellitus (Gauguier *et al.*, 1996). Formula linkage has been shown only for the major histocompatibility complex class II region, a variable number of tandem repeats 5' to the insulin gene and the CTLA-4 gene, coding for the ligand B7, an important accessory molecule for T cell activation (Nisticò *et al.*, 1996). Studies comparing different racial groups have shown evidence of appreciable genetic heterogeneity between ethnic groups. Much work is needed before the complex genetic susceptibility of insulin dependent diabetes mellitus is understood and the functional role of the products of these genes in pathogenesis is clarified (Peres *et al.*, 2017).

Type 2 Diabetes mellitus: More than 250 candidate genes have been tested for association with or linkage to non-insulin dependent diabetes mellitus, but none has shown consistent results in different study populations (Horikawa *et al.*, 2000). Most success for the candidate gene approach has been provided in the study of maturity onset diabetes in young people, an autosomal dominant form of early onset non-insulin dependent diabetes mellitus (Yamagata *et al.*, 1996). Although maturity onset diabetes in young people may seem to be a homogeneous phenotype, four genetically and pathogenetically distinct forms have been described. The first form is caused by a mutation in the transcription factor hepatic nuclear factor 4 (Yamagata *et al.*, 1996), the second results from different mutations in the glucokinase gene affecting β cell glucose sensing (A.D.A., 2014) the third is caused by mutations in the gene encoding for hepatic nuclear factor 1 and the fourth results from mutation of the insulin promoter factor-I. The precise role of the hepatic nuclear factor 4 and 1 mutations is not known, but it is suspected that mutations in these transcription factors affect the βcell response to glucose (A.D.A., 2017).These genes account for a small percentage of non-insulin dependent diabetes and underline the genetic complexity behind apparently homogeneous diabetic phenotypes (A.D.A., 2017). However, these discoveries may lead to new drugs that modify the β cell response to glucose, and to the development of pharmacological principles for treating common non-insulin dependent diabetes. Linkage studies in non-insulin dependent diabetes are hampered by a general lack of parents of patients with diabetes of adult onset. Very large numbers of affected sibling pairs are needed for linkage studies, and this calls for international collaboration (Mandrup-Poulsen, 2017).

Metabolic consequences of hyperglycaemia: A common health aim is to reduce the morbidity and mortality associated with diabetes (Ottosson-Laakso *et al.*, 2017). Risk factors for macro vascular disease are well established in the non - diabetic population and include hypercholesterolemia, increased plasma fibrinogen, smoking, obesity and hypertension (Zelihic *et al.*, 2015). The evidence is that in the diabetic population the same risk factors operate but, if anything, diabetes has an additive effect with them (Zelihic *et al.*, 2015). In addition, in type 2 diabetes mellitus there is tendency for some of these risk factors to aggregate; these patients are particularly at risk of obesity, dyslipidaemia and hypertension (Corsino *et al.*, 2017). A common feature of these changes is their association with insensitivity to insulin, an observation which has led to the proposal that insulin resistance is the single unifying factor causing an excess risk of macro vascular disease has been called the New World syndrome, the metabolic syndrome or syndrome X (Furukawa *et al.*, 2017).

The cause of microvascular disease is not clearly defined but the belief is that hyperglycaemia is a major factor (Packer, 2018). Microvascular-complications are not simply genetically determined since the non - diabetic twins of diabetic patients do not get them. For complications to develop, hyperglycaemia must be present (Packer, 2018). The cause of hyperglycaemia is irrelevant since microvascular complications are a feature of all types of diabetes mellitus (Magri *et al.*, 2018). As diabetes is defined by hyperglycaemia, it is reasonable to anticipate that; these microvascular complications should result from this hyperglycaemia. It is clear that the risk of diabetic complications is related to the duration of the disease (Targher *et al.*, 2018). A number of studies have demonstrated a relationship between the level of blood glucose and the risk of developing complications (Targher *et al.*, 2018). Thus, in one study, average blood glucose more than 50% above the normal range was associated with a 49% risk of developing severe retinopathy at 14 years: in contrast, this risk was only 5% in those patients with blood glucose levels close to the normal range (Cooper *et al.*, 2017).

Pregnancy: It is known that hyperglycaemia can influence foetal development (Berry *et al.*, 2016). The incidence of major and minor congenital anomalies in children of patients with diabetes mellitus is between 6% and 9% that is up to 3 times greater than in the general population (Basu and Garg, 2018). The most prevalent congenital anomalies in children of diabetic patients include caudal regression syndrome, neural tube defects and cardiac anomalies (Basu and Garg, 2018). The excess in malformations is confined to patients whose diabetes mellitus antedates their pregnancy (Ornoy *et al.*, 2015). In addition, the malformations arise form developmental changes likely to have occurred before the seventh week of gestation (Monteiro *et al.*, 2016). It was proposed that the excess congenital anomalies in children of patients with diabetes mellitus were due to hyperglycaemia in early fetal life (Monteiro *et al.*, 2016). Measuring glycated haemoglobin, an index of blood glucose levels, over the previous two months, tested this hypothesis (Ribeiro *et al.*, 2018). Children of patients with high glycated haemoglobin levels had a striking excess of congenital anomalies, which reached 22% if the glycated haemoglobin was greater than 10% (Ribeiro *et al.*, 2018). The risk of major malformations can be reduced to non - diabetic levels ifthe diabetic mother is treated to obtain normal glycated haemoglobin levels before

conception (Mañé *et al.*, 2019). The mechanism of this embryopathy is not clear. Pregnancy counselling is now routine in diabetic clinics and patients are advised to obtain near normal fasting plasma glucose levels before conception (Mañé *et al.*, 2019).

Mechanisms of diabetes complications: Exposure to hyperglycaemia can cause acute reversible metabolic changes and, if prolonged, cumulative irreversible changes (Gioacchini *et al.*, 2018). Broad mechanisms have been described for glucose-induced damage (Habib and Ali, 2018). First, glucose and other sugars can bond with any exposed lysine residues or (in the case of haemoglobin) valine, or any protein (Góralczyk *et al.*, 2016). This process of glycation can alter the structure and function of protein (Giri *et al.*, 2018). Further changes can lead to glycation products with extensive cross - linkage called advanced glycation end products - an irreversible change (Giri *et al.*, 2018). These molecules may lead to the production of free oxygen radicals, which could themselves cause tissue damage (Zhang *et al.*, 2018b). The second mechanism is the production of excess sorbitol through a normally redundant pathway involving the enzyme aldose reductase (Negahdar *et al.*, 2015). Sorbitol cannot readily leave a cell and accumulation of the alcohol sugar could lead to osmotically driven over hydration of the tissue and damage (Negahdar *et al.*, 2015).

The third mechanism involves the direct competition between glucose and myoinositol (Ivanov Kavkova *et al.*, 2019). Myoinositol is an important substrate in cellular energy production, and its structure is very similar to that of glucose. Excess glucose can therefore compete for myoinositol uptake by a cell, leading to myoinositol depletion (Zhan *et al.*, 2015). Although, diabetes control and complications trial has identified, hyperglycaemia as a significant risk factor for the development of diabetic complications (Wessells *et al.*, 2018). The full spectrum of the pathophysiological mechanisms of chronic diabetic complications has not been thoroughly elucidated. Some equally tenable hypotheses for the origin of complications are oxidative stress damage (Kortenkamp *et al.*, 2011; Wessells *et al.*, 2018), advanced glycation end products (AGEs) hypotheses (King and Brownlee, 1996), aldose reductase pathway (Yabuuchi *et al.*, 1995) reductive stress (pseudohypoxia) (Oldham *et al.*, 2015), true hypoxia (Sansome *et al.*, 2001), carbonyl stress (Takeda *et al.*, 2015), altered lipoprotein metabolism (Rye and Barter, 2014), increased protein kinase - c activity (Newton *et al.*, 2014), and altered growth factors and- cytokine activities (Sun *et al.*, 2017). The various hypotheses overlap and intersect with one another; AGE formation and altered polyol pathway activity may lead to oxidative stress, oxidative stress may accelerate AGE formation, reductive stress may lead to activation of protein Kinase - C, AGEs may induce growth factors and cytokine production and so on (Baynes and Thorpe, 1999). Among Chronic Complications of DM as a result to endothelial injury include; Nephropathy, Retinopathy, Diabetic Neuropathy, Peripheral vascular disease, and Coronary artery diseasesChange in endothelial function is considered an early pivotal step in the development of vascular disease (Kanters *et al.*, 2001). The role of circulating (cFn) in the diagnosis of vascular endothelial injury in rheumatoid vasculitis, pre-eclamptic women, have been documented (Kanters *et al.*, 2001). Vessel wall damage with characteristic endothelial extracellular matrix changes is also found in subjects with diabetes.

Several reports have implicated DM in the pathogenesis of vessel wall damage with extracellular matrix changes (Peters *et al.*, 1989).

Justification: Cellular FN level assessment could be useful as an adjuvant in predicting early vascular complications of DM. The paucity of data on circulating (cFn) in only DM in Nigeria, is the main thrust for this study. The anticipated data could provide useful adjuvant in predicting early vascular complication of DM patients.

Aim: The aim of the present study was to determine the levels of plasma cFn among DM patients attending diabetic clinic in Specialist Hospital, Sokoto.

Objectives: To determine the levels of plasma cellular fibronectin (cFn), nitric oxide (NO), glycated haemoglobin (HbA1c), von Willebrand factor (VWF) and TNFα in DM patients and apparently healthy individuals (controls). To compare levels of plasma cFn, NO, HbA1c, VWF and TNFα among DM patients and controls. To determine effects of duration treatment of DM on cFn, NO, HbA1c, VWF and TNFα level. To compare levels of plasma cFn, NO, HbA1c, VWF and TNFα among DM patients with/ without complications.

MATERIALS AND METHODS

The research was carried out in Sokoto State, basically in the Department of Chemical Pathology and Immunology, College of Health Sciences (CHS) and Department of Medicine Specialist Hospital Sokoto. A total of 200 participants were consecutively selected for the study. Only diabetic and apparently healthy individuals who fulfilled the inclusion criteria and agreed to participate in the study were selected. Diabetic subjects were selected from diabetic clinics in the Department of Medicine Specialist Hospital, Sokoto. Preliminary information's such as age, sex, height, weight of the patients, duration of the disease and medications were obtained using a questionnaire. Patients that were only dieting as means of diabetic controls were also noted.The control subjects were one hundred (100) apparently healthy individuals, of both genders. Those with history of liver diseases and cigarette smoking were excluded from the study. Both type 1& 2 diabetic patients and apparently healthy individuals aged 18 years to 60years were recruited into the study. Diabetic patient with hypertension, Diabetic patient with coexisting other endocrine disorders and Diabetic patient that consume alcohol were excluded from the study. Individuals who were non-diabetic and who have never had any family history of diabetes were included in the study as controls. Participants (Diabetics patients and apparently healthy controls) were fully informed, and their consent were obtained before the commencement of the research. Participants were allowed to withdraw from the study at any time and for any reason. Approval was obtained from the Ethics and Research Committee of the Specialist Hospital Sokoto. The study was a descriptive cross-sectional study, which was performed on Diabetic subjects attending Diabetic Clinic at Specialist Hospital Sokoto, for a period of 12 months. The diabetic patients were categorized into 2; group A based on duration of treatment and group B, which served as control. Group A was further subclassified into 5.

Group A1. Treatment naïve
Group A2. on treatment for less than a year (<1yr)
Group A3. on treatment for one to less than two years (1 - <2yr) Group A4. on treatment two to less than five years (2 - <5yr) Group A5. On treatment for five years and above (≥ 5yr). From each selected subject, a total of five milliliters (5mls) of fasting venous blood specimen were collected using a sterile syringe and needle. From the 5mls, three mls was placed in EDTA bottles for cFn, HbA1c, VWF and TNFα assay and two ml was be placed into a fluoride oxalate container for Fasting blood glucose assay. The blood sample was centrifuged to separate plasma from the cells immediately. The plasma was refrigerated at -20^{o}c

Statistical analysis: The data obtained were analyzed using Microsoft Office Excel 2007 and SPSS software version 20.0 of 2016. The results of plasma fasting glucose and lipid profile obtained from diabetic subjects were compared with the controls using pair two-tailed student's t-test for matched samples, while analysis of variance (ANOVA) was used to for comparisons of three (3) or more mean values of the parameters in the various groups. In each case where there was significant difference, a post-hoc analysis was carried out using Bonferroni multiple comparisons test. A p-value of less than or equal to 0.05 ($P \leq 0.05$) was considered as statistically significant.

RESULTS

A total of two hundred (200) subjects participated in this study. Of this number, 100 were diabetic patients, 45 males (45%) and 55 females (55%) with their age ranged between 20 and 60years and mean age and standard error of mean of (49.53±0.92). The remaining 100 were age and sex matched apparently healthy individual comprised of 48 males (48%) and 52 female (52%) who served as controls. The diabetic patients comprised of 4 type I diabetes and 96 type II diabetes. The diabetic patients were categorized into 3; group A based on duration of treatment, group B and group C. Group A was further subclassified into 5 and group B into 2.Group A1. Treatment naïve 10 (10%), Group A2. on treatment for less than a year (<1yr)12 (12%), Group A3. on treatment for one to less than two years (1 - <2yr) 18 (18%), Group A4. on treatment two to less than five years (2 - <5yr) 20 (20%), Group A5. On treatment for five years and above (≥ 5yr) 40 (40%). Group B1. Diabetic with complication. Group B2. Diabetic patients without complication. Group served as control. Table; 1 shows the results of socio-demographic and diabetics related characteristics of the study subjects including marital status, tribe, and level of education. The anthropometric data of the diabetic subjects were summarized in table 2, the age, body weight, BMI and diastolic blood pressure of the diabetic were found to be similar with the control ($p>0.05$). However, the systolic blood pressure and height of the diabetic subjects was significantly higher than the control ($p<0.05$). The mean concentration of cellular fibronectin, glycated haemoglobin, nitric oxide, von Willebrand factor and tumour necrosis factor alpha were shown in table 3; There were significantly increased in the mean concentration of cellular fibronectin (13.21±1.62mg/L), mean concentration of glycated haemoglobin (9.47±0.31%), mean concentration of von Willebrand factor alpha (35.58±3.94ng/ml) and mean concentration tumor necrosis factor alpha (93.52±20.23) ($p<0.05$) in diabetic subjects compared to control.

Furthermore, mean nitric oxide level was significantly higher in control compared to diabetic subjects (106.42±13.62µmol/L) ($p<0.05$). There were also no significantly difference in SBP and DBP as ($p>0.05$) in all the classes of duration of diabetes.Table 4. shows the results of plasma concentration of endothelial function biomarker in diabetic subjects according to the duration of treatment of diabetes in years. The mean concentration of plasma cellular fibronectin in group A5 (43.30±2.02mg/L) were significantly higher ($p<0.05$) compared to group A1, A2, A3 and A4 of the diabetic subjects and also in the control subjects. The mean plasma concentration of Nitric oxide was significantly lower in group A5 of the diabetic subjects (52.42±9.21µmol/L) compare to group A1, A2, A3, A4 and the control subjects ($p<0.05$). However, the plasma level of glycated haemoglobin (5.71±0.37%) was only significantly lower ($p<0.05$) in the control subjects compared to all the groups in the diabetic subjects. The mean concentration of the von willebrand factor was significantly higher in group A5 (35.09±4.70ng/L) and group A2 (42.80±8.40ng/L) ($p<0.05$) compared the controls.
The mean plasma concentration of tumor necrosis factor alpha in group A5 was significantly higher compared to all the groups ($p<0.05$).

Table 5; shows the effect of erectile dysfunction on endothelial function biomarkers in diabetic subjects. The mean concentration of cellular fibronectin in diabetic subjects with erectile dysfunction (49.00±3.76mg/L) were significantly higher ($p<0.05$) compared to diabetic subjects with no erectile dysfunction (32.40±2.31mg/L). There were also, a significantly difference ($p<0.05$) between the mean concentration of nitric oxide in diabetic subjects with erectile dysfunction (45.21±3.77µmol/L) and diabetic subjects with no erectile dysfunction (49.59±4.40µmol/L). There was no significantly relationship between the mean concentration of glycated haemoglobin in diabetic subjects with erectile dysfunction (9.84±0.53%) compared to diabetic subjects with no erectile dysfunction (10.34±0.71%). There were no significantly ($p>0.05$) difference between the mean concentration of von willebrand factor in diabetic subjects with erectile dysfunction (42.93±8.03ng/L) compared to diabetic subjects with no erectile dysfunction (34.67±7.95ng/L). There was no significantly ($p>0.05$) difference between the mean concentration of tumor necrosis factor alpha in diabetic subjects with erectile dysfunction (91.50±40.46ng/L) and diabetic subjects with no erectile dysfunction (52.93±13.41ng/L). Table 6; shows the effect of blurring of vision on markers used for assessing endothelial function in diabetic subjects. The mean concentration of cellular fibronectin in diabetic subjects with blurring of vision (45.52±3.32mg/L) were significantly higher ($p<0.05$) compared to diabetic subjects with no blurring of vision (34.00±1.91mg/L). There was no significantly difference ($p>0.05$) between the mean concentration of nitric oxide in diabetic subjects with blurred vision (53.91±3.86µmol/L) compared to diabetic subjects with no blurred vision (57.94±4.84µmol/L). There was no significantly ($p>0.05$) relationship between the mean concentration of glycated haemoglobin in diabetic subjects with blurred vision (9.76±0.44%) compared to diabetic subjects with no blurring of vision (9.37±0.39%). There were no significantly ($p>0.05$) difference between the mean concentration of von willebrand factor in diabetic subjects with blurred vision (32.17±6.45ng/L) compared to diabetic subjects with no blurred vision (36.72±4.81ng/L).

Table 1. Socio-Demographic Characteristics of the Diabetic Subjects

Characteristics	Number of Subjects	Percentage (%)
Marital Status	100	100
Married	79	
Single	1	
Widowed	15	
Divorced	5	
Tribe	100	
Hausa	77	
Fulani	17	
Yoruba	2	
Igbo	2	
Other	2	
Level of education	100	
Primary School Cert.	10	
Secondary School Cert.	9	
Tertiary	12	
Non-formal/ Quranic Sch.	69	
Duration of DM	100	
1-11Months	15	
12-23Months	13	
24-59Months	24	
60 Months and above	48	
Types of DM	100	
Type 1	4	
Type 2	96	
Duration of treating DM	100	
Treatment naïve	10	
Less than a year	12	
1yr – less than 2yrs	18	
2yrs – Less than 5yrs	20	
5years and above	40	

Majority of the diabetic subjects in the population had type II diabetes mellitus (96%),

Table 2. Anthropometric Data of the Diabetic Subjects (Mean ± SEM)

Characteristics	Group A Diabetic Patients (n=100)	Group B Controls (n=100)	P value
Male	45	48	
Female	55	52	
Age (Years)	49.53 ± 0.92	45.9 ± 1.48	0.457
Sbp (mmHg)	115.59 ± 0.84^{b}	111.30 ± 1.54^{a}	0.009
Dbp (mmHg)	74.24±0.77	73.24±1.10	0.460
Body Weight (Kg)	68.11±1.70	65.98±2.62	0.485
Height(m)	1.62 ± 0.01^{b}	1.72 ± 0.01^{a}	0.000
BMI(Kgm^{-2})	27.19 ± 0.63	25.14 ± 0.82	0.056

Values are expressed as Mean ± SEM; Values of the group with superscript "a" are statistically significantly ($p<0.05$) and different from group A. Values of the group with superscript "b" are statistically significantly ($p<0.05$) and different from group B.

Table 3. Endothelial functions Biomarkers in Diabetics Subjects (Mean ± SEM)

Characteristics	Group A Diabetic Patients (n=100)	Group B Controls (n=100)	p value
Cellular Fibronectin (mg/L)	31.21 ± 1.62^{b}	13.91 ± 1.20^{a}	0.000
Glycated Haemoglobin (%)	9.47 ± 0.31^{b}	5.71 ± 0.06^{a}	0.000
Nitric Oxide (µmol/L)	56.93 ± 3.75^{b}	106.42 ± 13.62^{a}	0.000
Von willebrand Factor (ng/ml)	35.58 ± 3.94^{b}	23.56 ± 3.11^{a}	0.018
Tumor Necrosis Factor (ng/L) Alpha	93.52 ± 20.23^{b}	44.49 ± 6.48^{a}	0.045

Values are expressed as Mean ± SEM; Values of the group with superscript "a" are statistically significantly ($p<0.05$) and different from group A. Values of the group with superscript "b" are statistically significantly ($p<0.05$) and different from group B.

Table 4. Effect of treatment Duration in Diabetes Mellitus on endothelial function biomarkers on diabetic subjects

Charac Teristics	N	Fibronectin (mg/L)	Nitric Oxide (µmol/L)	Glycated Haemoglobin (%)	Von willebrand Factor(ng/L)	Tumor Necrosis Factor (ng/L)
Group A1	10	30.29 ± 2.17^{eq}	64.22 ± 14.31^{eq}	9.41 ± 0.48^{eq}	40.73 ± 6.90^{eq}	71.81 ± 36.29^{eq}
Group A2	12	36.44 ± 3.62^{ae}	67.39 ± 16.49^{eq}	9.53 ± 0.68^{eq}	42.80 ± 8.40^{eq}	77.83 ± 43.30^{eq}
Group A3	18	27.08 ± 3.88^{ae}	57.92 ± 17.71^{eq}	9.82 ± 0.73^{eq}	30.98 ± 9.03^{eq}	54.70 ± 46.51^{eq}
Group A4	20	29.62 ± 2.86^{aceq}	58.89 ± 13.03^{eq}	9.30 ± 0.53^{eq}	34.55 ± 6.64^{eq}	97.27 ± 34.23^{eq}
Group A5	40	43.30 ± 2.02^{abcdq}	52.42 ± 9.21^{abcdq}	10.44 ± 0.38^{abcdq}	35.09 ± 4.70^{abcdq}	173.05 ± 24.20^{abcdq}
Group C (Control)	100	13.91 ± 1.98^{bcde}	106.42 ± 9.03^{abcde}	5.71 ± 0.37^{bcde}	13.12 ± 4.60^{be}	44.49 ± 23.72^{bcde}

Values are expressed as Mean ± SEM; Values of the group with superscript "a" are statistically significantly ($p<0.05$) and different from group A1. Values of the group with superscript "b" are statistically significantly ($p<0.05$) and different from group A2. Values of the group with superscript "c" are statistically significantly ($p<0.05$) and different from group A3. Values of the group with superscript "d" are statistically significantly ($p<0.05$) different from group A4. Values of the group with superscript "e" are statistically significantly ($p<0.05$) different from group A5. Values of the group with superscript "q" are statistically significantly ($p<0.05$) different from group C.

Table 5. Endothelial function biomarker in diabetes subjects with erectile dysfunction

Characteristics	Erectile dysfunction n=20	No erectile dysfunction n=25	P value
Fibronectin (mg/L)	49.00±3.76[b]	32.40±2.31[a]	0.000
Nitric oxide (µmol/L)	45.21±3.77[b]	49.59±4.40[a]	0.002
Glycated haemoglobin (%)	9.84±0.53	10.34±0.71	0.591
Von willebrand factor (ng/L)	42.93±8.03	34.67±7.95	0.475
Tumor necrosis factor (ng/L)	91.50±40.46	52.93±13.41	0.329

Values are expressed as mean ± SEM; Values of the group with superscript "a" are statistically significantly ($p<0.05$) different from group A. Values of the group with superscript "b" are statistically significantly ($p<0.05$) different from group B.

Table 6. Endothelial function biomarker in diabetes subjects with blurred vision

Characteristics	Blurring of vision n=25	No blurring of vision n=75	P value
Fibronectin (mg/L)	45.52±3.32[b]	34.00±1.91[a]	0.003
Nitric oxide (µmol/L)	53.91±3.86	57.94±4.84	0.645
Glycated haemoglobin (%)	9.76±0.44	9.37±0.39	0.602
Von willebrand factor (ng/L)	32.17±6.45	36.72±4.81	0.620
Tumor necrosis factor (ng/L)	92.89±32.96	93.72±24.94	0.986

Values are expressed as mean ± SEM; Values of the group with superscript "a" are statistically significantly ($p<0.05$) different from group A. Values of the group with superscript "b" are statistically significantly ($p<0.05$) different from group B.

There was no significantly ($p>0.05$) difference between the mean concentration of tumor necrosis factor alpha in diabetic subjects with blurred vision (92.89±32.96ng/L) compared to diabetic subjects with no blurred vision (93.72±24.94ng/L).

DISCUSSION

Diabetes mellitus is a common group of metabolic disorders that are characterized by chronic hyperglycaemia resulting from relative insulin deficiency, insulin resistance or both (Tuomilehto, 2019). The clinical complications associated with diabetes are most likely the consequences of hyperglycaemia through altered metabolism, non-enzymatic glycation of protein and advanced glycosylated end -products (AGEs) that accumulated in long-lived proteins such as vascular collagen and reduce the elasticity of vessel walls (Bruce and Mallika, 2019). Endothelial dysfunction in diabetes originates from hyperglycaemia and its immediate biochemical sequelae directly alter endothelial function or influence endothelial cell functioning indirectly by synthesis of cellular fibronectin, growth factors, cytokine and vasoactive agents in other cells (Tabit *et al.*, 2010).

In this present study, out of 100 diabetic subjects recruited for the research 55 (55%) were females this indicates that, most of the patients that visit diabetic clinic in Specialist Hospital, Sokoto were female. Though, the prevalence of diabetes mellitus is common amongst male (Dabelea *et al.*, 2014), however, several studies revealed that, the risk of having diabetes mellitus in female, is inversely related to their socio-economic status (Uloko *et al.*, 2018), this may be why most of the diabetes mellitus patients that visits specialist hospital, Sokoto were female and had no formal education. Another reason, may be because most of the women that visit the hospital seeking medical attention are full time house wife and therefore, can have enough time to go to hospital. This tallied with a study carried out by Uloko *et al.* (2018) which revealed that majority of diabetic patients in Nigeria are female this may not be unconnected that, female are at risk of developing obesity which is an important risk factor of type II diabetes mellitus (A.D.A., 2017). Obesity is a major factor and can potentiate type 2 diabetes mellitus in genetically susceptible individuals (Costanzo *et al.*, 2015). This present study revealed that the plasma level of cellular fibronectin, glycated haemoglobin, von willebrand factor and tumor necrosis factor alpha which could be used as endothelial function biomarkers were significantly increased in the diabetic subjects compared to control. This may not be unconnected that, diabetic subjects with poor, long standing glucose control have tendencies of developing diabetic complications like retinopathy and nephropathy, due to endothelial injury (Azeze *et al.*, 2018). Risk factors for macro vascular disease are well established in the non - diabetic population and include hypercholesterolemia, increased plasma fibrinogen, smoking, obesity and hypertension (Zelihic *et al.*, 2015). The evidence is that in the diabetic population the same risk factors operate but diabetes has an additive effect with them (Zelihic *et al.*, 2015). In addition, in type 2 diabetes mellitus, there is tendency for some of these risk factors to aggregate; these patients are particularly at risk of obesity, dyslipidaemia and hypertension (Corsino *et al.*, 2017). A common feature of these changes is their association with insensitivity to insulin, an observation which has led to the proposal that insulin resistance is the single unifying factor causing an excess risk of macro vascular disease (Furukawa *et al.*, 2017). The cause of microvascular disease is not clearly defined but the belief is that hyperglycaemia is a major factor (Packer, 2018). Microvascular complications are not simply genetically determined since the non - diabetic twins of diabetic patients do not get them. For complications to develop, hyperglycaemia must be present (Packer, 2018). The cause of hyperglycaemia is irrelevant since microvascular complications are a feature of all types of diabetes mellitus (Magri *et al.*, 2018). As diabetes is defined by hyperglycaemia, it is reasonable to anticipate that; these microvascular complications should result from this hyperglycaemia. It is clear that the risk of diabetic complications is related to the duration of the disease (Targher *et al.*, 2018). In a study carried out in Netherland that assess the plasma levels of cellular fibronectin in diabetes patients, shows that circulating cellular fibronectin were significantly higher in diabetic patients compared to ischaemic stroke patients (Kanters *et al.*, 2001). Similar finding was also obtained in China, though, von willebrand factor was only used for the research, by Chen *et al.* (2013) it revealed that type II diabetic patients older than 60years of age had increased levels of VWF, VWF activation and VWF propeptide compared to younger patients who are non-diabetics. They also found that the total active VWF was associated with the time of being diagnosed with diabetes, indicating that probably the total active VWF is the best

marker in this patient population for endothelial activation and endothelial damage (Chen *et al.*, 2013). However, the mean nitric oxide level was significantly lower in control compared to diabetic subjects. This may be due to impaired conversion of arginine to NO due oxidative stress in type II diabetes mellitus (Tessari *et al.*, 2010). Though many researches show that nitric was significantly increase in diabetic subjects with complication (Chen *et al.*, 2018). Nitric Oxide (NO) is a crucial player in vascular homeostasis, is synthesized within endothelial cells, during conversion of L-arginine to L-citrulline by endothelial nitric oxide synthase (eNOS) (Sambe *et al.*, 2018). It is released from endothelial cells mainly in response to shear stress elicited by the circulating blood or receptor-operated substances such as acetylcholine, bradykinin, or serotonin. NO diffuses to vascular smooth muscle cells (VSMC) and activates soluble guanylate cyclase (sGC), yielding increased levels of cyclic guanosine-3,5-monophosphate (cGMP) and relaxation of VSMC. Additionally, NO also prevents leukocyte adhesion and migration, smooth muscle cell proliferation, platelet adhesion and aggregation, and opposes apoptosis and inflammation having an overall antiatherogenic effect (Todd Milne *et al.*, 2017). This also tally with research carried out by Chen *et al.* (2018),s which show that, there is decreases bioavailability of NO as result of oxidative stress cause by hyperglycaemia. The results of plasma concentration of endothelial function biomarkers in diabetic subjects based on the treatment duration of diabetes mellitus in years, this present study shows that, the mean concentration of plasma cellular fibronectin in diabetes subjects with a treatment duration of ≥5years where significantly higher compared to diabetes subjects with <5years duration of treatment. A long period of diabetes and poor glucose control is associated with an increased production of glycosylation end products, metabolic derangements, endothelial cell activation, endothelial injury and reactive oxygen species production with increased level of cellular fibronectin (Kanters *et al.*, 2001). poor compliance of diabetic drugs might be another factor that could result to endothelial injury and consequently increase in plasma level of cellular fibronectin, this is in agreement with research carried out by (Alghadir *et al.*, 2016).

Also, in this study we observed significantly lower plasma NO levels in subjects living with diabetes for more than 5years compared to subjects having diabetes for less than 5years and also in control. This corresponds with a study carried out by Assmann *et al.* (2016). However, in another study carried out in Indian in type II diabetes mellitus subjects revealed higher NO levels with hyperglycaemia (Adela *et al.*, 2015). This study also shows a significant decreased NO level, in diabetes mellitus subjects with erectile dysfunction compared to diabetic subjects without the complication. NO might have both beneficial and harmful effects depending upon its concentration (Adela *et al.*, 2015). On the other hand, NO causes relaxation of blood vessel reducing blood pressure, prevent platelet aggregation and adhesion, limits oxidation of LDL cholesterol, inhibits proliferation of smooth muscle cells, and decrease the expression of pro-inflammatory genes that are associated with atherogenesis (Yang *et al.*, 2010). However, NO interact with O_2^-leading to NO inactivation and production of peroxynitrite, which post-transcriptionally modify proteins and negatively affects their function (Cosentino *et al.*, 1997). This may contribute to endothelial dysfunction by in active eNOS dimers, a zinc ion is held by four thiols, two from each monomer.

Physiological relevant concentrations of peroxynitrite oxidize the zinc thiolate center in eNOS, releasing zinc and oxidizing the thiols(cite). Upon thiol reduction, eNOS dimers dissociate into monomers. This modification reduces NO bioactivity and enhances O_2^- production, which reacts with NO, further generating more peroxynitrite, in a process called as eNOS uncoupling (Aydın *et al.*, 2001). Increase NO concentrations or its reduced bioactivity due to eNOS uncoupling may not only be relevant to the development of endothelial dysfunction and atherosclerotic complications in diabetes mellitus, but may also affect insulin-mediated postprandial glucose disposal and possibly contribute to the development of insulin resistance (Domingueti *et al.*, 2016). However, the plasma level of glycated haemoglobin in this study was found to be on the increasing site as duration of diabetes is increases. This correlate with a study carried out by Verma *et al.* (2006) in Indian, which revealed that amount of carbohydrate attached to the HbA1c increases with increasing duration of the disease. Also, in another study, it revealed that diabetic subjects with poorly controlled glucose showed a significant correlation between HbA1c and duration of diabetes (van Steen *et al.*, 2018). The mean concentration of the von willebrand factor was significantly higher in subjects living with diabetes mellitus for more than 5years compared to subjects with diabetes for less five years and also in controls. This is agreement with study carried out in china by Chen *et al.* (2013),which said there is positive correlation between the period of suffering from diabetes and von willebrand factor related parameters. Indicating that the duration of the diabetic process influences the endothelium and there by promotes VWF secretion (Chen *et al.*, 2013). The mean plasma concentration of tumor necrosis factor alpha was significantly elevated in diabetic subjects with the disease for more than 5years compared to subjects living the disease for less than 5years, this similar to a study done in japan (Yano *et al.*, 2004) revealed that circulating levels of TNF-α increases in diabetic with increase in duration of disease.

This is because hyperglycaemia stimulates TNF-α secretion from monocytes and endothelial cells (Yano *et al.*, 2004). However, TNF-α can induce vascular injury by affecting the balance between coagulation and fibrinolysis (Margetic, 2012). TNF-α stimulate the expression of tissue factor that is the initiator of blood coagulation activation and the secretion of plasminogen activator inhibitor-1 that inhibits fibrinolysis (Margetic, 2012). This present study shows that, the mean concentration of cellular fibronectin, glycated haemoglobin, von willebrand factor and tumor necrosis factor alpha in diabetic subjects with erectile dysfunction were significantly higher compared to diabetic subjects with no erectile dysfunction. Endothelial dysfunction is the hallmark of erectile dysfunction in diabetes mellitus subjects and might be through PKC activation, activation of the hexosamine and polyol pathways and formation of advanced glycation end product (Brownlee, 2001). This is in agreement with research carried out by (Malavige and Levy, 2009). However, the mean concentration of nitric oxide in diabetic subjects with erectile dysfunction were significantly lower compared to diabetic subjects with no erectile dysfunction. This study revealed that the mean concentration of cellular fibronectin, glycated haemoglobin, von willebrand factor and tumor necrosis factor alpha in diabetic subjects with blurred vision were significantly higher compared to the diabetic subjects with no blurred vision. This is because microangiopathy is one of the complications that could be sequel to chronic diabetes mellitus

and eye is not exceptional organ. This was proved right in a research carried out in by (Khan *et al.*, 2017). Hyperglycaemia is the hallmark of diabetic retinopathy starting from advanced glycosylation products and activation of cytokines, could result to retinal pigment epithelium, microaneurysm, inter-retinal oedema, haemorrhage, exudate and intraocular neovascularization (Wan *et al.*, 2015).

Conclusions and Recommendations

Conclusions

Endothelial function biomarkers cellular fibronectin, von Willebrand factor and tumour necrosis factor alpha were significantly higher in diabetic subjects compared to control.
Endothelial function biomarkers nitric oxide was significantly lower in diabetic subjects compared to control. Diabetic subject with duration of the disease for more than five years had increase level of endothelial function biomarkers compared to diabetic subject living with diseases for less than five years. Diabetic subjects with complications had higher level of cellular fibronectin compared to diabetic subjects without complication.

Recommendations

Endothelial function biomarker especially cellular fibronectin, nitric oxide, von willebrand factor and tumour necrosis factor alpha should include in the routine investigation in the management of diabetic patients.

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REFERENCES

A.D.A. 2010. American Diabetes Association,
Diagnosis and classification of diabetes mellitus. *Diabetes care,* 33**,** S62-S69.

A.D.A. 2014. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care,* 37**,** S81-S90.

A.D.A. 2015. American Diabetes Association, Children and adolescents. *Diabetes Care,* 38**,** S70-S76.

A.D.A. 2016. American Diabetes Association, Standards of medical care in diabetes—2016 abridged for primary care providers. *Clinical diabetes: a publication of the American Diabetes Association,* 34**,** 3.

A.D.A. 2017. American Diabetes Association, Standards of medical care in diabetes—2017 abridged for primary care providers. *Clinical Diabetes,* 35**,** 5-26.

Adela, R., Nethi, S. K., Bagul, P. K., Barui, A. K., Mattapally, S., Kuncha, M., Patra, C. R., Reddy, P. N. C. & Banerjee, S. K. 2015. Hyperglycaemia enhances nitric oxide production in diabetes: a study from South Indian patients. *PloS One,* 10**,** e0125270.

Akenami, F. O., Koskiniemi, M., Siimes, M. A., Ekanem, E. E., Bolarin, D. M. & Vaheri, A. 1997. Assessment of plasma fibronectin in malnourished Nigerian children. *Journal of Pediatric Gastroenterology and Nutrition,* 24**,** 183-188.

Alberti, K. G. M. M. & Zimmet, P. F. 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic Medicine,* 15**,** 539-553.

Alexander, C. M., Landsman, P. B., Teutsch, S. M. & Haffner, S. M. 2003. NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes,* 52**,** 1210-1214.

Alghadir, A. H., Gabr, S. A. & Al-Eisa, E. 2016. Cellular fibronectin response to supervised moderate aerobic training in patients with type 2 diabetes. *Journal of Physical Therapy Science,* 28**,** 1092-1099.

Asmat, U., Abad, K. & Ismail, K. 2016. Diabetes mellitus and oxidative stress—a concise review. *Saudi Pharmaceutical Journal,* 24**,** 547-553.

Assmann, T. S., Brondani, L. A., Boucas, A. P., Rheinheimer, J., De Souza, B. M., Canani, L. H., Bauer, A. C. & Crispim, D. 2016. Nitric oxide levels in patients with diabetes mellitus: a systematic review and meta-analysis. *Nitric Oxide,* 61**,** 1-9.

Aydın, A., Orhan, H., Sayal, A., Özata, M., Şahin, G. & Işımer, A. 2001. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. *Clinical Biochemistry,* 34**,** 65-70.

Ayodele, A., Fox, N. S., Gupta, S., Spiegelman, J., Saltzman, D. H., Booker, W. & Rebarber, A. 2017. The Association between Fetal Fibronectin, Cervical Length, and Amniotic Fluid Sludge with Histological Indicators of Placental Inflammation in Twin Gestations. *American Journal of Perinatology*.

Azeze, T. K., Sisay, M. M. & Zeleke, E. G. 2018. Incidence of diabetes retinopathy and determinants of time to diabetes retinopathy among diabetes patients at Tikur Anbessa Hospital, Ethiopia: a retrospective follow up study. *BMC Research Notes,* 11**,** 542.

Basu, M. & Garg, V. 2018. Maternal hyperglycemia and fetal cardiac development: Clinical impact and underlying mechanisms. *Birth Defects Research,* 110**,** 1504-1516.

Baynes, J. W. & Thorpe, S. R. 1999. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes,* 48**,** 1-9.

Beaumont, R. N., Horikoshi, M., Mccarthy, M. I. & Freathy, R. M. 2017. How Can Genetic Studies Help Us to Understand Links Between Birth Weight and Type 2 Diabetes? *Current Diabetes Reports,* 17**,** 22.

Becke, T. D., Ness, S., Gürster, R., Schilling, A. F., Di Guilmi, A.-M., Sudhop, S., Hilleringmann, M. & Clausen-Schaumann, H. 2018. Single Molecule Force Spectroscopy Reveals Two-Domain Binding Mode of Pilus-1 Tip Protein RrgA of Streptococcus Pneumoniae to Fibronectin. *ACS Nano*.

Berry, D. C., Boggess, K. & Johnson, Q. B. 2016. Management of pregnant women with type 2 diabetes mellitus and the consequences of fetal programming in their offspring. *Current Diabetes Reports,* 16**,** 36.

Bogoslovsky, T., Qu, B.-X., Cota, M., Latour, L. & Diaz-Arrastia, R. 2015. Von Willberand Factor And Cellular Fibronectin Plasma Concentrations Are Increased In Mild Traumatic Brain Injury (TBI) With MRI Evidence Of Microvascular Injury.(P7. 164). *Neurology,* 84**,** P7. 164.

Bouguerra, R., Alberti, H., Salem, L. B., Rayana, C. B., Atti, J., Gaigi, S., Slama, C. B., Zouari, B. & Alberti, K. 2007. The global diabetes pandemic: the Tunisian experience. *European Journal of Clinical Nutrition,* 61**,** 160.

Brownlee, M. 2001. Biochemistry and molecular cell biology of diabetic complications. *Nature,* 414**,** 813.

Bruce, M. P. & Mallika, M. V. 2019. Prevalence of complications of diabetes among patients with diabetes mellitus attending a tertiary care centre in Tamil Nadu. *International Journal Of Community Medicine And Public Health*.

Burgio, E., Lopomo, A. & Migliore, L. 2015. Obesity and diabetes: from genetics to epigenetics. *Molecular Biology Reports,* 42**,** 799-818.

Cabello, J. R., De Torre, I., Cipriani, F. & Poocza, L. 2018. Elastin-like materials for tissue regeneration and repair. *Peptides and Proteins as Biomaterials for Tissue Regeneration and Repair*. Elsevier.

Cappellari, G. G., Barazzoni, R., Cattin, L., Muro, A. F. & Zanetti, M. 2016. Lack of Fibronectin Extra Domain A Alternative Splicing Exacerbates Endothelial Dysfunction in Diabetes. *Scientific Reports,* 6.

Chen, J.-Y., Ye, Z.-X., Wang, X.-F., Chang, J., Yang, M.-W., Zhong, H.-H., Hong, F.-F. & Yang, S.-L. 2018. Nitric oxide bioavailability dysfunction involves in atherosclerosis. *Biomedicine & Pharmacotherapy,* 97**,** 423-428.

Chen, S., Mukherjee, S., Chakraborty, C. & Chakrabarti, S. 2003. High glucose-induced, endothelin-dependent fibronectin synthesis is mediated via NF-κB and AP-1. *American Journal of Physiology-Cell Physiology,* 284**,** C263-C272.

Chen, S. F., Xia, Z. L., Han, J. J., Wang, Y. T., Wang, J. Y., Pan, S. D., Wu, Y. P., Zhang, B., Li, G. Y. & Du, J. W. 2013. Increased active von Willebrand factor during disease development in the aging diabetic patient population. *Age,* 35**,** 171-177.

Cheok, A., Rodriguez-Mateos, A., George, T. & Caton, P. 2018. Effect of betalain (betanin) supplementation on endothelial dysfunction and vascular tone in human umbilical vascular endothelial cells. *Proceedings of the Nutrition Society,* 77.

Chiang, G. C., Chang, E., Pandya, S., Kuceyeski, A., Hu, J., Isaacson, R., Ganzer, C., Schulman, A., Sobel, V. & Vallabhajosula, S. 2017. Cognitive deficits in non-demented diabetic elderly appear independent of brain amyloidosis. *Journal of the Neurological Sciences,* 372**,** 85-91.

Choy, H., Yung, S., Li, N., Cheung, K. & Chan, D. EDA+ Fibronectin in Tubulo-interstitial Injury in Lupus Nephritis. 12th International Congress on SLE & 7th Asian Congress on Autoimmunity, 2017.

Cooper, M. N., De Bock, M. I., Carter, K. W., De Klerk, N. H., Jones, T. W. & Davis, E. A. 2017. Incidence of and risk factors for hospitalisations due to vascular complications: A population-based type 1 diabetes cohort (n= 1316) followed into early adulthood. *Journal of Diabetes and its Complications,* 31**,** 843-849.

Corsino, L., Dhatariya, K. & Umpierrez, G. 2017. Management of diabetes and hyperglycemia in hospitalized patients. *Endotext [Internet]*. MDText. com, Inc.

Cosentino, F., Hishikawa, K., Katusic, Z. S. & LüScher, T. F. 1997. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation,* 96**,** 25-28.

Costanzo, P., Cleland, J. G., Pellicori, P., Clark, A. L., Hepburn, D., Kilpatrick, E. S., Perrone-Filardi, P., Zhang, J. & Atkin, S. L. 2015. The obesity paradox in type 2 diabetes mellitus: relationship of body mass index to prognosis: a cohort study. *Annals of Internal Medicine,* 162**,** 610-618.

Crusell, M., Damm, P., Hansen, T., Pedersen, O., Glümer, C., Vaag, A. & Lauenborg, J. 2017. Ponderal index at birth associates with later risk of gestational diabetes mellitus. *Archives of Gynecology and Obstetrics***,** 1-8.

Cucca, F., Lampis, R., Frau, F., Macis, D., Angius, E., Masile, P., Chessa, M., Frongia, P., Silvetti, M. & Cao, A. 1995. The distribution of DR4 haplotypes in Sardinia suggests a primary association of type I diabetes with DRB1 and DQB1 loci. *Human Immunology,* 43**,** 301-308.

Dabelea, D., Mayer-Davis, E. J., Saydah, S., Imperatore, G., Linder, B., Divers, J., Bell, R., Badaru, A., Talton, J. W. & Crume, T. 2014. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. *Jama,* 311**,** 1778-1786.

De Oliveira, V. L. P., Martins, G. P., Mottin, C. C., Rizzolli, J. & Friedman, R. 2018. Predictors of long-term remission and relapse of type 2 diabetes mellitus following gastric bypass in severely obese patients. *Obesity Surgery,* 28**,** 195-203.

Defronzo, R. A., Ferrannini, E., Groop, L., Henry, R. R., Herman, W. H., Holst, J. J., Hu, F. B., Kahn, C. R., Raz, I. & Shulman, G. I. 2015. Type 2 diabetes mellitus. *Nature Reviews Disease Primers,* 1**,** 15019.

Doddapattar, P., Jain, M., Dhanesha, N., Lentz, S. R. & Chauhan, A. K. 2018. Fibronectin Containing Extra Domain A Induces Plaque Destabilization in the Innominate Artery of Aged Apolipoprotein E–Deficient Mice. *Arteriosclerosis, Thrombosis, and Vascular Biology***,** ATVBAHA. 117.310345.

Domingueti, C. P., Dusse, L. M. S. A., Das Graças Carvalho, M., De Sousa, L. P., Gomes, K. B. & Fernandes, A. P. 2016. Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. *Journal of Diabetes and its Complications,* 30**,** 738-745.

Dubridge, R. B., Lemon, B. D., Austin, R. J., Evnin, L. & Guenot, J. 2019. Single domain serum albumin binding protein. Google Patents.

Duque-Guimarães, D. & Ozanne, S. 2017. Early nutrition and ageing: can we intervene? *Biogerontology***,** 1-8.

Duran-Salgado, M. B. & Rubio-Guerra, A. F. 2014. Diabetic nephropathy and inflammation. *World Journal of Diabetes,* 5**,** 393.

Eimont, R., Vailionytė, A., Cėpla, V., Druceikaitė, K., Inokaitis, H., Dabkevičiūtė, L., Sukackaitė, E., Masilionis, I., Ulčinas, A. & Samanta, A. Extracellular matrix mimetics by crosslinked peptide hydrogels: application to neural 3D cell cultures. Vita Scientia: International conference „Vita Scientia “: conference book: 3rd January, 2018, Vilnius, 2018. Vita est Scientia, 59-59.

Enander, R., Adolfsson, P., Bergdahl, T., Forsander, G., Ludvigsson, J. & Hanas, R. 2018. Beta cell function after intensive subcutaneous insulin therapy or intravenous insulin infusion at onset of type 1 diabetes in children without ketoacidosis. *Pediatric Diabetes,* 19**,** 1079-1085.

Filla, M. S., Dimeo, K. D., Tong, T. & Peters, D. M. 2017. Disruption of fibronectin matrix affects type IV collagen, fibrillin and laminin deposition into extracellular matrix of human trabecular meshwork (HTM) cells. *Experimental Eye Research,* 165**,** 7-19.

Frank, J. A., Yushchenko, D. A., Hodson, D. J., Lipstein, N., Nagpal, J., Rutter, G. A., Rhee, J.-S., Gottschalk, A., Brose, N. & Schultz, C. 2016. Photoswitchable diacylglycerols enable optical control of protein kinase C. *Nature Chemical Biology*.

Fu, C., Hao, J., Zeng, M., Song, Y., Jiang, W., Zhang, P., Luo, A., Cao, Z., Belardinelli, L. & Ma, J. 2017. Modulation of late sodium current by Ca2+-calmodulin-dependent protein kinase II, protein kinase C and Ca2+ during hypoxia in rabbit ventricular myocytes. *Experimental Physiology,* 102**,** 818-834.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M. & Shimomura, I. 2017. Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of Clinical Investigation,* 114**,** 1752-1761.

Gauguier, D., Froguel, P., Parent, V., Bernard, C., Bihoreau, M.-T., Portha, B., James, M. R., Penicaud, L., Lathrop, M. & Ktorza, A. 1996. Chromosomal mapping of genetic loci associated with non-insulin dependent diabetes in the GK rat. *Nature Genetics,* 12**,** 38-43.

Gil, F., Amezqueta, A., Martinez, D., Aznal, E., Etayo, V., Durá, T. & Sánchez-Valverde, F. 2017. Association between Caesarean Delivery and Isolated Doses of Formula Feeding in Cow Milk Allergy. *International Archives of Allergy and Immunology,* 173**,** 147-152.

Gingras, V., Leroux, C., Fortin, A., Legault, L. & Rabasa-Lhoret, R. 2017. Predictors of cardiovascular risk among patients with type 1 diabetes: A critical analysis of the metabolic syndrome and its components. *Diabetes & Metabolism,* 43**,** 217-222.

Gioacchini, F. M., Albera, R., Re, M., Scarpa, A., Cassandro, C. & Cassandro, E. 2018. Hyperglycemia and diabetes mellitus are related to vestibular organs dysfunction: Truth or suggestion? A literature review. *Acta Diabetologica,* 55**,** 1201-1207.

Giri, B., Dey, S., Das, T., Sarkar, M., Banerjee, J. & Dash, S. K. 2018. Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: an update on glucose toxicity. *Biomedicine & Pharmacotherapy,* 107**,** 306-328.

Gjesing, A. P., Rui, G., Lauenborg, J., Have, C. T., Hollensted, M., Andersson, E., Grarup, N., Sun, J., Quan, S. & Brandslund, I. 2017. High Prevalence of Diabetes-Predisposing Variants in MODY Genes Among Danish Women With Gestational Diabetes Mellitus. *Journal of the Endocrine Society,* 1**,** 681-690.

Góralczyk, K., Szymańska, J., Szot, K., Fisz, J. & Rość, D. 2016. Low-level laser irradiation effect on endothelial cells under conditions of hyperglycemia. *Lasers in Medical Science,* 31**,** 825-831.

Gormsen, L. C., Svart, M., Thomsen, H. H., Søndergaard, E., Vendelbo, M. H., Christensen, N., Tolbod, L. P., Harms, H. J., Nielsen, R. & Wiggers, H. 2017. Ketone Body Infusion With 3□Hydroxybutyrate Reduces Myocardial Glucose Uptake and Increases Blood Flow in Humans: A Positron Emission Tomography Study. *Journal of the American Heart Association,* 6**,** e005066.

Gospin, R., Leu, J. P. & Zonszein, J. 2017. Diagnostic Criteria and Classification of Diabetes. *Principles of Diabetes Mellitus***,** 1-16.

Grigoriou, E., Cantini, M., Dalby, M. J., Petersen, A. & Salmeron-Sanchez, M. 2017. Cell migration on material-driven fibronectin microenvironments. *Biomaterials Science*.

Habib, S. & Ali, A. 2018. An Overview on Nitric Oxide and Energy Metabolism. *Sustained Energy for Enhanced Human Functions and Activity.* Elsevier.

Hales, C. N. & Barker, D. J. 2001. The thrifty phenotype hypothesis: Type 2 diabetes. *British Medical Bulletin,* 60**,** 5-20.

Hamidi, H. & Ivaska, J. 2017. Vascular Morphogenesis: An Integrin and Fibronectin Highway. *Current Biology,* 27**,** R158-R161.

Hecker, M., Mäder, U. & Völker, U. 2018. From the genome sequence via the proteome to cell physiology–Pathoproteomics and pathophysiology of Staphylococcus aureus. *International Journal of Medical Microbiology*.

Horikawa, Y., Oda, N., Cox, N. J., Li, X., Orho-Melander, M., Hara, M., Hinokio, Y., Lindner, T. H., Mashima, H. & Schwarz, P. E. 2000. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nature Genetics,* 26**,** 163-175.

Hu, X., Bai, T., Xu, Z., Liu, Q., Zheng, Y. & Cai, L. 2017. Pathophysiological Fundamentals of Diabetic Cardiomyopathy. *Comprehensive Physiology,* 7**,** 693-711.

Huang, Q., Yu, M., Chen, H., Zeng, M., Sun, Y., Saha, T. T. & Chen, D. 2018. LRFN (leucine-rich repeat and fibronectin type-III domain-containing protein) recognizes bacteria and promotes hemocytic phagocytosis in the Pacific oyster Crassostrea gigas. *Fish & Shellfish Immunology,* 72**,** 622-628.

Isakov, N. Protein kinase C (PKC) isoforms in cancer, tumor promotion and tumor suppression. Seminars in Cancer Biology, 2017. Elsevier.

Ismail, A. E., Fabian, F. M., Wang, O., Lei, Y., Carlson, M. A., Burgess, W. H. & Velander, W. H. 2018. The isolation of a plasma-derived γγ'fibrinogen: Fibronectin mixture that forms a novel polymeric matrix. *Process Biochemistry,* 75**,** 257-265.

Ivanov Kavkova, E., Blöchl, C. & Tenhaken, R. 2019. The Myo□inositol pathway does not contribute to ascorbic acid synthesis. *Plant Biology,* 21**,** 95-102.

Jun, J.-I. & Lau, L. F. 2018. Resolution of organ fibrosis. *The Journal of Clinical Investigation,* 128**,** 97-107.

Kanters, S. D., Banga, J.-D., Algra, A., Frijns, R. C., Beutler, J. J. & Fijnheer, R. 2001. Plasma levels of cellular fibronectin in diabetes. *Diabetes Care,* 24**,** 323-327.

Kaspar, M., Schwager, K. & Trachsel, E. 2017. Antigen associated with rheumatoid arthritis. Google Patents.

Kassas, I., Tran, K.-V., Fitzgibbons, T., Chen, K., Craige, S., Kant, S., Bote, J., Girgis, S., Aurigemma, G. & Keaney, J. 2016. Endothelial Dysfunction and Inflammation in Stress Cardiomyopathy. Am Heart Assoc.

Khan, A., Petropoulos, I., Ponirakis, G. & Malik, R. 2017. Visual complications in diabetes mellitus: beyond retinopathy. *Diabetic Medicine,* 34**,** 478-484.

King, G. L. & Brownlee, M. 1996. The cellular and molecular mechanisms of diabetic complications. *Endocrinology and Metabolism Clinics,* 25**,** 255-270.

King, S. L., Joshi, H. J., Schjoldager, K. T., Halim, A., Madsen, T. D., Dziegiel, M. H., Woetmann, A.,

Vakhrushev, S. Y. & Wandall, H. H. 2017. Characterizing the O-glycosylation landscape of human plasma, platelets, and endothelial cells. *Blood Advances,* 1**,** 429-442.

Klemis, V., Ghura, H., Federico, G., Würfel, C., Bentmann, A., Gretz, N., Miyazaki, T., Gröne, H.-J. & Nakchbandi, I. A. 2017. Circulating fibronectin contributes to mesangial expansion in a murine model of type 1 diabetes. *Kidney International,* 91**,** 1374-1385.

Koivusalo, S. B., Rönö, K., Klemetti, M. M., Roine, R. P., Lindström, J., Erkkola, M., Kaaja, R. J., Pöyhönen-Alho, M., Tiitinen, A. & Huvinen, E. 2016. Gestational diabetes mellitus can be prevented by lifestyle intervention: the Finnish Gestational Diabetes Prevention Study (RADIEL): a randomized controlled trial. *Diabetes Care,* 39**,** 24-30.

Kornblihtt, A., Pesce, C., Alonso, C., Cramer, P., Srebrow, A., Werbajh, S. & Muro, A. 1996. The fibronectin gene as a model for splicing and transcription studies. *The FASEB Journal,* 10**,** 248-257.

Kortenkamp, A., Martin, O., Faust, M., Evans, R., Mckinlay, R., Orton, F. & Rosivatz, E. 2011. State of the art assessment of endocrine disrupters. *Final Report,* 23.

Kratochvilova, H., Lacinova, Z., Klouckova, J., Kavalkova, P., Cinkajzlova, A., Trachta, P., Krizova, J., Benes, M., Dolezalova, K. & Fried, M. 2019. Neudesin in obesity and type 2 diabetes mellitus: the effect of acute fasting and weight reducing interventions. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy,* 12**,** 423.

Kumar, V., Abbas, A. K., Fausto, N. & Aster, J. C. 2014. *Robbins and Cotran Pathologic Basis of Disease, Professional Edition E-Book*, Elsevier Health Sciences.

Kumra, H. & Reinhardt, D. P. 2016. Fibronectin-targeted drug delivery in cancer. *Advanced Drug Delivery Reviews,* 97**,** 101-110.

Lalia, A. Z., Dasari, S., Johnson, M. L., Robinson, M. M., Konopka, A. R., Distelmaier, K., Port, J. D., Glavin, M. T., Esponda, R. R. & Nair, K. S. 2016. Predictors of whole-body insulin sensitivity across ages and adiposity in adult humans. *The Journal of Clinical Endocrinology & Metabolism,* 101**,** 626-634.

Lamb, M. M., Miller, M., Seifert, J. A., Frederiksen, B., Kroehl, M., Rewers, M. & Norris, J. M. 2015. The effect of childhood cow's milk intake and HLA□DR genotype on risk of islet autoimmunity and type 1 diabetes: The Diabetes Autoimmunity Study in the Young. *Pediatric Diabetes,* 16**,** 31-38.

Lenselink, E. A. 2015. Role of fibronectin in normal wound healing. *International Wound Journal,* 12**,** 313-316.

Leslie, R. D., Palmer, J., Schloot, N. C. & Lernmark, A. 2016. Diabetes at the crossroads: relevance of disease classification to pathophysiology and treatment. *Diabetologia,* 59**,** 13-20.

Li, C., Yang, M., Hou, G., Liu, S., Huan, Y., Yu, D., Sun, S., Liu, Q., Yan, S. & Shen, Z. 2017a. A human glucagon-like peptide-1-albumin recombinant protein with prolonged hypoglycemic effect provides efficient and beneficial control of glucose metabolism in diabetic mice. *Biological and Pharmaceutical Bulletin***,** b17-00169.

Li, M., Qian, M. & Xu, J. 2017b. Vascular endothelial regulation of obesity-associated insulin resistance. *Frontiers in Cardiovascular Medicine,* 4**,** 51.

Magri, C. J., Mintoff, D., Camilleri, L., Xuereb, R. G., Galea, J. & Fava, S. 2018. Relationship of Hyperglycaemia, Hypoglycaemia, and Glucose Variability to Atherosclerotic Disease in Type 2 Diabetes. *Journal of Diabetes Research,* 2018.

Maione, A. G., Smith, A., Kashpur, O., Yanez, V., Knight, E., Mooney, D. J., Veves, A., Tomic□Canic, M. & Garlick, J. A. 2016. Altered ECM deposition by diabetic foot ulcer□derived fibroblasts implicates fibronectin in chronic wound repair. *Wound Repair and Regeneration,* 24**,** 630-643.

Makusidi, M. A., Liman, H. M., Yakubu, A., Isah, M., Jega, R., Adamu, H. & Chijioke, A. 2013. Prevalence of non-communicable diseases and its awareness among inhabitants of Sokoto metropolis: Outcome of a screening program for hypertension, obesity, diabetes mellitus and overt proteinuria. *Arab Journal of Nephrology and Transplantation,* 6**,** 189-191.

Malavige, L. S. & Levy, J. C. 2009. Erectile dysfunction in diabetes mellitus. *The Journal of Sexual Medicine,* 6**,** 1232-1247.

Mandrup-Poulsen, T. 2003. Apoptotic signal transduction pathways in diabetes. *Biochemical Pharmacology,* 66**,** 1433-1440.

Mandrup-Poulsen, T. 2017. Granzyme A in the Pathogenesis of Type 1 Diabetes: The Yes and the No. *Diabetes,* 66**,** 2937-2939.

Mañé, L., Flores-Le Roux, J. A., Pedro-Botet, J., Gortazar, L., Chillarón, J. J., Llauradó, G., Payà, A. & Benaiges, D. 2019. Is fasting plasma glucose in early pregnancy a better predictor of adverse obstetric outcomes than glycated haemoglobin? *European Journal of Obstetrics & Gynecology and Reproductive Biology,* 234**,** 79-84.

Margetic, S. 2012. Inflammation and hemostasis. *Biochemia Medica: Biochemia Medica,* 22**,** 49-62.

Marzona, I., Avanzini, F., Lucisano, G., Tettamanti, M., Baviera, M., Nicolucci, A. & Roncaglioni, M. 2017. Risk & prevention collaborative group. Are all people with diabetes and cardiovascular risk factors or microvascular complications at very high risk? Findings from the rsk and prevention study. *Acta Diabetol,* 54**,** 123-131.

Matsugasumi, M., Hashimoto, Y., Okada, H., Tanaka, M., Kimura, T., Kitagawa, N., Tanaka, Y., Fukuda, Y., Sakai, R. & Yamazaki, M. 2018. The Association between Taste Impairment and Serum Zinc Concentration in Adult Patients with Type 2 Diabetes. *Canadian Journal of Diabetes*.

Mehta, B. B., Sharma, S., Vasishta, R. K., Sen, R. K., Sharma, A. & Luthra-Guptasarma, M. 2018. Blocking osteopontin-fibronectin interactions reduce extracellular fibronectin deployment and arthritic immunopathology. *International Immunopharmacology,* 55**,** 297-305.

Misra, R., Patel, T., Kotha, P., Raji, A., Ganda, O., Banerji, M., Shah, V., Vijay, K., Mudaliar, S. & Iyer, D. 2010. Prevalence of diabetes, metabolic syndrome, and cardiovascular risk factors in US Asian Indians: results from a national study. *Journal of Diabetes and its Complications,* 24**,** 145-153.

Monteiro, L. J., Norman, J. E., Rice, G. E. & Illanes, S. E. 2016. Fetal programming and gestational diabetes mellitus. *Placenta,* 48**,** S54-S60.

Moreira, M. B., Garcia-Cardeña, G., Saffi, M. a. L. & Libby, P. 2018. Endothelium: A Coordinator of Acute and Chronic Inflammation. *Endothelium and Cardiovascular Diseases.* Elsevier.

Moses, H. L., Roberts, A. B. & Derynck, R. 2016. The discovery and early days of TGF-β: a historical

perspective. *Cold Spring Harbor Perspectives in Biology,* 8**,** a021865.

Murphy, P. A., Butty, V. L., Boutz, P. L., Begum, S., Kimble, A. L., Sharp, P. A., Burge, C. B. & Hynes, R. O. 2018. Alternative RNA splicing in the endothelium mediated in part by Rbfox2 regulates the arterial response to low flow. *Elife,* 7**,** e29494.

Negahdar, L., Hausoul, P. J., Palkovits, S. & Palkovits, R. 2015. Direct cleavage of sorbitol from oligosaccharides via a sequential hydrogenation-hydrolysis pathway. *Applied Catalysis B: Environmental,* 166**,** 460-464.

Néri, J. D. S. V., Lomba, E., Karam, A. M., De Almeida Reis, S. R., Marchionni, A. M. T. & Medrado, A. R. a. P. 2017. Ozone therapy influence in the tissue repair process: A literature review. *Journal of Oral Diagnosis,* 2**,** 1-6.

Newton, K., Dugger, D. L., Wickliffe, K. E., Kapoor, N., De Almagro, M. C., Vucic, D., Komuves, L., Ferrando, R. E., French, D. M. & Webster, J. 2014. Activity of protein kinase RIPK3 determines whether cells die by necroptosis or apoptosis. *Science,* 343**,** 1357-1360.

Newton, K. P., Hou, J., Crimmins, N. A., Lavine, J. E., Barlow, S. E., Xanthakos, S. A., Africa, J., Behling, C., Donithan, M. & Clark, J. M. 2016. Prevalence of prediabetes and type 2 diabetes in children with nonalcoholic fatty liver disease. *JAMA Pediatrics,* 170**,** e161971-e161971.

Nisticò, L., Buzzetti, R., Pritchard, L. E., Van Der Auwera, B., Giovannini, C., Bosi, E., Martinez Larrad, M. T., Serrano Rios, M., Chow, C. & Cockram, C. S. 1996. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. *Human Molecular Genetics,* 5**,** 1075-1080.

Oldham, W. M., Clish, C. B., Yang, Y. & Loscalzo, J. 2015. Hypoxia-mediated increases in L-2-hydroxyglutarate coordinate the metabolic response to reductive stress. *Cell Metabolism,* 22**,** 291-303.

Ornoy, A., Reece, E. A., Pavlinkova, G., Kappen, C. & Miller, R. K. 2015. Effect of maternal diabetes on the embryo, fetus, and children: congenital anomalies, genetic and epigenetic changes and developmental outcomes. *Birth Defects Research Part C: Embryo Today: Reviews,* 105**,** 53-72.

Osawa, S., Kurachi, M., Yamamoto, H., Yoshimoto, Y. & Ishizaki, Y. 2017. Fibronectin on extracellular vesicles from microvascular endothelial cells is involved in the vesicle uptake into oligodendrocyte precursor cells. *Biochemical and Biophysical Research Communications,* 488**,** 232-238.

Ottosson-Laakso, E., Krus, U., Storm, P., Prasad, R. B., Oskolkov, N., Ahlqvist, E., Fadista, J., Hansson, O., Groop, L. & Vikman, P. 2017. Glucose-induced changes in gene expression in human pancreatic islets: causes or consequences of chronic hyperglycemia. *Diabetes,* 66**,** 3013-3028.

Packer, M. 2018. Have we really demonstrated the cardiovascular safety of anti□hyperglycaemic drugs? Rethinking the concepts of macrovascular and microvascular disease in type 2 diabetes. *Diabetes, Obesity and Metabolism,* 20**,** 1089-1095.

Park, S. J., Kang, H. J., Na, S., Lee, S. H. & Baik, M. 2018. Differential expression of extracellular matrix and integrin genes in the longissimus thoracis between bulls and steers and their association with intramuscular fat contents. *Meat Science,* 136**,** 35-43.

Peres, L. C., Risch, H., Terry, K. L., Webb, P. M., Goodman, M. T., Wu, A. H., Alberg, A. J., Bandera, E. V., Barnholtz-Sloan, J. & Bondy, M. L. 2017. Racial/ethnic differences in the epidemiology of ovarian cancer: a pooled analysis of 12 case-control studies. *International Journal of Epidemiology*.

Peters, J. H., Maunder, R. J., Woolf, A. D., Cochrane, C. G. & Ginsberg, M. H. 1989. Elevated plasma levels of ED1+ ("cellular") fibronectin in patients with vascular injury. *Translational Research,* 113**,** 586-597.

Piperigkou, Z., Manou, D., Karamanou, K. & Theocharis, A. D. 2018. Strategies to Target Matrix Metalloproteinases as Therapeutic Approach in Cancer. *Proteases and Cancer*. Springer.

Piprek, R. P., Kolasa, M., Podkowa, D., Kloc, M. & Kubiak, J. Z. 2018. Transcriptional profiling validates involvement of extracellular matrix and proteinases genes in mouse gonad development. *Mechanisms of Development,* 149**,** 9-19.

Pociot, F. & Lernmark, Å. 2016. Genetic risk factors for type 1 diabetes. *The Lancet,* 387**,** 2331-2339.

Prakash, P., Kulkarni, P. P., Lentz, S. R. & Chauhan, A. K. 2015. Cellular fibronectin containing extra domain A promotes arterial thrombosis in mice through platelet Toll-like receptor 4. *Blood,* 125**,** 3164-3172.

Qiao, L., Gao, H., Zhang, T., Jing, L., Xiao, C., Xiao, Y., Luo, N., Zhu, H., Meng, W. & Xu, H. 2014. Snail modulates the assembly of fibronectin via α5 integrin for myocardial migration in zebrafish embryos. *Scientific Reports,* 4.

Ragnarson Tennvall, G. & Apelqvist, J. 2004. Health-economic consequences of diabetic foot lesions. *Clinical Infectious Diseases,* 39**,** S132-S139.

Raitman, I., Huang, M. L., Williams, S. A., Friedman, B., Godula, K. & Schwarzbauer, J. E. 2017. Heparin-fibronectin interactions in the development of extracellular matrix insolubility. *Matrix Biology*.

Rathmann, W. & Giani, G. 2004. Global Prevalence of Diabetes: Estimates for the Year 2000 and Projections for 2030: Response to Wild et al. *Diabetes Care,* 27**,** 2568-2569.

Rewers, M. & Ludvigsson, J. 2016. Environmental risk factors for type 1 diabetes. *The Lancet,* 387**,** 2340-2348.

Ribeiro, L., Cruz, S., Alves, J., Gomes-Filho, I. & Passos-Soares, J. 2018. High Level of Maternal Glycated Hemoglobin and Low Birth Weight. *Epidemiology (Sunnyvale),* 8**,** 2161-1165.1000337.

Roy, L.-O., Poirier, M.-B. & Fortin, D. 2015. Transforming growth factor-beta and its implication in the malignancy of gliomas. *Targeted Oncology,* 10**,** 1-14.

Rye, K.-A. & Barter, P. J. 2014. Regulation of high-density lipoprotein metabolism. *Circulation Research,* 114**,** 143-156.

Sackey-Aboagye, B., Olsen, A. L., Mukherjee, S. M., Ventriglia, A., Yokosaki, Y., Greenbaum, L. E., Lee, G. Y., Naga, H. & Wells, R. G. 2016. Fibronectin Extra Domain A Promotes Liver Sinusoid Repair following Hepatectomy. *PloS One,* 11**,** e0163737.

Sambe, T., Mason, R. P., Dawoud, H., Bhatt, D. L. & Malinski, T. 2018. Metformin treatment decreases nitroxidative stress, restores nitric oxide bioavailability and endothelial function beyond glucose control. *Biomedicine & Pharmacotherapy,* 98**,** 149-156.

Sankiewicz, A., Romanowicz, L., Pyc, M., Hermanowicz, A. & Gorodkiewicz, E. 2018. SPR imaging biosensor for the quantitation of fibronectin concentration in blood

samples. *Journal of Pharmaceutical and Biomedical Analysis,* 150**,** 1-8.

Sansome, C., Zaika, A., Marchenko, N. D. & Moll, U. M. 2001. Hypoxia death stimulus induces translocation of p53 protein to mitochondria. *FEBS Letters,* 488**,** 110-115.

Sattar, N. & Gill, J. M. 2014. Type 2 diabetes as a disease of ectopic fat? *BMC Medicine,* 12**,** 123.

Sauder, K., Hockett, C., Ringham, B., Glueck, D. & Dabelea, D. 2017. Fetal overnutrition and offspring insulin resistance and β□cell function: the Exploring Perinatal Outcomes among Children (EPOCH) study. *Diabetic Medicine*.

Scanzello, C. R., Markova, D. Z., Chee, A., Xiu, Y., Adams, S. L., Anderson, G., Zgonis, M., Qin, L., An, H. S. & Zhang, Y. 2015. Fibronectin splice variation in human knee cartilage, meniscus and synovial membrane: observations in osteoarthritic knee. *Journal of Orthopaedic Research,* 33**,** 556-562.

Sena, C. M., Pereira, A. M. & Seiça, R. 2013. Endothelial dysfunction—a major mediator of diabetic vascular disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* 1832**,** 2216-2231.

Shih, I.-F., Paul, K., Haan, M., Yu, Y. & Ritz, B. 2018. Physical activity modifies the influence of apolipoprotein E ε4 allele and type 2 diabetes on dementia and cognitive impairment among older Mexican Americans. *Alzheimer's & Dementia: the Journal of the Alzheimer's Association,* 14**,** 1-9.

Stewart, R. L. & O'connor, K. L. 2015. Clinical significance of the integrin α6β4 in human malignancies. *Laboratory Investigation,* 95**,** 976-986.

Sun, L., Qi, Y., Fredericks, J., Jergens, A., Wannemuehler, M. J. & Wang, Q. 2017. Effect of Cytokines On the Growth of Miniguts of Altered Schaedler Flora and Conventional Mice. *Gastroenterology,* 152**,** S352.

Tabit, C. E., Chung, W. B., Hamburg, N. M. & Vita, J. A. 2010. Endothelial dysfunction in diabetes mellitus: molecular mechanisms and clinical implications. *Reviews in Endocrine and Metabolic Disorders,* 11**,** 61-74.

Takeda, M., Ohnuma, T., Takeuchi, M., Katsuta, N., Maeshima, H., Takebayashi, Y., Higa, M., Nakamura, T., Nishimon, S. & Sannohe, T. 2015. Altered serum glyceraldehyde-derived advanced glycation end product (AGE) and soluble AGE receptor levels indicate carbonyl stress in patients with schizophrenia. *Neuroscience Letters,* 593**,** 51-55.

Takii, M., Suehiro, T., Shima, A., Yotsueda, H., Hisano, S. & Katafuchi, R. 2017. Fibronectin glomerulopathy complicated with persistent cloaca and congenital esophageal atresia: a case report and literature review. *BMC Nephrology,* 18**,** 288.

Tanoue, H., Morinaga, J., Yoshizawa, T., Yugami, M., Itoh, H., Nakamura, T., Uehara, Y., Masuda, T., Odagiri, H. & Sugizaki, T. 2018. Angiopoietin-like protein 2 promotes chondrogenic differentiation during bone growth as a cartilage matrix factor. *Osteoarthritis and Cartilage,* 26**,** 108-117.

Targher, G., Lonardo, A. & Byrne, C. D. 2018. Nonalcoholic fatty liver disease and chronic vascular complications of diabetes mellitus. *Nature Reviews Endocrinology,* 14**,** 99.

Tessari, P., Cecchet, D., Cosma, A., Vettore, M., Coracina, A., Millioni, R., Iori, E., Puricelli, L., Avogaro, A. & Vedovato, M. 2010. Nitric oxide synthesis is reduced in subjects with type 2 diabetes and nephropathy. *Diabetes,* 59**,** 2152-2159.

Tilg, H., Moschen, A. R. & Roden, M. 2017. NAFLD and diabetes mellitus. *Nature Reviews Gastroenterology & Hepatology,* 14**,** 32.

Todd Milne, G., Sandner, P., Lincoln, K. A., Harrison, P. C., Chen, H., Wang, H., Clifford, H., Qian, H. S., Wong, D. & Sarko, C. 2017. Abstracts from the 8th International Conference on cGMP Generators, Effectors and Therapeutic Implications: Bamberg, Germany. 23-25 June, 2017.

Tuomilehto, J. 2019. Definitions of intermediate hyperglycaemia and progression to type 2 diabetes. *The Lancet Diabetes & Endocrinology,* 7**,** 243-245.

Uloko, A. E., Musa, B. M., Ramalan, M. A., Gezawa, I. D., Puepet, F. H., Uloko, A. T., Borodo, M. M. & Sada, K. B. 2018. Prevalence and risk factors for diabetes mellitus in Nigeria: A systematic review and meta-analysis. *Diabetes Therapy,* 9**,** 1307-1316.

Van Dijk, S. J., Molloy, P., Varinli, H., Morrison, J., Muhlhausler, B., Buckley, M., Clark, S., Mcmillen, I., Noakes, M. & Samaras, K. 2015. Epigenetics and human obesity. *International Journal of Obesity,* 39**,** 85.

Van Dongen, J., Willemsen, G., Heijmans, B. T., Neuteboom, J., Kluft, C., Jansen, R., Penninx, B. W., Slagboom, P. E., De Geus, E. J. & Boomsma, D. I. 2015. Longitudinal weight differences, gene expression and blood biomarkers in BMI-discordant identical twins. *International Journal of Obesity,* 39**,** 899-909.

Van Steen, S. C., Woodward, M., Chalmers, J., Li, Q., Marre, M., Cooper, M. E., Hamet, P., Mancia, G., Colagiuri, S. & Williams, B. 2018. Haemoglobin glycation index and risk for diabetes-related complications in the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial. *Diabetologia,* 61**,** 780-789.

Verma, M., Paneri, S., Badi, P. & Raman, P. 2006. Effect of increasing duration of diabetes mellitus type 2 on glycated hemoglobin and insulin sensitivity. *Indian Journal of Clinical Biochemistry,* 21**,** 142.

Viola, M., Brüggemann, K., Karousou, E., Caon, I., Caravà, E., Vigetti, D., Greve, B., Stock, C., De Luca, G. & Passi, A. 2017. MDA-MB-231 breast cancer cell viability, motility and matrix adhesion are regulated by a complex interplay of heparan sulfate, chondroitin−/dermatan sulfate and hyaluronan biosynthesis. *Glycoconjugate Journal,* 34**,** 411-420.

Vranka, J. A. & Acott, T. S. 2017. Pressure-induced expression changes in segmental flow regions of the human trabecular meshwork. *Experimental Eye Research,* 158**,** 67-72.

Wan, T.-T., Li, X.-F., Sun, Y.-M., Li, Y.-B. & Su, Y. 2015. Recent advances in understanding the biochemical and molecular mechanism of diabetic retinopathy. *Biomedicine & Pharmacotherapy,* 74**,** 145-147.

Wang, K., Seo, B. R., Fischbach, C. & Gourdon, D. 2016. Fibronectin mechanobiology regulates tumorigenesis. *Cellular and Molecular Bioengineering,* 9**,** 1-11.

Wang, L.-J., Wu, Z.-H., Zheng, X.-T., Long, J.-Y., Dong, Y.-M. & Fang, X. 2017. Zinc Finger E-Box Binding Protein 2 (ZEB2) Suppress Apoptosis of Vascular Endothelial Cells Induced by High Glucose Through Mitogen-Activated Protein Kinases (MAPK) Pathway Activation. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research,* 23**,** 2590.

Wessells, H., Braffett, B. H., Holt, S. K., Jacobson, A. M., Kusek, J. W., Cowie, C., Dunn, R. L., Sarma, A. V. &

Group, D. E. S. 2018. Burden of Urological Complications in Men and Women With Long-standing Type 1 Diabetes in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Cohort. *Diabetes Care,* 41**,** 2170-2177.

Wilding, J. 2014. The importance of weight management in type 2 diabetes mellitus. *International Journal of Clinical Practice,* 68**,** 682-691.

Willemsen, G., Ward, K. J., Bell, C. G., Christensen, K., Bowden, J., Dalgård, C., Harris, J. R., Kaprio, J., Lyle, R. & Magnusson, P. K. 2015. The concordance and heritability of type 2 diabetes in 34,166 twin pairs from international twin registers: The Discordant Twin (DISCOTWIN) Consortium. *Twin Research and Human Genetics,* 18**,** 762-771.

Xi, G., Shen, X., Wai, C., White, M. F. & Clemmons, D. R. 2019. Hyperglycemia induces vascular smooth muscle cell dedifferentiation by suppressing insulin receptor substrate-1–mediated p53/KLF4 complex stabilization. *Journal of Biological Chemistry,* 294**,** 2407-2421.

Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H. & Nishiuchi, Y. 1995. Transfer of two Burkholderia and an Alcaligenes species to Ralstonia gen. Nov. *Microbiology and Immunology,* 39**,** 897-904.

Yada, T., Shimokawa, H. & Tachibana, H. 2018. Endothelium□dependent hyperpolarization□mediated vasodilatation compensates nitric oxide□mediated endothelial dysfunction during ischemia in diabetes□induced canine coronary collateral microcirculation in vivo. *Microcirculation,* 25**,** e12456.

Yamagata, K., Oda, N., Kaisaki, P. J., Menzel, S., Furuta, H., Vaxillaire, M., Southam, L., Cox, R. D., Lathrop, G. M. & Boriraj, V. V. 1996. Mutations in the hepatocyte nuclear factor-1α gene in maturity-onset diabetes of the young (MODY3). *Nature,* 384**,** 455.

Yan, W., Liu, H., Deng, X., Jin, Y., Wang, N. & Chu, J. 2017. Acellular dermal matrix scaffolds coated with connective tissue growth factor accelerate diabetic wound healing by increasing fibronectin through PKC signalling pathway. *Journal of Tissue Engineering and Regenerative Medicine*.

Yang, P., Cao, Y. & Li, H. 2010. Hyperglycemia induces inducible nitric oxide synthase gene expression and consequent nitrosative stress via c-Jun N-terminal kinase activation. *American Journal of Obstetrics and Gynecology,* 203**,** 185. e5-185. e11.

Yang, T.-L., Lee, P.-L., Lee, D.-Y., Wang, W.-L., Wei, S.-Y., Lee, C.-I. & Chiu, J.-J. 2018. Differential regulations of fibronectin and laminin in Smad2 activation in vascular endothelial cells in response to disturbed flow. *Journal of Biomedical Science,* 25**,** 1.

Yang, Y., Kim, S. C., Yu, T., Yi, Y.-S., Rhee, M. H., Sung, G.-H., Yoo, B. C. & Cho, J. Y. 2014. Functional roles of p38 mitogen-activated protein kinase in macrophage-mediated inflammatory responses. *Mediators of Inflammation,* 2014.

Yano, Y., Gabazza, E. C., Kitagawa, N., Bruno, N. E., Matsumoto, K., Nakatani, K., Araki, R., Katsuki, A., Adachi, Y. & Sumida, Y. 2004. Tumor necrosis factor-α is associated with increased protein C activation in nonobese type 2 diabetic patients. *Diabetes Care,* 27**,** 844-845.

Yuksel, N., Takmaz, T., Ozel Turkcu, U., Ergin, M., Altinkaynak, H. & Bilgihan, A. 2017. Serum and Aqueous Humor Levels of Fetuin-A in Pseudoexfoliation Syndrome. *Current Eye Research***,** 1-4.

Zeebaree, B. K., Kwong, W. Y., Mann, G. E., Gutierrez, C. G. & Sinclair, K. D. 2018. Physiological responses of cultured bovine granulosa cells to elevated temperatures under low and high oxygen in the presence of different concentrations of melatonin. *Theriogenology,* 105**,** 107-114.

Zelihic, E., Poneleit, B., Siegmund, T., Haller, B., Sayk, F. & Dodt, C. 2015. Hyperglycemia in emergency patients–prevalence and consequences: results of the GLUCEMERGE analysis. *European Journal of Emergency Medicine,* 22**,** 181-187.

Zhan, M., Usman, I. M., Sun, L. & Kanwar, Y. S. 2015. Disruption of renal tubular mitochondrial quality control by Myo-inositol oxygenase in diabetic kidney disease. *Journal of the American Society of Nephrology,* 26**,** 1304-1321.

Zhang, W.-J., Wang, X.-H., Gao, S.-T., Chen, C., Xu, X.-Y., Zhou, Z.-H., Wu, G.-Z., Yu, Q., Xu, G. & Yao, Y.-Z. 2018a. Tumor-associated macrophages correlate with phenomenon of epithelial-mesenchymal transition and contribute to poor prognosis in triple-negative breast cancer patients. *Journal of Surgical Research,* 222**,** 93-101.

Zhang, Y., Liu, X., Wang, M., Song, Y., Zhang, L., You, Y., Su, Y., Liu, Y. & Kou, C. 2018b. Risk of Hyperglycemia and Diabetes after Early-Life Famine Exposure: A Cross-Sectional Survey in Northeastern China. *International Journal of Environmental Research and Public Health,* 15**,** 1125.

Zheng, Y., Ley, S. H. & Hu, F. B. 2018. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology,* 14**,** 88.

Zollinger, A. J. & Smith, M. L. 2017. Fibronectin, the extracellular glue. *Matrix Biology,* 60**,** 27-37.

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