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Changes in circulating levels of endothelial progenitor cells in young women with type 1 diabetes during the menstrual cycle: an observational study

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DOTTORATO DI RICERCA IN MEDICINA TRASLAZIONALE

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SUMMARY

CHAPTER 1

TYPE 1 DIABETES MELLITUS

1.1 Introduction

Diabetes is a serious, long-term condition that occurs when the body cannot produce any or enough insulin or cannot effectively use the insulin it produces. Insulin, the essential hormone produced in the pancreas, allows the entrance of glucose from the bloodstream into the body's cells, thus producing energy. Increased levels of blood glucose due to lack of insulin can cause long term damage to many of the body's organs. This damage will eventually, if untreated, lead to disabling and life-threatening health complications such as cardiovascular diseases, nerve damage, kidney damage and eye disease. in the contrary, with appropriate treatment, these serious complications can be delayed or prevented altogether[1]

Type 1 diabetes mellitus (T1DM) is caused by an autoimmune process in which the body's immune system attacks the insulin producing beta-cells of the pancreas. As a result, the body produces very little or no insulin. The causes of this destructive process are not fully understood but a likely explanation is that the combination of genetic susceptibility (conferred by a large number of genes) and an environmental trigger such as a viral infection, initiate the autoimmune reaction. The condition can develop at any age, although T1DM occurs most frequently in children and young adults. T1DM is one of the most common chronic diseases in childhood. Type 2 diabetes is also seen in older children and is increasing in some countries as childhood overweight and obesity become more common[2].

1.2 Epidemiology

Findings of the current 10th edition confirm that diabetes is one of the fastest growing global health emergencies of the 21st century (Figure 1.1). In 2021, it is estimated that 537 million people have diabetes, and this number is projected to reach 643 million by 2030, and 783 million by 2045. In addition, 541 million people are estimated to have impaired glucose tolerance in 2021. It is also estimated that over 6.7 million people aged 20–79 will die from diabetes-related causes in 2021. The number of children and adolescents (i.e., up to 19 years old) living with diabetes increases annually. In 2021, over 1.2 million children and adolescents have type 1 diabetes. Direct health expenditures due to diabetes are already close to one trillion USD and will exceed this figure by 2030 [2].

Wide variations exist between the incidence rates of different populations, incidence is lowest in China and Venezuela (0.1 per 100 000 per year) and highest in Finland and Sardinia (37 per 100 000 per year). In most populations girls and boys are equally affected. In general, the incidence increases with age; the incidence peak is at puberty. After the pubertal years, the incidence rate significantly drops in young women, but remains relatively high in young adult males up to the age 29–35 years.

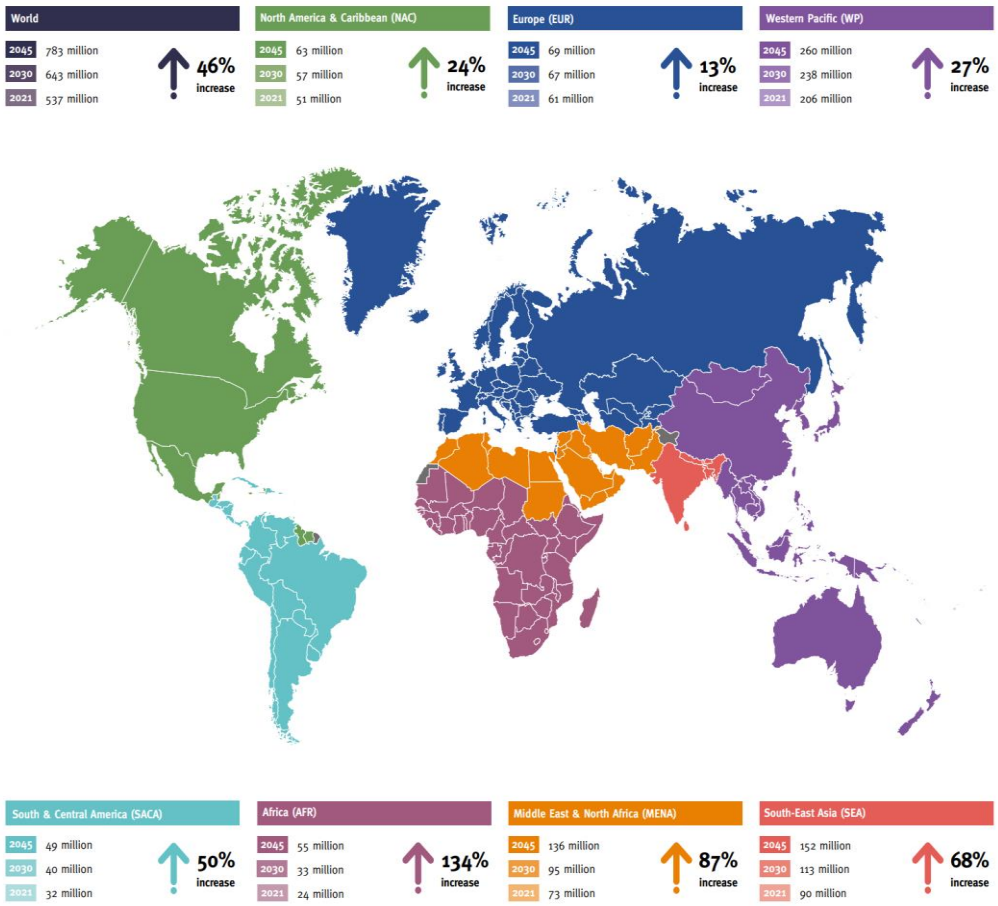


Figure 1.1- *Number of people with diabetes worldwide and per IDF Region in 2021–2045 (20–79 years).*

Diabetes estimates for 2021 show increasing prevalence of diabetes by age. Similar trends are predicted for 2045. Prevalence is lowest among adults aged 20–24 years (2.2% in 2021) (Figure 1.2). Among adults aged 75–79 years diabetes prevalence is estimated to be 24.0% in 2021 and predicted to rise to 24.7% in 2045. The aging of the world's population will produce an increasing proportion of those with diabetes being over the age of 60 years.

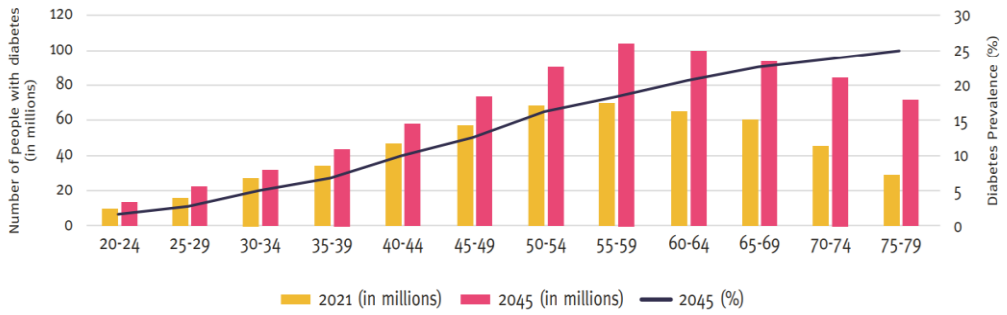


Figure 1.2*- Number of people with diabetes in adults (20–79 years) by age group in 2021 (columns) and estimated prevalencei across age groups in 2045 (black line).*

The estimated prevalence of diabetes in women aged 20–79 years is slightly lower than in men (10.2% vs 10.8%). In 2021, there are 17.7 million more men than women living with diabetes (Figure 1.3).

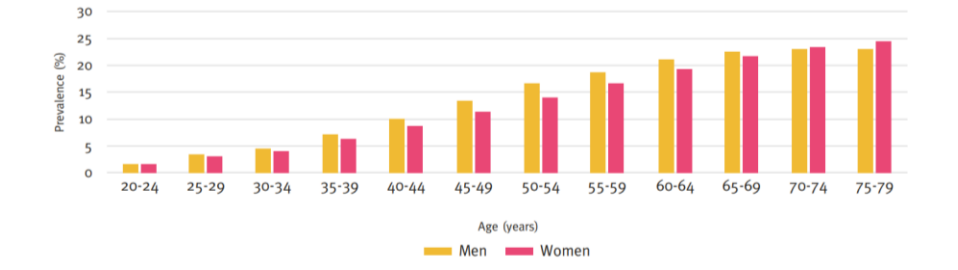


Figure 1.3*- Prevalence of diabetes among men and women (20–79 years), 2021.*

In 2021, more people with diabetes live in urban (360.0 million) than in rural (176.6 million) areas – the prevalence in urban areas being 12.1% and in rural areas 8.3%. The number of people with diabetes living in urban areas is expected to increase to 596.5 million in 2045, as a result of global urbanization. By 2045, the predicted prevalence of diabetes in urban areas is estimated to increase to 13.9%, due to population ageing (Figure 1.4)[2].

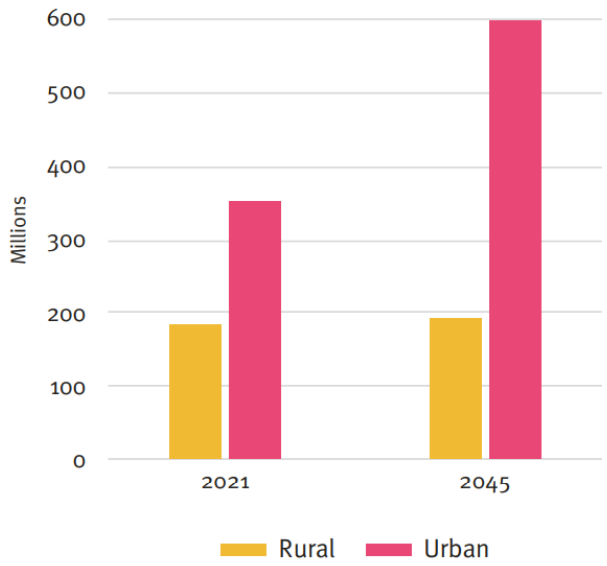


Figure 1.4*-Number of people with diabetes in adults (20–79 years) living in urban and rural areas in 2021 and 2045.*

The incidence of T1DM varies around the world, with some regions having much higher incidences than others. The countries with the largest numbers of adults with diabetes aged 20–79 years in 2021 are China, India and Pakistan. They are anticipated to remain so in 2045 (Table 1.1). The countries that have the highest number of people with diabetes do not necessarily have the highest prevalence. The highest comparative diabetes prevalence rates in 2021 are reported in Pakistan (30.8%), French Polynesia (25.2%) and Kuwait (24.9%). These countries are also expected to have the highest overall comparative diabetes prevalence in 2045, with figures in Pakistan reaching 33.6%, Kuwait 29.8% and French Polynesia 28.2%.

***Table 1.1**- Top 10 countries or territories for number of adults (20–79 years) with diabetes in 2021 and 2045.*

2021			2045		
Rank	**Country or territory**	**Number of people with diabetes (millions)**	**Rank**	**Country or territory**	**Number of people with diabetes (millions)**
1	China	140.9	1	China	174.4
2	India	74.2	2	India	124.9
3	Pakistan	33.0	3	Pakistan	62.2
4	United States of America	32.2	4	United States of America	36.3
5	Indonesia	19.5	5	Indonesia	28.6
6	Brazil	15.7	6	Brazil	23.2
7	Mexico	14.1	7	Bangladesh	22.3
8	Bangladesh	13.1	8	Mexico	21.2
9	Japan	11.0	9	Egypt	20.0
10	Egypt	10.9	10	Turkey	13.4

In total, 1,211,900 children and adolescents younger than 20 years are estimated to have type 1 diabetes globally. It is estimated that around 108,200 children and adolescents under 15 years are diagnosed each year. This number rises to 149,500 when the age range extends to those under 20 years (Table 1.2)[3].

***Table 1.2**-Global estimates for type 1 diabetes in children and adolescents (0–14 years and 0–19 years) in 2021.*

Global population (0–14 years)	1.99 billion
Global population (0–19 years)	2.61 billion
Type 1 diabetes in children and adolescents (0–14 years)	
Number of prevalent (existing) cases of type 1 diabetes	651,700
Number of incident (new) cases of type 1 diabetes per year	108,300
Type 1 diabetes in children and adolescents (0–19 years)	
Number of prevalent (existing) cases of type 1 diabetes	1,211,900
Number of incident (new) cases of type 1 diabetes per year	149,500

1.3 Aetiology and Risk Factors for T1DM

T1DM can be defined as a polygenic multifactorial disease due to the interaction between multiple predisposing genetic factors in the presence of permissive environmental factors.

1.3.1 Genetics factors

T1DM is a polygenic disease linked with over 60 loci across the genome including strong association in the HLA class II region[4]. Genetic risk factors that have been identified to be associated with T1DM include HLA-DR3-DQ2 and HLA-DR4-DQ8 haplotypes, non-HLA single nucleotide polymorphism (SNP) and a family history of T1DM [5]. HLA class II molecules perform antigen presentation and the haplotypes HLA-DR3-DQ2 and HLA-DR4-DQ8 are linked to approximately 50% of T1DM[6]. HLA-DR4-DQ8 confers the highest risk for T1DM development with an 11.37 odds ratio[7]. Indeed, 90% of children diagnosed with T1DM in Scandinavia have either HLA-DR3-DQ2 or HLA-DR4-DQ8 haplotypes or both of these haplotypes [4]. Some other HLA class II haplotype are associated with protection from T1DM e.g., HLA-DR2-DQ6[7]. Non-HLA single nucleotide polymorphism (SNP) that have been associated with autoantibody production and T1DM, include PTPN22 (an Immune regulator with gene located at 1p13), insulin gene and Cytotoxic T-lymphocytes-associated protein 4 (CTLA4) a T-cell inhibitory receptor[8.] Probably these genetic factors also predispose these patients to other autoimmune disorders, as these patients are also prone to diseases such as Grave's disease, Addison's disease, autoimmune hepatitis, myasthenia gravis and

pernicious and celiac disease. The summation of these genetic risk factors into a single continuous variable or polygenic risk score is likely to be useful for T1DM prediction, discrimination from other diabetes and classification of T1DM [4]. Also these HLA and non-HLA genetic factors represent potential targets for the prevention and therapy of T1DM.

1.3.2 Environmental factors

Infections

Early ecological reports, seroepidemiological studies, and case reports have drawn attention to viral infections as a potential cause of T1DM. Several viruses have been implicated, with enteroviruses having the strongest evidence from studies in animal models and in human beings. These viruses have a tropism to human pancreatic islets in vivo and in vitro, and have been detected in the pancreas of patients recently diagnosed with T1DM. Recent findings consistent with a persistent enteroviral infection in patients at diagnosis of T1DM include: more frequent detection of enteroviral VP1 protein immunoreactivity in the β cells of children with T1DM than in age-matched controls[9].

Intestinal microbiota

Some of the candidate environmental factors for T1DM (eg, caesarean delivery, early childhood diet, and use of antibiotics) are intertwined with the development and function of the human microbiome. Gut microbes influence lipid and glucose

metabolism, as well as immunity and systemic inflammation outside of the intestine. Commensal microbiota might modulate the risk of T1DM, but studies so far have been underpowered and focused on taxa diversity. Some have reported lower microbial diversity in children with islet autoimmunity before progression to diabetes, compared with healthy controls[9].

Breastfeeding

Many case-control studies suggested that short breastfeeding and/ or early cow's milk introduction increases T1DM risk [10-11] including data from a large study of randomly selected children from Southeast Sweden [12-13]. The mechanism of cow's milk effect was postulated as related to development of human antibodies to bovine insulin[14]. One study found that increased amounts of cow's milk ingestion, but not age at introduction, was associated with T1DM risk[15], while another found increased cow's milk intake to be protective against T1DM[16].

Vitamin D

Other nutritional factors may modulate the T1DM process as well. Much attention has been paid to the role of vitamin D in protection against T1DM. Vitamin D may act by modulating immune cell function[17-18]. Case-control studies from both Norway (545 cases, 1668 controls) and the EURODIAB-2 study (820 cases, 2335 controls) suggested that early vitamin D supplementation decreased T1DM risk[19]. The first prospective study of Vitamin D protection from T1DM

included 12,055 pregnant women living in the far north of Finland[20]. Vitamin D supplementation was determined from medical records, usually at multiple time points. Most children were given regular or occasional Vitamin D supplements as recommended to prevent rickets. The relative risk of developing T1D was 0.12 and 0.16 in those given regular and occasional Vitamin D supplements, respectively, as compared to children not given supplements. The relative risk of developing T1DM was 0.12 and 0.16 in those given regular and occasional Vitamin D supplements, respectively, as compared to children not given supplements. The protective effect was also dose-related (>2,000 IU/day, RR 0.14; exactly 2,000 IU/day, RR 0.22; both compared to supplementation with <2,000 IU/day). These highly significant and very convincing results suggest that infant supplementation with Vitamin D, at least in northern locations and in high doses, protects against T1DM[20].

1.4 Pathogenesis of T1DM

The underlying pathogenesis of T1DM is the targeted autoimmune destruction of insulin secreting pancreatic beta cell by islet-specific $CD4^+$, $CD8^+$ and B cell[21](Figure 1.5). Histopathological examination of T1DM pancreas shows loss of insulin producing beta cells and immune infiltration of the islet, known as insulitis[22]. This infiltration is mostly consisting of $CD8^+$ T lymphocytes, however other types of leucocytes may also be present including $CD4^+$ lymphocytes, B-cells and macrophages[22]. It is thought that the destruction of beta cells is initiated when APCs present an unknown antigen that might be beta cell peptide (auto-antigen) or viral antigen. The APCs with these antigens migrate to the pancreatic lymph nodes

where they interact with $CD4^+$ T lymphocytes. This interaction causes activation of $CD4^+$ T lymphocytes which then activate $CD8^+$ T lymphocytes. Activated $CD8^+$ T lymphocytes then move into the islet and cause lysis of beta cells expressing these antigens on MHC class I molecules[6]. Proinflammatory cytokines, defects in regulatory T lymphocyte function, innate cells like macrophages, natural killer cells and neutophils and reactive oxygen species they produce, contribute to the destruction of beta cells[6]. In addition, B cells are activated to produce auto-antibodies against insulin and beta cell proteins. The measurement of these auto-antibodies in peripheral blood serves as biomarkers of asymptomatic T1DM.

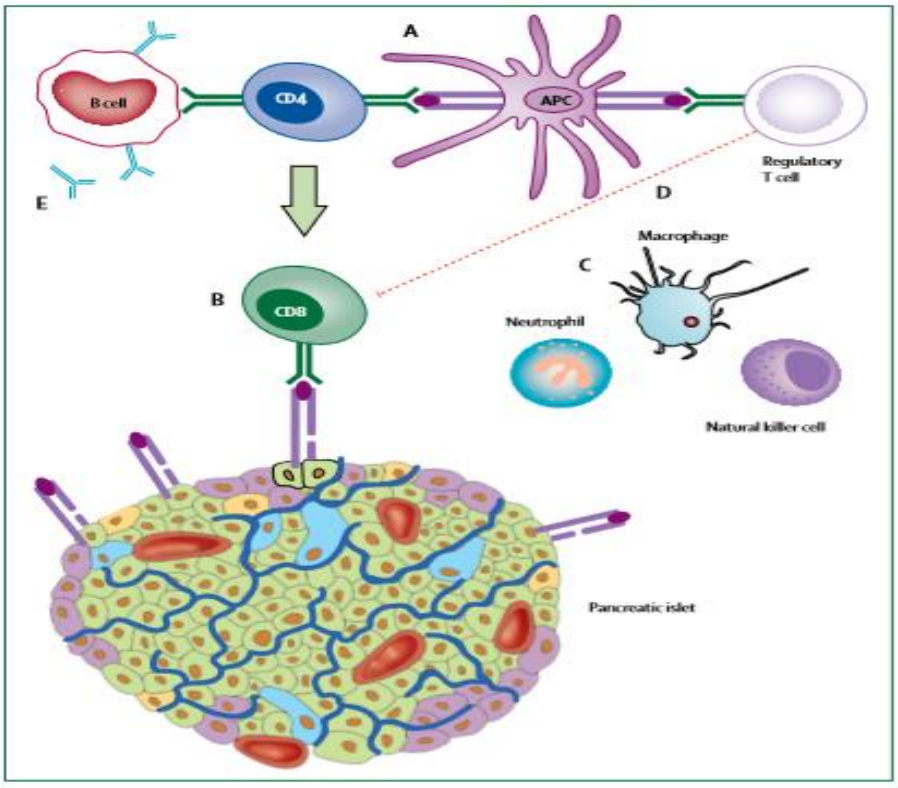


Figure 1.5- *The immunopathogenesis of type 1 diabetes.*

These T1DM associated auto-antibodies include Islet-cell cytoplasmic autoantibodies (ICA), insulin auto-antibodies (IAA), glutamic acid decarboxylase

(GAD), insulinoma-associated autoantigen 2 (IA2) and Zinc transporter 8 (ZnT8). Greater than 90 percent of patients with recent T1DM diagnosis have one or more of these auto-antibodies; actually, these auto-antibodies appear early in life with a peak incidence before 2 years of age in genetically susceptible individuals [23]. Thus, these auto-antibodies are present long before symptomatic onset of T1DM however, not all individuals with these auto-antibodies progress to symptomatic T1DM. Having just one of the four antibodies is associated with a small increase in risk however the risk for T1DM is markedly increased when there are two or more islet auto-antibodies, with an increase in risk with an increase in the number of antibodies.

Therefore, the highest risk is when all four antibodies are present. In a study of 13,337 children, Ziegler et al found an 84% risk of progression to clinical diabetes after islet autoantibody seroconversion in children with multiple autoantibodies at 15 years follow up [24]. Due to the high risk of progression to clinical diabetes with hyperglycaemia individual with two or more auto-antibodies the presence of these auto-antibodies can be considered as pre-symptomatic T1DM.

Symptomatic onset of T1DM only occurs when a critical mass of beta cells is destroyed. At least >75% of beta cell mass is destroyed before onset of symptoms [22]. The rate of beta cell destruction differs and is usually more rapid in children and slower in adults [25].

Figure 1.6 shows the natural history of T1DM and the different stages. Symptomatic T1DM also known as stage 3 occurs after a long silent phase lasting several years considered as pre-symptomatic T1DM. Pre-symptomatic T1DM refers to stages 1 and 2. Given that, the progression from onset of beta cell

autoantibody production (stage 1 T1DM) to symptomatic onset (stage 3 T1DM) takes several months or years, it allows a window for potential immunotherapy or preventive therapy to stop beta cell destruction below critical mass [26].

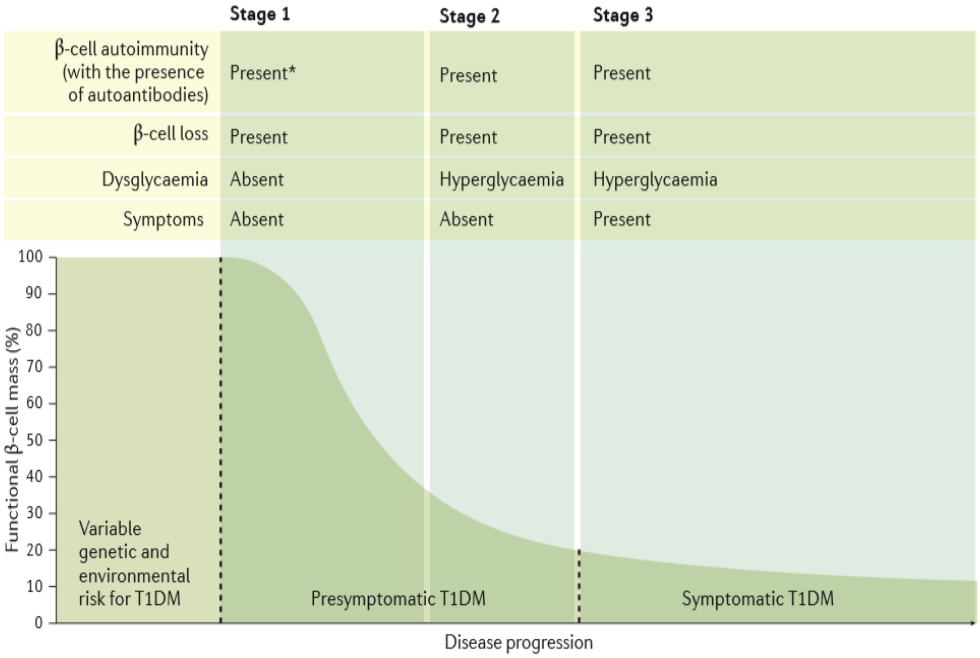


Figure 1.6 *–Variable factors contributing to T1DM, disease progression and stages of T1DM.*

1.5 Diagnosis of T1DM

T1DM is usually diagnosed in stage 3 when the patient presents with the symptoms of T1DM. The clinical presentations of T1DM included polydipsia, polyuria, fatigue, polyphagia, ketoacidosis, blurred vision and weight loss. These manifestations of T1DM are due to insulin deficiency and hyperglycaemia, after a critical mass of beta cells has been lost. At this stage, the patient requires exogenous insulin therapy. The onset of T1DM frequently occurs in the setting of an intercurrent illness, which may support the theory that it may be triggered by an

infection [25]. Children usually present with more severe symptoms than adults, and adults are sometimes misclassified as having type 2 diabetes [27]. Infact as many as 50% of adults with T1DM might be initially misdiagnosed as have type 2 diabetes (T2DM) [6]. Also, more than 50% of patients diagnosed with T1DM after age 35 were shown to have T2DM in long term follow up [27]. This is an indication that there is a challenge distinguishing between T1DM and T2DM in adults, probably because of the much higher prevalence of type 2 diabetes compared to T1DM in adults.

The American Diabetes Association [28] and the World Health Organization [29], in their diagnostic criteria do not distinguish between T1DM and T2DM i.e. the diagnostic criteria for T1DM and T2DM are the same.

The American Diabetes Association criteria for diagnosis of diabetes [28] are:

1. A1C ≥6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*

   or

2. FPG ≥126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.*

   or

3. 2-h plasma glucose ≥200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water. *

   or

4. In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose ≥200 mg/dl (11.1 mmol/l).

*In the absence of unequivocal hyperglycaemia, criteria 1–3 should be confirmed by repeat testing.

Types 1 and 2 diabetes are classified and distinguished based on accessing biomarkers such as auto-antibodies, C-peptide concentration, presence of risk factors for T1DM and clinical features of the patient, e.g. young age at diagnosis and low body mass index[6]. However, no single clinical feature can perfectly distinguish between T1DM and other types of diabetes. Distinguishing between T1DM and T2DM is important for the management of T1DM patient and avoidance of dangerous complications like diabetic ketoacidosis

Clinical features like physical appearance and patient age give a clue to the diagnosis of the types of diabetes but cannot be relied on, as can sometimes be misleading. For instance, in obese adolescents with clinical diagnosis of T2DM, 10% were pancreatic auto-antibodies positive suggesting T1DM is the likely diagnosis[31]. The increasing incidence of obesity especially in youth could complicate the diagnosis T1DM [31] hence, though T1DM patients are usually not obese, the presence of obesity should not preclude the diagnosis of T1DM.

Another feature that can be used to distinguish between type 1 and type 2 diabetes is the presence of beta cell auto-antibodies because more than 90% of patients with recently diagnosed T1DM have one or more of these auto-antibodies at disease onset [23]. It should be noted that measuring beta cell auto-antibodies can sometime give a false negative result if auto-antibody testing is done after long duration of diabetes as antibody titers diminish over time. 5 years after diagnosis,

almost 20% of individual had neither GAD nor IA2 (antibody-negative); this percentage increased to 40% after 6-13 years[32].

C-peptide level, a marker for insulin secretion can be used to distinguish T1DM (low C-peptide concentration) from other diabetes however, it is inadvisability to use C-peptide alone to differentiate between type 1 diabetes and other forms of diabete[33]. Residual insulin secretion though unexpected in T1DM is present in one out of three individuals after 3 or more year from diagnosis[33].

By taking into account biomarkers, risk factors and clinical features of the patient T1DM can be classified. An indication that a patient has been classed wrongly is when hyperglycaemia persists even after treatment with noninsulin agent used in the therapy of T2DM; in this situation T1DM should be considered. Monogenic forms of diabetes are diagnosed with genetic testing.

1.6 Therapy of T1DM

T1DM is a chronic disease that eventually leads to complete loss of insulin due to destruction of β cells. Also, the lack of appropriate islet cell repair mechanisms which ultimately affects glycaemic control. As a result, insulin replacement therapy is currently the first-line therapeutic option for treating T1DM. The use of exogenous insulin as a therapy for T1DM was first described by Banting and Best in 1921, who used crude extracts of animal pancreas to achieve glucose-lowering actions.[34] Soon after in 1922, crude insulin preparations of animal origins were commercialised for clinical use. However, there were problems associated with pharmacokinetics of insulin, prominently due to insulin absorption. In addition,

inefficient action of administered insulin was also reported, which led to either inconsistent glucose-lowering effects or prolonged periods of hypoglycaemia. These remained hindrances for achieving long-term glycaemic control and for prevention of diabetic complications.[35] Since then, many humanised insulin analogues have emerged (Table 1.3), which not only mimic the biologic actions of endogenous insulin but also have enhanced the pharmacokinetic profile.[36] Nevertheless, clinical insights in the past decades have highlighted limitations of insulin replacement therapy, especially the failure of insulin preparations to fully replicate biological actions of endogenous insulin.[37] Together with increasing number of T1DM cases, there is a need to identify novel therapeutic approaches to restore normoglycaemia. The current treatment regimen for T1DM focusses on combining intensive diet treatments coupled with lifelong exogenous insulin administration, either using multiple daily injections or by insulin pumps.[38] In addition, there have been advances in the development of genetically modified insulin analogues (Aspart, Lispro, and Delgludec insulins), which are fast acting and long acting. These provide a more physiological glycometabolic control compared with traditional insulins.[39] The current insulin centric therapeutic approach renders a T1DM patient susceptible to severe episodes of hypoglycaemia, lifelong dependency on exogenous insulin, insulin resistance, mild obesity, and psychiatric conditions.[38,40,41] Such observations highlight the importance of developing alternative strategies to restore glycaemic control and complete insulin independence.

***Table 1.3**- List of different categories of insulin available in the National Health Service formulary.*

TYPE	BRAND NAMES	TIME ACTION PROFILE	DOSE
Rapid acting	Insulin aspart (Novorapid, Fiasp), insulin lispro (Humalog), insulin glulisine (Apidra)	Usually 4-20 min after s.c. injection with peak at 20-30 min	Three times a day up to 15 min before food intake
Short acting	Actrapid (Novo Nordisk), Humulin S (Lilly), Insuman Rapid (Aventis)	Begins from 30 min after s.c. injection with peak action reaching 2-4 h	Three times a day, 30 min before food intake
Long acting	Levemir (Novo Nordisk), ABASAGLAR (Lilly), Lantus (Aventis), Toujeo (Aventis), Tresiba (Novo Nordisk)	Beyond 24 h and up to 36 h	Once daily s.c., usually at the same time everyday with minimum 8 h interval between consecutive doses
Intermediate acting	Insulatard (Novo Nordisk), Insuman Basal (Aventis)	Peak onset from 4-6 h, with duration of action until 14-16 h	Once or twice daily s.c.

1.6.1 Future perspectives on insulin replacement therapy

The widespread use of self-monitoring devices for measuring blood glucose and non-enzymatically glycosylated haemoglobin A1C (HbA1c) has enhanced the therapeutic applicability of commercial insulin preparations.[42] This has resulted in the generation of a range of insulin analogues (Table 1.3). These modified insulins are rapid acting (biological activity begins from 4min and lasts for 30min), short acting (regular insulin – biological activity beginning from 30min and lasting for 4h), intermediate acting (Insulatard, Insuman – peak onset from 4 to 6h), long acting (Glargine and Detemir – biological activity from 24 to 36h), and ultra-long acting (Degludec – onset from 30 to 90min and lasts until 42h). However, even such preparations are dependent on delivery systems, including syringes, glucose sensor-augmented insulin infusion pumps, supersonic injectors, and pens.[43]

The use of these traditional delivery systems involves an invasive procedure and treatment fails to provide long-term insulin independence.[37] Consequently, research is being carried out to identify alternative means of insulin replacement therapy. A novel approach for oral insulin delivery utilises an ingestible self-orienting

millimetre-scale applicator (SOMA).[44] The device autonomously positions itself to engage with gastro-intestinal tissue and deploys milliposts directly through the gastric mucosa while avoiding perforation.[44]

CHAPTER 2

COMPLICATIONS OF TYPE 1 DIABETES

2.1 Acute Complications of T1DM

T1DM has been linked to several complications that can be microvascular or macrovascular, or acute complications such as diabetic ketoacidosis and hypoglycaemia. Hypoglycaemia and diabetic ketoacidosis (DKA) are serious acute life-threatening complication of T1DM which are preventable. Acute hypoglycaemia can lead to confusion, seizures, loss of consciousness and even death. An estimated 10% of deaths in patients with T1DM are caused by hypoglycaemia. Recurrent hypoglycaemia leads to decreased hypoglycaemia awareness known as hypoglycaemia unawareness, and can result in subsequent severe hypoglycaemic event because each hypoglycaemic event reduces the glucose concentration that triggers the counter-regulatory responses to return to euglycaemia [45].

Severe hypoglycaemia occurs when low blood glucose level requires the assistance of others for treatment or when patients with hypoglycaemia of <70mg/dl but ≥40mg/dl have a coma or seizure [6,46]. Severe hypoglycaemia a complication of insulin therapy, most commonly occurs in children aged less than six years of age[47]. This is worrying because young children who have developing brains are more prone to repeated severe hypoglycaemic events which has been linked to adverse

effects on various cognitive domains like long term memory, attention and verbal IQ[46,48]. Hypoglycaemia can significantly impact the quality of life T1DM patient. Some of these patients constantly live with the fear of hypoglycaemia, nocturnal hypoglycaemia can impact sleep and one's sense of well-being the next day, can reduces productivity, and some of these patients have been found to struggle with chronic mood disorders like depression and anxiety [45].

DKA accounts for 13-19% of mortality in T1DM [6] and it is characterised by the presence of elevated ketones in the blood known as ketosis, hyperglycaemia and metabolic acidosis. DKA most commonly occurs in adolescents and is associated with higher haemoglobin A1c (HbA1c) [47]. Karges B et la found the highest rate of DKA was observed in patients with HbA1c of ≥9.0% [49]. The incidence of DKA is higher in females than in males and higher in migrants than non-migrants[49.] Probably due to poorer health services available to migrants and their social economic status.

2.2 Macrovascular Complications of T1DM

Microvascular and macrovascular complications are long term complications that affect the quality of life and life expectancy of T1DM patients. Macrovascular complications are cardiovascular, cerebrovascular and peripheral vascular diseases.

Hyperglycaemia is the primary risk factor for microvascular disease which can be reduced by reducing HbA1c through intensive blood glucose control. However, for macrovascular complications of T1DM, the risk of cardiovascular disease does not seem to be considerably reduced by intensive blood glucose control [6].

Type 1 diabetes patients are at higher risk of cardiovascular disease than the general population. Despite recent advance in the management of these patients, cardiovascular disease still remain the major cause of morbidity and pre mature mortality in T1DM. The extent of coronary artery disease was correlated with HbA1c over 18 years of follow-up in the Oslo Study and a 1% increase in mean HbA1c was associated with a 6.4% increase in coronary vessel stenosis [50].

An estimate of life expectancy in a Scottish cohort of national population with T1DM between the years 2008 to 2010 showed that at the age of 20 years the average man with T1DM had an estimated life expectancy loss of about 11 years and women had an estimated loss of about 13 years [51]. In the general population without T1DM in Scotland, 76% of men and 83% of women survived to age 70 years compared with 47% of men and 55% of women with T1DM [51]. Intensive glycaemic control is now the standard of care for T1DM patients compared to conventional diabetes therapy for the prevention of cardiovascular disease, due to the findings of the Diabetes Control and Complication Trial (DCCT) and Epidemiology of Diabetes Interventions and Complication (EDIC) follow up study which compared intensive glycaemic control with conventional management, and demonstrated intensive glycaemic therapy reduces the incidence of cardiovascular disease [52,53]. Although the incidence of cerebrovascular disease (stroke) is less than that of cardiovascular disease it is still a disturbing cause of morbidity and mortality in T1DM. Compared to the general populations the incidence of cerebrovascular disease in T1DM patient is much higher. The European Diabetes (EURODIAB) Study reported an incidence of 0.74% for cerebrovascular disease per year

compared to the incidence reported in the general population, which are reported at approximately 0.2% to 0.3% per year [25].

Peripheral artery disease can result in peripheral arterial calcification and non-traumatic amputation [54]. In a meta-analysis by Adler et la, each one percentage increase in HbA1c was associated with between 26-36% increase in the risk of lower extremity amputation. The DCCT/EDIC study reported that intensive treatment is associated with reduced incidence rate of peripheral arterial calcification [55].

2.3 Microvascular Complications of Type 1 Diabetes

Microvascular complications of T1DM include nephropathy, retinopathy and neuropathy. Clinically evident diabetes-related vascular complications are rare in childhood and adolescence. However, early functional and structural abnormalities may be present a few years after the onset of the disease and often progress during puberty[56]

Diabetic nephropathy is a major cause of morbidity and mortality amongst young adults with type 1 diabetes. The changes occurring in the kidney in patients with type 1 diabetes are generally classified into five stages, reflecting specific and progressive alterations in renal morphology and function. The earliest stage is characterized by glomerular hypertrophy, hyperfiltration, and hyperperfusion. This is followed by a stage of subclinical morphological changes and increases in albumin excretion rates (AER) within the normal range[57].Further increases in albumin excretion, with an AER between 30 and 300 mg/24 h or 20 and 200 μg/min in a 24-hour or timed urine collection, indicate the development of albuminuria

(formerly “microalbuminuria”) (stage 3), which may further progress to overt proteinuria (formerly termed “macroalbuminuria)” (AER >200 μg/min or >300 mg/24 h) (stage 4) and, without any treatment, to end-stage renal disease (ESRD) (stage 5) [58] .

Diabetic retinopathy (DR) is one of the major complications of diabetes. Diabetes has been one of the most common causes for death in adults aged 20–74 years old[59]. DR is resulted from long-term accumulated damages by hyperglycaemia or other factors (such as hypertension) to the microvessels in the retina[60]. It is a major cause of blindness and other relevant retinal diseases (such as diabetic macular edema and DME) and has received particular attention[61].

The underlying molecular mechanisms associated with vascular dysfunction, especially endothelial dysfunction, in DR are multifactorial. Extensive studies have been performed to identify factors that are associated with endothelial dysfunction in DR, such as advanced glycosylation end products (AGEs) and receptors (RAGE), disruption of peroxisome proliferator-activated receptor-γ (PPARγ), chronic inflammation, leukotasis[62,63], oxidative stress, and dysregulated growth factors, cytokines, and microRNA (miRNA) networks[64].

Diabetic neuropathy is the most common type of neuropathies. It affects patients with both type 1 and type 2 diabetes, but it progresses more rapidly and its manifestations are more severe in type 1 diabetes. Although there has been a significant progress in the understanding of the clinical aspects of these conditions, many questions remain unanswered. Peripheral and autonomic neuropathy are strong risk markers for future mortality. Diabetic autonomic neuropathy is a serious and common complication of diabetes. DAN frequently coexists with other

peripheral neuropathies and other diabetic complications, but DAN may be isolated, frequently preceding the detection of other complications. The presence of the autonomic diabetic neuropathy significantly influences the regulatory function of microcirculation, which may predispose to the occurrence of different late diabetic complications[65].

Since childhood and adolescence is a period during which intensive education and treatment may prevent or delay the onset and progression of complications, efforts should be steered to screening for early signs of diabetic complications and modifiable risk factors.[66]

CHAPTER 3

FEMALE REPRODUCTIVE SYSTEM

3.1 Reproductive system and menstrual cycle

The reproductive system in females is responsible for producing gametes, certain sex hormones, and maintaining fertilized eggs as they develop into a mature fetus and become ready for delivery. A female's reproductive years are between menarche (the first menstrual cycle) and menopause (cessation of menses for 12 consecutive months)[67].

The reproductive 'axis'depends on the dynamic interplay between neural and hormonal signals originating from three primary sources: the anterior hypothalamus, where GnRH is synthesised and secreted in pulses; the anterior pituitary, where GnRH pulses stimulate pituitary gonadotrophin (luteinising hormone (LH) and follicle-stimulating hormone (FSH)) secretion; and the gonads, which respond to the trophic actions of gonadotrophins by secreting sex steroids and producing gametes[68]. Menstrual cycles are measurable vital signs of women's reproductive health[69] and are repeated up to about 500 times within a healthy woman's 35- to 40-year reproductive lifespan, from menarche to menopause. Menstrual cycles are indicative of not only women's reproductive health but also functioning of other body organs and systems. For example, bleeding problems and menstrual irregularities are common in hypothyroid women[70]. Women's reproductive dysfunction has been linked to long-term risk of developing chronic

diseases including osteoporosis, some types of cancer, and cardiovascular diseases[71,72].

The reproductive cycle is divided into a follicular phase, an ovulatory phase and a luteal phase (Figure3.1). The first day of menses is the beginning of the follicular phase, during the first half of which concentrations of estrogens and progesterone are stable and low[73]. Approximately 7–8 days before the preovulatory luteinizing hormone (LH) surge, the second half of the follicular phase begins, which is characterized by an increase in estradiol concentrations[73]. Progesterone concentrations do not increase during this period. During the ovulatory phase, there is a rapid increase in plasma LH levels eventually leading to the rupture of the mature follicle, which marks the beginning of the luteal phase[74]. Representing the hallmark of the luteal phase is an increase in progesterone levels, which reaches maximum concentrations approximately 8 days after the LH peak[74]. At this time, estradiol and progesterone concentrations increase in parallel[73].

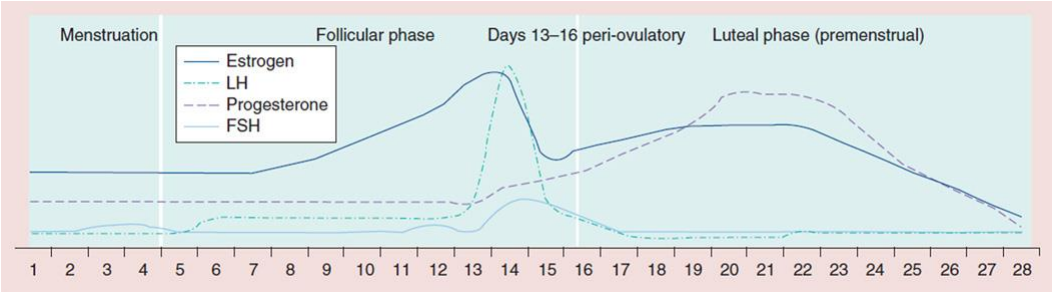


Figure 3.1*- Changes in reproductive hormone concentrations across the menstrual cycle.*

Luteinising hormone (LH) and follicle stimulating hormone (FSH) are important pituitary hormones, required for reproductive processes in both males and females.

The reference range for FSH and LH in adult females is

Phase	FSH (IU/L)	LH (IU/L)
Early follicular	3 – 10	2 - 8
Mid-cycle peak	4 – 25	10 - 75
Post-menopausal	>20	>15
Pregnancy	<1	2 – 9

Oestradiol is the principal oestrogen in females who are ovulating and the dominant ovarian hormone during the follicular (first) phase of the menstrual cycle. The concentration of oestradiol varies throughout the menstrual cycle and. The adult female reference range for oestradiol is:

Phase	Oestradiol (pmol/L)
Early follicular	< 300
Ovulatory surge	< 500 – 3000
Luteal surge	150 – 1400
Post-menopausal	< 200

Progesterone is the dominant ovarian hormone secreted during the luteal (second) phase of the menstrual cycle.

The reference range for progesterone in adult females is:

0–6 nmol/L	ovulation unlikely
7–25 nmol/L	ovulation possible
>25 nmol/L	ovulation likely

3.2 Reproductive system: role of insulin

Reproductive dysfunction is a common but little studied complication of diabetes. Depending on the age at diagnosis of diabetes, reproductive problems can manifest early on in puberty, emerge later when fertility is desired, or occur during the climacteric period.

Insulin is the other major hormone involved in the crosslink between reproduction and metabolism[75]. The insulin effect on GnRH neurons seems to be indirect, via kisspeptins, AgRP, and proopiomelanocortin (POMC) neurons[76]. Insulin possibly also modulates the GnRH receptors in the pituitary and increases LH secretion[77]. Moreover, insulin (and glucose) exerts modulating actions in the ovaries (demonstrated in vitro) by enhancing both FSH and LH-stimulated steroidogenesis in the granulosa and theca cells, insulin receptors having been identified in the granulosa and theca cells/oocytes. Interestingly, the in vivo data indicate that insulin may also have an inhibiting effect (dependent on glucose uptake by the ovaries) on ovarian steroidogenesis (E2)[78].

Maintenance of a functional reproductive system is reflected in the presence of regular ovulatory cycles. Menstrual irregularities, however, are common in women

with type 1 diabetes mellitus. Approximately, one-third of these women have irregular cycles during their reproductive years[79]. A higher prevalence of oligomenorrhea is seen in women with poor metabolic control as indicated by HbA1c[80]. There are many theories as to the cause of ovulatory dysfunction in women with type 1 diabetes mellitus. The data (discussed previously) suggest that although hyperglycaemia likely contributes to these manifestations, other factors may be involved in the pathophysiology. States of chronic disease and physiologic stress, such as malnutrition, are associated with delayed puberty and menarche. It is possible that the metabolic stress of the onset of type 1 diabetes mellitus affects age of menarche in the girls[79]. Increased catecholamine and dopamine release during periods of hyperglycemia may also suppress LH levels. Additionally, in mice with streptozotocin-induced diabetes, there is a hypogonadotrophic supresssion of LH and FSH. The mechanism for this effect is believed to involve abnormal gonadotropin-releasing hormone (GnRH) secretion[81].

Before the introduction of insulin therapy in 1923, primary and secondary amenorrhoea, infertility, and the absence of pubertal development were common in women with type 1 diabetes[82].

As early as 1925, Joslin described the important impact of insulin therapy on reproductive function in women with diabetes[83]. In women with type 1 diabetes who had pubertal delay and primary and secondary amenorrhoea, insulin therapy led to improvements in pubertal development and menstrual regularity. In the 1980s, hypogonadotropic hypogonadism was described in patients with type 1 diabetes and poor metabolic control, with decreased concentrations of luteinising hormone (LH), folliclestimulating hormone (FSH), and oestradiol[84].

CHAPTER 4

SEXUAL DYSFUNCTIONS

4.1 Diabetes and sexual dysfunctions

Diabetes is an increasingly prevalent problem that has been associated very strongly with sexual problems in both men and women. The presence of sexual dysfunction in type 1 diabetes has been associated with markedly lower quality of life and psychological distress[85].

4.1.1 Epidemiology

Diabetes is an established risk factor for sexual dysfunction in men; a threefold increased risk of erectile dysfunction (ED) was documented in diabetic compared with nondiabetic men. Among women, the evidence regarding the association between diabetes and sexual dysfunction is less conclusive although most studies have reported a higher prevalence of female sexual dysfunction (FSD) in diabetic women as compared with nondiabetic women[86,87].

Several studies have reported that FSD is more frequent in diabetic women than in control women. A meta-analysis that included 26 studies, 3,168 diabetic women, and 2,823 controls showed that FSD is more frequent, and is associated with a lower Female Sexual Function Index (FSFI) score in diabetic women than in controls. In

particular, the risk for FSD was 2.27 (95% confidence interval [CI]: 1.23–4.16) and 2.49 (95% CI: 1.55–3.99) in type 1 and type 2 diabetic women, respectively.

Furthermore, the risk for FSD was 2.02 (CI: 1.49–2.72) when considering "any diabetes" (which repre- sented the two forms of diabetes together)[88].

Another Italian study has shown a significantly higher prevalence of FSD in women with DM1 than in the control group (36.4% vs. 5.2%, respectively)[89]. According to a recent meta-analysis, involving 25 studies and 3892 women with an age range of 18–72 years, the overall prevalence of FSD in women DM2 was 68.6% (95% CI 61.6–75.3%)[90]. Results of meta-regression analysis have also shown a statistically significant increase in FSD prevalence in diabetic women over the years[90]. All the phases of sexual cycle response, including desire, arousal, lubrication, orgasm, and satisfaction, are impaired in diabetic women. Meeking and colleagues described a reduction in sexual desire (64%), loss of vaginal lubrication (70%), difficulty of achieving orgasm (50%), decrease satisfaction (47%), loss of genital sensation (36%), and dyspareunia (43%) in 270 women with diabetes aged 21–65[91].

4.1.2 Pathogenesis

FSD is a highly prevalent, age-related and progressive problem. It is characterized by disturbances in the psychophysiological changes associated with the sexual response cycle in women, and it includes disorders of sexual desire, arousal, orgasm, and pain. There are common risk factor categories associated with sexual dysfunction in women; these include aging, diabetes mellitus[87], CVD[92],

hypertension[93], concurrence of genitourinary disease[94], psychiatric/psychological disorders[95], cancer[96], and other chronic diseases. Moreover, limited social relations, financial difficulties, employment status, religious beliefs, educational background, and lack of exercise represent the sociocultural risk factors of FSD[97].

The normal female sexual response needs the integrity of the sensory and autonomic nervous systems in order to respond to erotic stimuli, as well as of the vascular districts that supply blood to the external genitalia and vagina. The regulation of blood flow and clitoral erectile function is governed by the same NO/ cGMP pathway in women as that involved in erectile function is in men. NO and PDE5 have been identified in human clitoral smooth muscle,115,116 indicating a key role of NO in female sexual function[98,99]. Normal levels of various hormones are also required for physiologic sexual activity. Diabetes may affect all of these integrated systems, leading to sexual dysfunction. The mechanisms involved include hyperglycemia, infections, vascular and neurological damage, and hormonal disorders[100]. Hyperglycemia reduces the hydration of the vaginal mucus membranes, producing poor vaginal lubrication and dyspareunia[101].

Diabetes induced vascular and nerve dysfunctions may impair the sexual response by producing structural and functional changes in the female genitalia. It has been hypothesized that FSD may be the consequence of an imbalance in the hormonal levels of diabetic women, as indicated by epidemiological studies showing a correlation between alterations in the levels of androgens, estrogens, as well as sex hormone-binding globulin and sexual problems in diabetic women[102].

Moreover, several endocrinological pathologies that may be associated with diabetes, such as thyroid disorders, hypothalamic pituitary dysfunctions and

polycystic ovarian syndrome, may further contribute to sexual dysfunctions in these women[103].

Depression seems to be the principally established determinant of sexual dysfunction in women with diabetes[104]. Diabetic complications may also affect health and relationship status, quality of life, and a woman's self-image, generating a vicious cycle that may have detrimental effects on sexual performance[105].FSD pathogenesis in diabetes is complex, and current studies have not yet clarified all of the pathological pathways involved.

4.1.3 Screening

Sexual function is assessed by completing the Female Sexual Function Index (FSFI), which is a validated 19-item self-report measure of female sexual function[106] (Figure 4.1). It is simple to administer and score and is unbiased with respect to age, ethnicity, education, and economic status. The 19 items are assigned to six separate domains of female sexual function. Four domains are related to the four major categories of sexual dysfunction: desire disorder, arousal disorder, orgasmic disorder, and sexual pain disorder. The fifth domain assesses the quality of vaginal lubrication, whether the sixth domain is related to global sexual and relationship satisfaction: it is viewed as the 'quality of life' domain of the scale. Each domain is scored on a scale of zero or 1–6, with higher score indicating better function. The full FSFI scale score, which could be 36 at the highest, was obtained by adding the six domain scores. The functional results are good when the FSFI

score was 30 or more, intermediate between 23 and 29, and poor below 23. Female sexual dysfunction is diagnosed with an FSFI result of less than 26.55[106].

Female Sexual Function Index (FSFI)

Subject Identifier _____ Age _____ Date _____

INSTRUCTIONS: These questions ask about your sexual feelings and responses during the past 4 weeks. Please answer the following questions as honestly and clearly as possible. Your responses will be kept completely confidential. In answering these questions the following definitions apply:

Sexual activity can include caressing, foreplay, masturbation and vaginal intercourse.

Sexual intercourse is defined as penile penetration (entry) of the vagina.

Sexual stimulation includes situations like foreplay with a partner, self-stimulation (masturbation), or sexual fantasy.

CHECK ONLY ONE BOX PER QUESTION.

Sexual desire or interest is a feeling that includes wanting to have a sexual experience, feeling receptive to a partner's sexual initiation, and thinking or fantasizing about having sex.

1. Over the past 4 weeks, how **often** did you feel sexual desire or interest?
- ▫ Almost always or always
- ▫ Most times (more than half the time)
- ▫ Sometimes (about half the time)
- ▫ A few times (less than half the time)
- ▫ Almost never or never

2. Over the past 4 weeks, how would you rate your **level** (degree) of sexual desire or interest?
- ▫ Very high
- ▫ High
- ▫ Moderate
- ▫ Low
- ▫ Very low or none at all

Sexual arousal is a feeling that includes both physical and mental aspects of sexual excitement. It may include feelings of warmth or tingling in the genitals, lubrication (wetness), or muscle contractions.

3. Over the past 4 weeks, how **often** did you feel sexually aroused ("turned on") during sexual activity or intercourse?
- ▫ No sexual activity
- ▫ Almost always or always
- ▫ Most times (more than half the time)
- ▫ Sometimes (about half the time)
- ▫ A few times (less than half the time)
- ▫ Almost never or never

4. Over the past 4 weeks, how would you rate your **level** of sexual arousal ("turn on") during sexual activity or intercourse?
- ▫ No sexual activity
- ▫ Very high
- ▫ High
- ▫ Moderate
- ▫ Low
- ▫ Very low or none at all

5. Over the past 4 weeks, how **confident** were you about becoming sexually aroused during sexual activity or intercourse?
- ▫ No sexual activity
- ▫ Very high confidence
- ▫ High confidence
- ▫ Moderate confidence
- ▫ Low confidence
- ▫ Very low or no confidence

6. Over the past 4 weeks, how **often** have you been satisfied with your arousal (excitement) during sexual activity or intercourse?
- ▫ No sexual activity
- ▫ Almost always or always
- ▫ Most times (more than half the time)
- ▫ Sometimes (about half the time)
- ▫ A few times (less than half the time)
- ▫ Almost never or never

7. Over the past 4 weeks, how **often** did you become lubricated ("wet") during sexual activity or intercourse?
- ▫ No sexual activity
- ▫ Almost always or always
- ▫ Most times (more than half the time)
- ▫ Sometimes (about half the time)
- ▫ A few times (less than half the time)
- ▫ Almost never or never

8. Over the past 4 weeks, how **difficult** was it to become lubricated ("wet") during sexual activity or intercourse?
- ▫ No sexual activity
- ▫ Extremely difficult or impossible
- ▫ Very difficult
- ▫ Difficult
- ▫ Slightly difficult
- ▫ Not difficult

9. Over the past 4 weeks, how often did you **maintain** your lubrication ("wetness") until completion of sexual activity or intercourse?
- ▫ No sexual activity
- ▫ Almost always or always
- ▫ Most times (more than half the time)
- ▫ Sometimes (about half the time)
- ▫ A few times (less than half the time)
- ▫ Almost never or never

10. Over the past 4 weeks, how **difficult** was it to maintain your lubrication ("wetness") until completion of sexual activity or intercourse?
- ▫ No sexual activity
- ▫ Extremely difficult or impossible
- ▫ Very difficult
- ▫ Difficult
- ▫ Slightly difficult
- ▫ Not difficult

11. Over the past 4 weeks, when you had sexual stimulation or intercourse, how **often** did you reach orgasm (climax)?
- ▫ No sexual activity
- ▫ Almost always or always
- ▫ Most times (more than half the time)
- ▫ Sometimes (about half the time)
- ▫ A few times (less than half the time)
- ▫ Almost never or never

12. Over the past 4 weeks, when you had sexual stimulation or intercourse, how **difficult** was it for you to reach orgasm (climax)?
- ▫ No sexual activity
- ▫ Extremely difficult or impossible
- ▫ Very difficult
- ▫ Difficult
- ▫ Slightly difficult
- ▫ Not difficult

13. Over the past 4 weeks, how **satisfied** were you with your ability to reach orgasm (climax) during sexual activity or intercourse?
- ▫ No sexual activity
- ▫ Very satisfied
- ▫ Moderately satisfied
- ▫ About equally satisfied and dissatisfied
- ▫ Moderately dissatisfied
- ▫ Very dissatisfied

14. Over the past 4 weeks, how **satisfied** have you been with the amount of emotional closeness during sexual activity between you and your partner?
- ▫ No sexual activity
- ▫ Very satisfied
- ▫ Moderately satisfied
- ▫ About equally satisfied and dissatisfied
- ▫ Moderately dissatisfied
- ▫ Very dissatisfied

15. Over the past 4 weeks, how **satisfied** have you been with your sexual relationship with your partner?
- ▫ Very satisfied
- ▫ Moderately satisfied
- ▫ About equally satisfied and dissatisfied
- ▫ Moderately dissatisfied
- ▫ Very dissatisfied

16. Over the past 4 weeks, how **satisfied** have you been with your overall sexual life?
- ▫ Very satisfied
- ▫ Moderately satisfied
- ▫ About equally satisfied and dissatisfied
- ▫ Moderately dissatisfied
- ▫ Very dissatisfied

17. Over the past 4 weeks, how **often** did you experience discomfort or pain during vaginal penetration?
- ▫ Did not attempt intercourse
- ▫ Almost always or always
- ▫ Most times (more than half the time)
- ▫ Sometimes (about half the time)
- ▫ A few times (less than half the time)
- ▫ Almost never or never

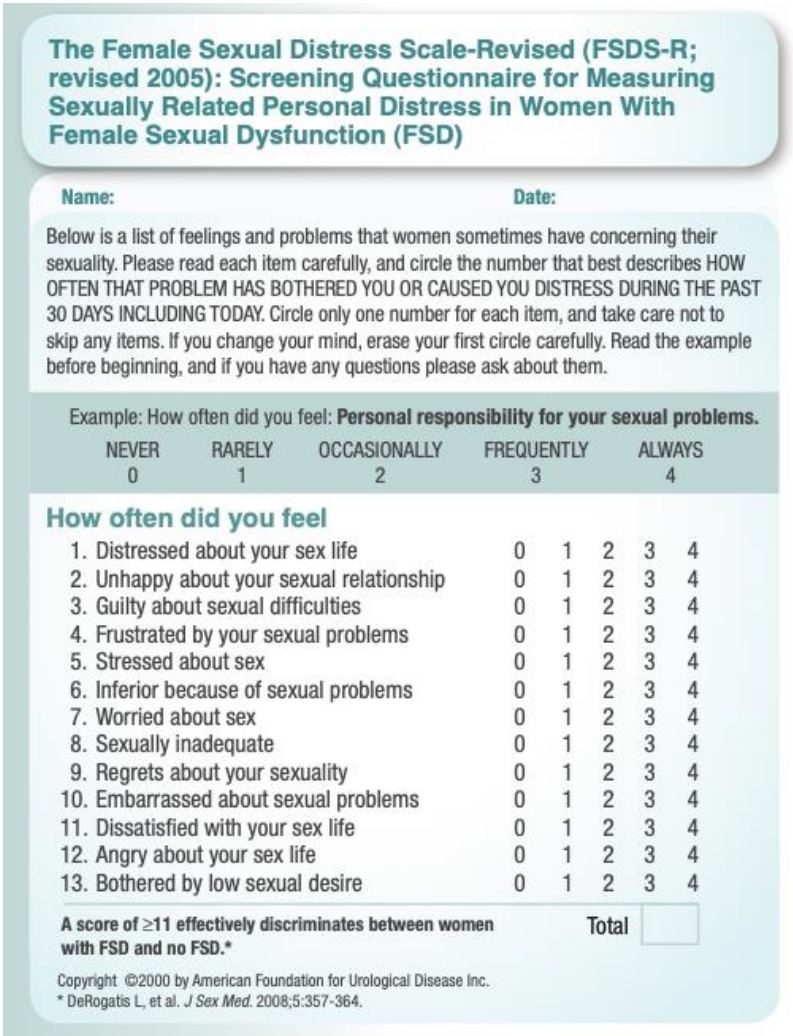
18. Over the past 4 weeks, how **often** did you experience discomfort or pain following vaginal penetration?
- ▫ Did not attempt intercourse
- ▫ Almost always or always
- ▫ Most times (more than half the time)
- ▫ Sometimes (about half the time)
- ▫ A few times (less than half the time)
- ▫ Almost never or never

19.Over the past 4 weeks, how would you rate your **level** (degree) of discomfort or pain during or following vaginal penetration?
- ▫ Did not attempt intercourse
- ▫ Very high
- ▫ High
- ▫ Moderate
- ▫ Low
- ▫ Very low or none at all

Thank you for completing this questionnaire.

Figure 4.1*- Female Sexual Function Index (FSFI) questionnaire.*

Another questionnaire to assess sexual function is the Female Sexual Distress Scale (FSDS) [107] that is a 12-item scale developed to assess sexual distress independent of specific domains of sexual function (e.g., erectile function, sexual desire) (Figure 4.2). Women were required to quantify the frequency of each area on a scale of 0–4 (0 = never, 4 = always). A total score higher than or equal to 15 indicated distress related to sexual life[107]. Each sexual function domain was considered altered if associated with personal distress, in accordance with the American Psychiatric Association guidelines[108].



The Female Sexual Distress Scale-Revised (FSDS-R; revised 2005): Screening Questionnaire for Measuring Sexually Related Personal Distress in Women With Female Sexual Dysfunction (FSD)

Name: Date:

Below is a list of feelings and problems that women sometimes have concerning their sexuality. Please read each item carefully, and circle the number that best describes HOW OFTEN THAT PROBLEM HAS BOTHERED YOU OR CAUSED YOU DISTRESS DURING THE PAST 30 DAYS INCLUDING TODAY. Circle only one number for each item, and take care not to skip any items. If you change your mind, erase your first circle carefully. Read the example before beginning, and if you have any questions please ask about them.

Example: How often did you feel: **Personal responsibility for your sexual problems.**

NEVER	RARELY	OCCASIONALLY	FREQUENTLY	ALWAYS
0	1	2	3	4

How often did you feel

1. Distressed about your sex life	0	1	2	3	4
2. Unhappy about your sexual relationship	0	1	2	3	4
3. Guilty about sexual difficulties	0	1	2	3	4
4. Frustrated by your sexual problems	0	1	2	3	4
5. Stressed about sex	0	1	2	3	4
6. Inferior because of sexual problems	0	1	2	3	4
7. Worried about sex	0	1	2	3	4
8. Sexually inadequate	0	1	2	3	4
9. Regrets about your sexuality	0	1	2	3	4
10. Embarrassed about sexual problems	0	1	2	3	4
11. Dissatisfied with your sex life	0	1	2	3	4
12. Angry about your sex life	0	1	2	3	4
13. Bothered by low sexual desire	0	1	2	3	4

A score of ≥11 effectively discriminates between women with FSD and no FSD.*

Total

* DeRogatis L, et al. *J Sex Med.* 2008;5:357-364.

Figure 4.2- *Female Sexual Distress Scale (FSDS) questionnaire.*

CHAPTER 5

MECHANISMS OF VASCULAR REPAIR: THE ROLE OF ENDOTHELIAL PROGENITOR CELLS

5.1 Endothelial progenitor cells (EPCs)

Endothelial progenitor cells (EPCs), are bone marrow-derived cells that contribute to vascular healing and remodeling under physiological and pathological conditions. They are indispensable for vascular homeostasis and maintenance of perivascular tissues. EPCs were first described by Asahara and coworkers in 1997. This group described a population of peripheral blood mononuclear cells that could differentiate into endothelial cells. These cells expressed the hematopoietic stem marker CD34 as well as the endothelial cell marker VEGFR2 and were shown to contribute to revascularization and the salvage of ischemic hindlimbs. Since then, various different markers have been used to identify this population, and the exact source for these cells remains controversial. EPCs are known to mobilize from the bone marrow into the peripheral blood in response to tissue ischemia/hypoxia and to differentiate into mature endothelial cells[109].

The identification of circulating EPCs in peripheral blood marked the beginning of a new era with enormous potential in the rapidly transforming regenerative field. Overwhelmed with the revelation, researchers across the globe focused on isolating, defining, and interpreting the role of EPCs in various physiological and pathological conditions. Consequently, controversies emerged regarding the isolation techniques and classification of EPCs.

In general, EPCs are characterized by the expression of 3 markers, CD133, CD34, and the vascular endothelial growth factor receptor-2. During differentiation, EPCs obviously lose CD133 and start to express CD31, vascular endothelial (VE) cadherin, and von Willebrand factor. CD133, an early hematopoietic stem-cell marker, is a 120- kDa transmembrane polypeptide, expressed on hematopoietic stem and progenitor cells from human bone marrow, fetal liver, and peripheral blood. A possible mix of both early progenitor and endothelial phenotype is CD133+/CD34/ VEGFR-2+ cells, which do not express vascular endothelial (VE) cadherin and von Willebrand factor. Cells with these characteristics are localized predominantly in the bone marrow. In the peripheral circulation of adults, more mature EPCs are found that obviously have lost CD133 but are positive for CD34 and VEGFR-2. Mature ECs show a high expression of VEGFR-2, VE-cadherin, and von Willebrand.

The mobilization of EPCs from the bone marrow is a complex process, regulated by a variety of factors. The activation of matrix metalloproteinase-9 (MMP-9), which promotes the transformation of membrane-bound Kit ligand (mKitL) to a soluble Kit ligand (sKitL), is an early step in this process. Subsequently, cKit-positive stem and progenitor cells, including also a common hematopoietic and angioblast precursor cell (hemangioblast, HABL), move to the vascular zone of the bone marrow microenvironment. This translocation activates the cells from a quiescent to a proliferative state. The signals, which initiate the diversion of the hemangioblast to either hematopoietic precursor cells or EPCs, are largely unknown at present but may include angiogenic growth factors from the periphery. For example, limb ischemia or vessel wall damage after coronary thrombosis, burn

injury, or coronary bypass surgery rapidly enhance the number of circulating EPCs. These events are associated also with elevated levels of endogenous VEGF, which has been shown to stimulate the release of EPCs from the bone marrow[110] (Figure 5.1).

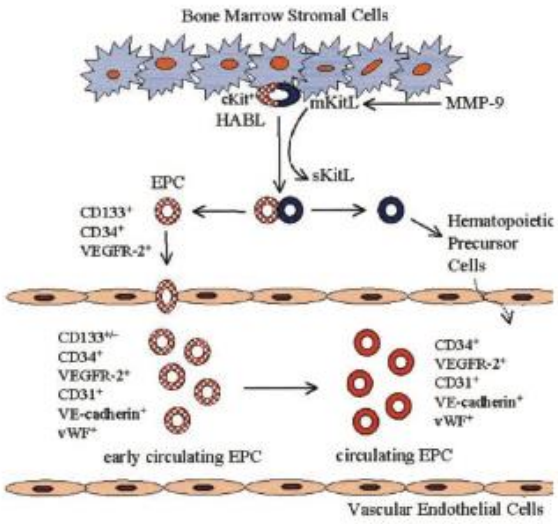


Figure 5.1*- The mobilization of EPCs from the bone marrow is a complex process, regulated by a variety of factors.*

5.1.1 Isolation of EPCs by various techniques

EPCs can be isolated from bone marrow or peripheral blood. In addition, EPCs have also been isolated from fetal liver or umbilical cord blood[111,112].

There are essentially two methods used to assess circulating EPCs: flow cytometry or fluorescence-activated sorting (FACS) system and cell culture. FACS works on the principle of excitation and emission of fluorochromes bonded to the antibody/protein. The flow cytometry protocol is based on a mononuclear cell analysis for size, nuclear complexity and binding of specific antibodies conjugated

to given fluorochromes Minimal antigenic profile in order to define the EPCs should include at least one stemness/immaturity (CD34 and/or CD133) marker and at least one vascular endothelial growth factor receptor 2 (VEGFR-2) marker. The classical isolation methods include the use of adherence culture of total peripheral blood mononuclear cells. After isolation, the cells are cultured in medium with specific growth factors (eg, VEGF, bovine brain extract, and epidermal growth factor), which facilitate the growth of endothelial-like cells. The incubation in vitro with a mixture of growth factors, the adhesion on specific substrates (eg, fibronectin), and the contact with the extracellular matrix or the surrounding mature ECs in vivo will probably influence the proliferation or differentiation of bone marrow– derived EPCs. After initial adhesion in vitro, EPCs begin to lose their progenitor characteristics and start to differentiate. EPCs form within 3 to 4 weeks monolayers with endothelial appearance[112,113].

5.1.2 EPC in Diabetes

Patients with type 1 and type 2 diabetes have fewer circulating EPCs compared with matched healthy subjects because of the reduced mobilization of EPCs from bone marrow either due to insufficient release of marrow-stimulating factors, such as VEGFR and SDF-1, which resulted in downregulation of hypoxia-induced factor (HIF-1) or through the PI3K-AKT-eNOS pathway[114]. Moreover, EPCs of diabetic patients exhibit reduced proliferation, adhesion, migration, and incorporation into tubular structures [115,116]. Also, many complications of diabetes, such as diabetic vasculopathy, cardiomyopathy, neuropathy, nephropathy, and retinopathy, are

closely linked to the problem of vascularization. Interestingly, among all these complications, there is a marked reduction in EPCs, except for retinopathy, which follows a reverse pattern[117].

Cindy J.M. Loomans et al showed that in T1DM, EPC numbers inversely correlate with HbA1c levels, demonstrating that the degree of glycemic dysregulation directly affects EPC proliferation or differentiation[118].

Longo M. et al have demonstrated that over a 2-year follow-up, young type 1 diabetic patients treated with intensive insulin regimen showed an increase in circulating EPCs levels, which correlates with the improvement in glucose variability[119].

5.2 Vascular repair mechanisms

It is accepted that generalized endothelial dysfunction precedes the development of atherosclerosis. When endothelium is exposed to hyperglycemia, an array of negative intracellular events promotes endothelial dysfunction. EPCs constitute a circulating pool of cells able to form a cellular patch at sites of endothelial injury, thus contributing directly to the homeostasis and repair of the endothelial layer. EPCs have now been recognized as playing a major role in cardiovascular biology: in fact, the extent of the circulating EPC pool is now considered a mirror of cardiovascular health[120].

Tissue ischemia is considered the strongest stimulus for EPC mobilization, through the activation of hypoxia-sensing systems, such as hypoxia inducible factor

(HIF)-1a[117].The resulting active HIF-1a binds to enhancer DNA regions and promotes the transcription of oxygen-sensible genes encoding, among others, vascular endothelial growth factor (VEGF), stromal derived factor-1a (SDF-1a), other genes involved in the angiogenic response, and erythropoietin[121].

Then, growth factors allow bone marrow EPCs to undergo transendothelial migration and to pass into the peripheral blood by means of attenuating stromal cell–stem cell interactions and by rearranging extracellular matrix. EPC mobilization is characterized by both a loss in cell-cell contacts and a desensitization of chemokine signaling, notably the SDF-1a/C-X-C chemokine receptor 4 (CXCR4) axis, the fundamental signaling pathway underlying stem cell mobilization and homing during tissue homeostasis and injury[122].

On the contrary, stem cell homing requires upregulation of cell adhesion molecules and activation of the SDF-1a/CXCR4 axis. The majority of cytokines that mediate stem cell migration do so via modulation either of SDF-1a or of its receptor, CXCR4. Under homeostatic conditions, most pro- genitors remain in the bone marrow compartment, retained by high SDF-1a expression, which is maintained by the hypoxic microenvironment. After an ischemic insult, soluble factors, such as SDF-1a, are released by injured tissue and stimulate mobilization of progenitor cells from the bone marrow. These cells are recruited to ischemic tissue by high local levels of SDF-1a, providing a permissive niche. Interestingly, SDF-1a is cleaved and inactivated by CD26/dipep- tidyl peptidase 4 (DPP-4). Zaruba et al.[123] showed that the pharmacological inhibition of DPP-4 increased myocardial homing of circulating $CXCR4^+$ stem cells, reduced cardiac remodeling, and improved heart function and survival. Against this background cardiovascular

health depends on a balance between damage and repair thanks to the contribution of endothelial progenitor cells (Figure 5.2).

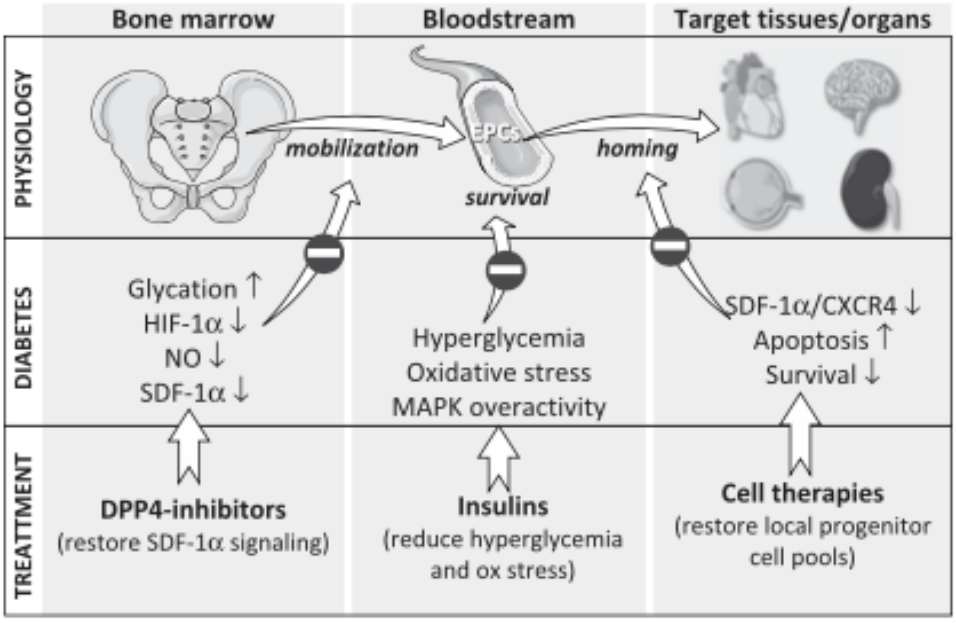


Figure 5.2*- Schematic biological role of endothelial progenitor cells and potentially available treatment aiming to restore their circulating concentration.*

CHAPTER 6

THE STUDY

6.1 Introduction and aim of the study

The overall global incidence of type 1 diabetes is rising, with an especially concerning rate in children under the age of 5 years[124], which translates to a lifetime of exposure and increased risk for early death from cardiovascular disease[125].

T1DM is associated with both macrovascular (including CVD) and microvascular (including retinopathy, nephropathy, and neuropathy) complications. People with diabetes are at a greater risk of developing CVDs, such as heart attack and stroke[126]. The risk of cardiovascular death remains 4.2 times higher in people with T1DM compared with non-diabetic controls[127,128].

Cardiovascular disease also disproportionally affects women with type 1 diabetes, which contrasts with a male predominance observed in the general population[129].

Diabetes attenuates the general female biological advantage by protecting against cardiovascular complications across all ages[129]. Reproductive factors, differences in experience, and presentation of symptoms or psychosocial stress may also play a role in the more unfavourable situation of diabetic women[130].

Hyperglycemia contributes significantly to endothelial damage, making itself responsible for a process of chronic inflammation, production of reactive oxygen species, alteration of hypoxia sensors. Circulating endothelial progenitor cells (EPCs) play a fundamental role in maintaining endothelial health, given their ability

to differentiate into mature endothelial cells and participate in the mechanisms of neo-angiogenesis and endothelial repair. EPCs show a wide heterogenic antigenic profile. Typical surface antigens to identify EPCs are CD34, CD133, and KDR[117]. There is evidence that circulating levels of EPCs are reduced in diabetic patients compared with age-matched subjects[131], representing one of the mechanisms linking increased vascular risk with diabetes mellitus[129].

During the menstrual cycle, the number of EPCs in peripheral blood exhibits a cyclic change. Robb et al have demonstrated that in healthy nulliparous, premenopausal, non-smoking women with regular menses, triple positive (CD34+CD133+KDR+) EPCs varied during the menstrual cycle with mid-follicular levels being 3-fold higher than peri-ovulatory levels[132]. Foresta et al observed a significant increase in the number of PCs (CD34+/CD133+) and EPCs (CD34+CD133+ KDR+) cells during ovulation in young fertile women. The levels of EPCs during menses in women with diabetes has never been investigated[133].

Female sexual dysfunctions (FSD) are complex conditions characterized by impairment of the female sexual cycle[134]. Higher prevalence of FSD has been found in women with diabetes, as compared with matched healthy controls. Sexual problems in women with diabetes can be explained by several mechanisms such as hyperglycemia, vascular and nervous dysfunctions, endocrinological disorders and psychological factors[131].

The objective of this longitudinal observational study is to evaluate the trend of circulating EPCs levels in the follicular, ovulatory and luteal phase of the menstrual cycle and the change of sexual function in young women with type 1

diabetes mellitus during the menses compared with non-diabetic women (controls) matched by age.

6.2 Materials and methods

6.2.1 Study design and participants

This is a single-center, longitudinal observational study conducted between January 2019 to March 2021. Type 1 diabetic women attending the Unit of Endocrinology and Metabolic Diseases at University Hospital "Luigi Vanvitelli'' (Naples, Italy) were consecutively screened for eligibility criteria: 1) age ≥ 18 and ≤35 years, 2) stable couple relationship or masturbation in the previous 6 months, 3) regular menses and 4) absence of oral contraceptive use. We excluded patients with major health problems including diabetic chronic complications, neoplasms, major depression or other psychiatric disorders, severe neurological diseases, drug or alcohol abuse, polycystic ovarian syndrome (PCOS), use of medication with recognized adverse effects on the female sexual function. Also excluded were pregnant or planning to become pregnant women and those who experienced gynecological surgery, lower urinary tract symptoms, and pelvic trauma in the last 6 months. The study was approved by the local ethical committee, and all participants signed an informed consent before enrolment.

6.2.2 Assessment of sexual function

Both patients and controls were asked to complete two validated multiple-choice questionnaires assessing sexual function [106] and the discomfort related to sexual activity [107] the score for each instrument was calculated by the recommended scoring system. Each questionnaire was administered after a short explanation.

Sexual function was assessed by completing the Female Sexual Function Index (FSFI) is a self-report questionnaire including 19 items subdivided into six domains (desire, arousal, lubrication, orgasm, satisfaction and pain) referring to sexual activity in the last 4 weeks. Each domain was scored on a scale of 0 or 1–6, with higher score indicating better function. For each of the six domains, a score was calculated and the total score was obtained by adding the six domains scores. The total score range was 2–36. An alteration of sexual function was evidenced by a score of 26.55 or less.

Sexual activity-related distress was assessed using the FSDS, a self-assessment questionnaire composed of 12 items. Women were required to quantify the frequency of each area on a scale of 0–4 (0 = never, 4 = always). A total score higher than or equal to 15 indicated distress related to sexual life. Each sexual function domain was considered altered if associated with personal distress, in accordance with the American Psychiatric Association guidelines. FSD was diagnosed according to a FSFI score lower than 26.55 and a FSDS score higher than 15[135].

6.2.3 Clinical measures and laboratory analyses

All patients underwent a full physical examination to assess weight and height, body mass index (BMI), and blood pressure. Height and weight were measured to the nearest 0.5 cm and 100 g, respectively, with participants wearing lightweight clothing and no shoes. BMI was calculated as weight (in kilograms) divided by standing height (in meters squared). Arterial blood pressure was measured three times, at the end of the physical examination with the subject in sitting position. Assays for fasting glucose, HbA1c, total cholesterol, low-density and high-density lipoprotein cholesterol, triglyceride levels, were performed in the hospital's chemistry laboratory. Blood samples were drawn in the follicular, ovulatory and luteal phases of the same menses to assess sexual hormones levels, including FSH, LH, progesterone and estradiol.

6.2.4 Assessment of Circulating Levels of EPCs

Peripheral blood cells were analyzed for the expression of surface antigens by direct flow cytometry, as previously described[119]. Briefly, fasting blood samples were processed after 1–2 hours. Mononuclear cells were isolated from peripheral venous blood by density centrifugation. Then, the isolated blood cells were stained for 30 minutes at 4°C in the dark with fluorescein isothiocyanate (FITC)-conjugated antihuman CD34 monoclonal antibody (mAb) (Becton Dickinson, Buccinasco, Bologna, Italy), phycoerythrin (PE)-conjugated antihuman KDR mAb (R&D

Systems, Minneapolis, MN, USA), and allophycocyanin (APC)-conjugated antihuman CD133 (Miltenyi Biotec, Calderara di Reno, Bologna, Italy). Isotope immunoglobulin IgG1 and IgG2a antibody was used to discriminate between signal range and baseline fluorescence within the samples. After incubation, quantitative analysis was performed on a BD FACSCalibur cytometer, and 1,000,000 cells were acquired in each sample. A morphological gate was used to exclude granulocytes. Then, we gated CD34+ or CD133+ peripheral blood cells in the mononuclear cell fraction and examined the resulting population for the dual expression of KDR. In the two-dimensional dotplot analysis, we identified CD34+ CD133+ cells. Triple-positive cells were identified by the dual expression of KDR and CD133 in the CD34+ gate. Data were processed with the use of the Macintosh CELLQuest software program (Becton Dickinson). The results from flow cytometry were expressed as the number of cells per 10^6 events.

6.2.5 Statistical analysis

Data in tables are presented as mean ± standard deviation, median and interquartile range or number and percentage. Descriptive statistics were used for demographic and baseline clinical characteristics of all participants in the study. Comparisons of baseline data between the patients' groups were performed by Student's *t* test or Mann–Whitney Rank Sum test, depending on the normality of sample distribution. The correlation between changes in levels of EPCs and corresponding changes in the sexual hormones was assessed using Spearman's coefficient of correlation. The χ^2 test was used for comparing dichotomous

variables. Data were analysed using Stata, version 16.0 (Stata Corp, College Station, Texas). P-value <0.05 was considered statistically significant.

6.3 Results

A total of 18 women with T1D and 8 healthy controls were enrolled. The clinical and metabolic characteristics of the overall study population are shown in Table 6.1. Mean age was 25 years and mean BMI was 23.4 Kg/m^2. The average age was not different between women with diabetes and control women, nor were different anthropometric parameters, lipid profile and blood pressure. As expected, women with diabetes had higher levels of fasting glucose (P = 0.003) and HbA1c (P < 0.001), compared with the control group. The overall prevalence of FSD, defined as the concomitant presence of pathological FSFI and FSDS scores, was 7 % (2/18) among women with diabetes and 12.5% (1/8) among control women.

Table 6.1*-Characteristics of participants in the study.*

Parameters	DM1	Controls	P
Age, years	25.0 ± 5.6	26.7 ± 2.3	0.062
Duration of diabetes, years	14.1 ± 4.8	-	-
Weight, Kg	65.9 ± 15.2	60.7 ± 7.3	0.375
BMI,Kg/m2	25.8 ± 5.0	22.9 ± 2.6	0.232
Fasting glucose, mg/dl	133.6 ± 42.9	76.0 ± 5.6	**0.003**
HbA1c, %	7.7 ± 1.1	5.0 ± 0.6	**< 0.001**
SBP, mmHg	110.0 (110.0, 112.5)	110.0 (105.0, 115.0)	0.876
DBP, mmHg	70.0 (65.0, 70.0)	60.0 (67.5, 62.5)	0.514
HR, bpm	81.3 ± 7.3	76.3 ± 6.9	0.137
Total cholesterol, mg/dl	168.5 ± 20.8	163.0 ± 20.4	0.536
HDL- cholesterol, mg/dl	60.7 ± 13.3	58.1 ± 8.4	0.835
LDL-cholesterol, mg/dl	96.3 ± 15.7	101.2 ± 26.0	0.303
Triglycerides, mg/dl	64.2 ± 24.5	72.5 ± 24.7	0.255
Creatinine, mg/dl	0.78 ± 0.1	0.85 ± 0.01	0.428

In the two groups, there was a normal cyclical variation in circulating pituitary and ovarian hormones with peri-ovulatory peaks in both serum LH and FSH concentrations, and peri-ovulatory and mid-luteal peaks in both serum estradiol and progesterone concentrations. There were no differences in sexual hormone levels during the different phases of menses between the two groups (Table 6.2).

Table 6.2*- Sexual hormone levels during follicular, ovulatory and luteal phases of the same mense in women with type 1 diabetes and healthy controls.*

	Follicular phase			Ovulatory phase			Luteal phase		
	Patients	**Controls**	**P**	**Patients**	**Controls**	**P**	**Patients**	**Controls**	**P**
FSH,mIU/ml	7.1 ± 1.8	6.8 ± 1.6	0.628	6.1 ± 2.2	6.1 ± 2.6	0.953	3.6 ± 1.5	4.0 ± 1.8	0.526
LH, mIU/ml	5.3 ± 1.5	5.7 ± 1.6	0.524	8.4 ± 4.1	10.5 ± 6.1	0.311	4.0 ± 1.5	3.7 ± 1.4	0.482
Progesterone, ng/mL	0.42 ± 0.2	0.36 ± 0.17	0.420	2.2 ± 3.9	1.1 ± 0.9	0.488	6.0 ± 4.8	4.8 ± 2.4	0.546
Estradiol, pg/mL	65.0 ± 61.1	77.4 ± 70.3	0.628	133.2 ± 72.7	129.6 ± 74.3	0.906	137.1 ± 77.9	140.0 ± 71.6	0.927

Table 6.3 shows the change in circulating levels of EPCs in the women with diabetes and controls during the three phases of the menstrual cycle. Compared with controls, women with diabetes showed a significantly lower levels of CD34+ cells in the ovulatory phase [cases vs control, median and IQR, 211 (171, 310) vs 361 (345, 437) P= 0.031], and CD34+KDR+ cells in both the ovulatory phase [22 (19, 26) vs 41(17, 251) P= 0.041] and in the luteal phase [19 (9, 27) vs 38 (28, 49) P= 0.002] (Table 6.3, Figure 6.1). No significant difference was recorded for the other EPC phenotypes between the two groups (Table 6.3).

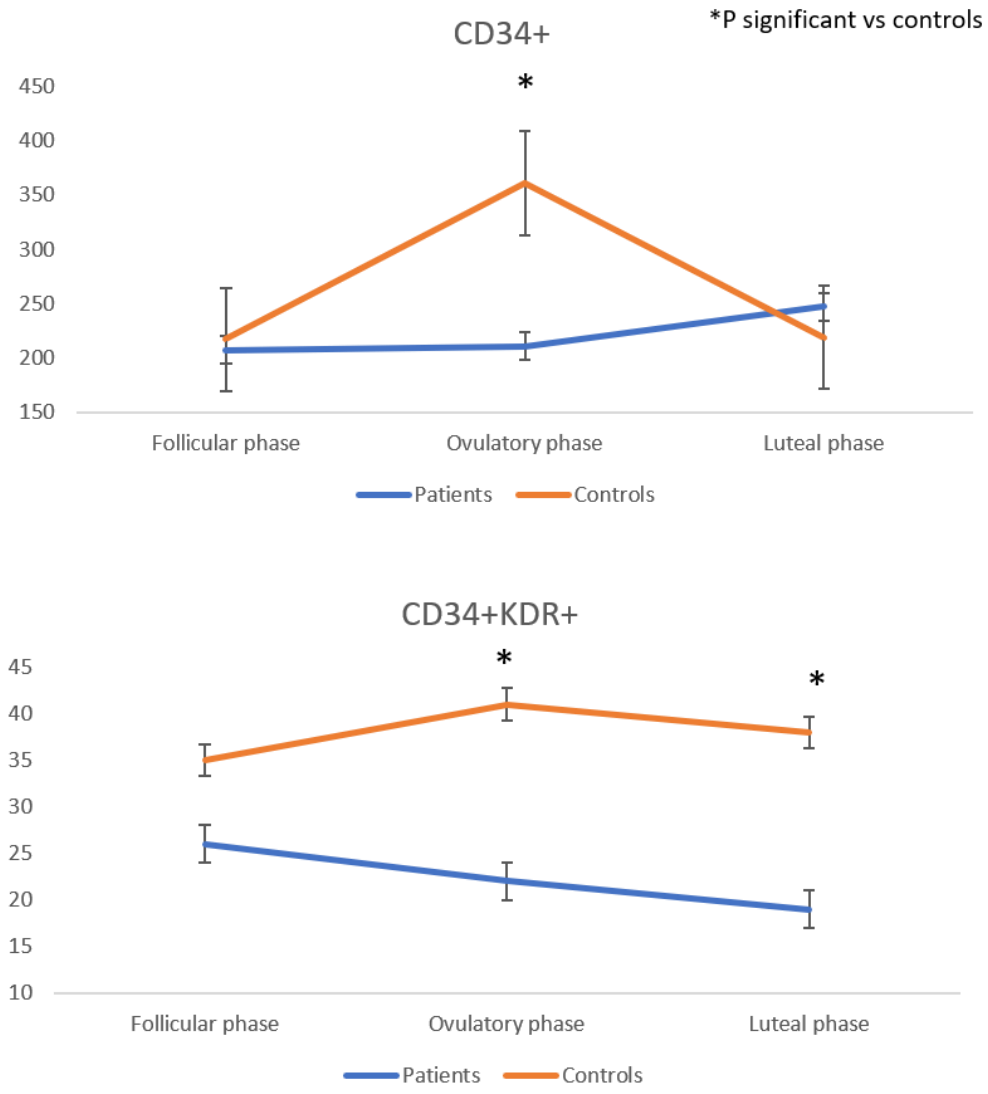


***Figure 6.1**- Circulating levels of CD34+ and CD34+KDR+ cells EPCs in the three phases of menses in women with diabetes and healthy controls.*

Table 6.3*-Circulating levels of EPCs in the follicular, ovulatory and luteal phases in diabetic and control women.*

CD34+	**Patients**	**Controls**	**P**
Follicular phase	207 (171,320)	217(147, 330)	0.842
Ovulatory phase	211 (171, 310)	361 (345, 437)	**0.031**
Luteal phase	247 (166, 267)	219 (142, 296)	0.765
CD34+CD133+			
Follicular phase	119 (90, 132)	124 (76, 198)	0.845
Ovulatory phase	111 (80, 113)	113 (110, 153)	0.278
Luteal phase	117 (80, 134)	116 (103, 119)	0.940
CD34+KDR+			
Follicular phase	16 (12, 32)	35 (23, 47)	0.215
Ovulatory phase	22 (19, 26)	41 (17, 251)	**0.041**
Luteal phase	19 (9, 27)	38 (28, 49)	**0.002**
CD34+KDR+CD133+			
Follicular phase	7 (2,10)	8 (6,11)	0.189
Ovulatory phase	4 (3, 6)	5 (4, 7)	0.275
Luteal phase	3 (2, 6)	7,5 (3,12)	0.073

Patients with diabetes showed a significantly lower total FSFI scores in all the three phases of the menses, as compared with control women (Table 6.4). We then evaluated the individual domains of sexual function in the different phases of the menstrual cycle comparing the FSFI single domain scores between women with diabetes and control subjects. Compared with control women, diabetic women showed significant lower scores in the domains of desire, arousal and pain in the follicular phase, significant lower scores in the domains of desire, arousal, lubrication, orgasm and pain in the ovulatory phase, and significant lower scores in

the domains of arousal, lubrication, orgasm and pain in the luteal phase (Table 6.4). and found a significant reduction in the score of all domains except that of satisfaction in diabetic women compared to controls in the ovulatory phase.

Table 6.4- *FSFI total score and single domains scores during the different phases of the menses in diabetic women and control subjects.*

DESIRE	**Patients**	**Controls**	**P**
Follicular phase	4.2 (3.6, 4.8)	5.1 (4.5, 5.7)	**0.005**
Ovulatory phase	4.2 (3.6, 4.2)	5.4 (4.9, 6)	**0.005**
Luteal phase	4.8 (3.6, 4.9)	5.4 (4.8, 6)	0.104
AROUSAL			
Follicular phase	5.1 (4.2, 5.4)	5.7 (5.5, 5.8)	**0.001**
Ovulatory phase	5.1 (4.2, 5.4)	6 (5.7, 6)	**<0.001**
Luteal phase	4.5 (4, 5.4)	6 (6, 6)	**0.002**
LUBRICATION			
Follicular phase	4.8 (3.6, 6)	5.7 (5.1, 6)	0.143
Ovulatory phase	5.4 (4.2, 5.4)	6 (5.7, 6)	**0.005**
Luteal phase	4.5 (3.6, 5.4)	6 (6, 6)	**0.006**
ORGASM			
Follicular phase	4.4 (3.2, 5.6)	4.8 (4.4, 5.4)	0.448
Ovulatory phase	4.8 (3.2, 5.2)	5.6 (5.6, 6)	**0.004**
Luteal phase	4.8 (4.5, 6)	6.6 (6, 6)	**0.002**
PAIN			
Follicular phase	5.6 (5.6, 6)	6 (5.8, 6)	**0.043**
Ovulatory phase	5.6 (4, 6)	6 (6, 6)	**0.006**
Luteal phase	5.2 (4.4, 5.6)	6 (6, 6)	**0.016**
SATISFACTION			
Follicular phase	4.6 ± 1.5	5.3 ± 0.6	0.256
Ovulatory phase	6 (4.8, 6)	6 (6, 6)	0.244
Luteal phase	5.6 (4, 6)	6 (6, 6)	0.069
FSFI TOTAL SCORE			
Follicular phase	28.4 (22, 32.8)	32.6 (31.5, 33.3)	**0.049**
Ovulatory phase	28.4 (26.8, 31.9)	35 (34.8, 35.7)	**0.002**
Luteal phase	29.4 (25, 29.9)	35.4 (34.8, 36)	**0.002**

Results from the univariate analysis, which tested the presence of an association between circulating levels of CD34+ and CD34+KDR+ cells and the concentrations of sex hormones in the different phases of the menstrual cycle in women with diabetes and control women, is showed in Table 6.5 and in Table 6.6.

In women with diabetes, there was a significant association between the number of CD34+ and FSH concentrations in the luteal phase (r = 0.600, P=0.013). Moreover, circulating levels of CD34+KDR+ cells were positively associated with progesterone in the follicular phase (r= 0.767, P=<0.001) and negatively associated with progesterone in the luteal phase (r = -0.738, P= <0.001).

***Table 6.5**- Correlation between circulating levels of CD34+ and CD34+KDR+ cells and the concentrations of sex hormones in the different phases of the menstrual cycle in women with diabetes.*

CD34+	**Follicular phase**		**Ovulatory phase**		**Luteal phase**	
	r_p	P	r_p	P	r_p	P
FSH	0.430	0.09	-0.337	0.202	**0.600**	**0.013**
LH	-0.314	0.274	-0.139	0.608	-0.364	0.165
Estradiol	-0.116	0.668	0.268	0.316	-0.227	0.398
Progesterone	0.373	0.154	-0.422	0.133	-0.185	0.526
CD34+KDR+	Follicular phase		Ovulatory phase		Luteal phase	
	r_p	P	r_p	P	r_p	P
FSH	0.288	0.279	-0.166	0.540	0.295	0.268
LH	0.145	0.620	0.206	0.445	0.018	0.945
Estradiol	-0.03	0.917	0.010	0.970	0.019	0.944
Progesterone	**0.767**	**<0.001**	0.203	0.486	**-0.738**	**<0.001**

In the control group, the number of CD34+ cells was positively associated with the levels of estradiol in the follicular phase (r=0.810, P= 0.014) and negatively associated with the levels of progesterone in luteal phase ((r = -0.967, P = 0.002). Moreover, CD34+KDR+ cell count was positively associated with the levels of progesterone in the follicular phase (r=0.892, P=0.003) and inversely correlated with the level of progesterone in the luteal phase (r = -0.894, P = 0.016).

Table 6.6*- Correlation between circulating levels of CD34+ and CD34+KDR+ cells and the concentrations of sex hormones in the different phases of the menstrual cycle in control women.*

CD34+	**Follicular phase**		**Ovulatory phase**		**Luteal phase**	
	r_p	P	r_p	P	r_p	P
FSH	-0.556	0.153	-0.215	0.728	-0.456	0.157
LH	-0.330	0.424	-0.110	0.860	-0.578	0.133
Estradiol	**0.810**	**0.014**	0.293	0.633	-0.185	0.662
Progesterone	0.130	0.759	-0.193	0.756	**-0.967**	**0.002**
CD34+KDR+	Follicular phase		Ovulatory phase		Luteal phase	
	r_p	P	r_p	P	r_p	P
FSH	-0.729	0.04	-0.359	0.553	-0.456	0.257
LH	0.277	0.507	-0.328	0.590	-0.578	0.133
Estradiol	0.229	0.585	0.139	0.824	-0.185	0.662
Progesterone	**0.892**	**0.003**	-0.357	0.556	**-0.894**	**0.016**

6.4 Discussion and conclusions

We showed for the first time a decrease in circulating levels of EPCs in young fertile women with diabetes during the menses. Our results are novel, as no previous studies evaluated the change in circulating progenitor cells in a population of young fertile women with type 1 diabetes in all phases of menstrual cycle. Moreover, young fertile diabetic women showed a worse sexual function and lower FSFI total score among the three phases of the menses compared with those of control subjects.

Women in the reproductive age are exposed to a lower cardiovascular risk than age-matched men[129]. This is generally attributed to the differences in sex hormones and, specifically, to the protective cardiovascular properties of female estrogens[130]. Besides the effects on plasma lipids and on the vessel wall, other mechanisms may link sexual hormones to a favourable cardiovascular profile, including the regulation of endothelial homeostasis. Interestingly, both quantitative and qualitative differences in EPCs between young men and women, together with the observation that EPCs are mobilized during the hormonal cycle, indicated that EPCs may be influenced by female sex hormones[136].

Indeed, there is evidence that in healthy normally menstruating women, a physiological role for the menstrual cycle in regulating the availability of EPCs emerged during the different phases[136,137]. Our results confirm these findings; however, we found significantly lower levels of CD34+ in the ovulatory phase associated with significant lower levels of CD34+KDR+ both in the ovulatory phase and in the luteal phase in diabetic women compared with control group, suggesting that the cyclic mobilization of EPCs during menstrual cycle observed in

healthy women is blunted in diabetic women, even in the context of a normal cyclical variation in circulating pituitary and ovarian hormones. The lack of previous studies evaluating the fluctuation of EPCs in young fertile women with type 1 diabetes in all phases of menstrual cycle does not allow to make comparisons.

A potential hypothesis explaining our findings may be the loss of regulating function of sexual hormones on the fluctuations of EPCs levels during the phases of the menses in women with diabetes. In type 2 diabetic women, an imbalance between ERα/ERβ distribution responsible for increased vasoconstriction and enhanced vascular inflammation has been described[138]. Moreover, in post-menopausal healthy women, with the cessation of the ovarian and endometrial activity, the number of EPCs are similar to those expressed in males[138]. Evidence on type 1 diabetic women are currently lacking. However, recent studies demonstrated that circulating levels of EPCs are lower in young people with type 1 diabetes as compared with healthy age-matched control[119,136]. Furthermore, we previously identified gender differences in the count of circulating EPCs in a population of young adult with type 1 diabetes favouring males[139], suggesting that women with diabetes may present additional detrimental conditions which further blunt the physiological mobilization from bone marrow of these staminal cells. Whether the gender gradient in EPCs number could reflect a vulnerability for diabetic women in terms of increased risk of vascular complications remains unknown.

Previous reports have shown an increased prevalence of sexual dysfunction among women with type 1 diabetes[140,141]. Our data confirm the observation that type 1 diabetes affects several aspects of female sexual function, including arousal,

lubrication, orgasm, desire and pain, but not satisfaction. Indeed, we found a significant reduction in the score of all domains except that of satisfaction in diabetic women compared with controls in the ovulatory phase. The FSFI total score as well as the scores related to desire, arousal, lubrication and pain were lower in diabetic women as compared with those of control subjects in the three phases of menses, even they can not be considered severely alterated.

Major strengths of this study include the use of a validated tool for the evaluation of sexual dysfunction, the assessment of four different EPC phenotypes by flow cytometry in different phases of the same menstrual cycle. This study has also limitations. Due to the observational nature of this study, we cannot make inference regarding cause and effect. Moreover, a major limitation of the study relates to the limited number of subjects investigated, which needs confirmation.

In conclusion, women with type 1 diabetes show lower levels of EPCs during the ovulatory and luteal phases of the menstrual cycle, associated with a worse sexual function, as compared with healthy controls. Further longitudinal studies are needed to clarify whether these dysfunctions can be related to the increase in cardiovascular risk of women with type 1 diabetes.

Bibliography

1. Diagnosis and classification of diabetes mellitus. association, American Diabetes. s.l. : Diabetes care , 2021.
2. International Diabetes Federation. IDF Diabetes Atlas 10a ed. 2021.
3. Tuomilehto J, Ogle GD, Lund-Blix N, Stene LC. Epidemiology of childhood type 1 diabetes. Pediatr Endocrinol Rev 2020;17(Suppl 1):198-209.
4. Sharp SA, Weedon MN, Hagopian WA, Oram RA. Clinical and research uses of genetic risk scores in type 1 diabetes. Curr Opin Genet Dev. 2018; 50:96-102.
5. Pociot F, Lernmark Å. Genetic risk factors for type 1 diabetes. Lancet. 2016;387(10035):2331-2339.
6. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. Lancet. 2018;391(10138):2449-2462.
7. Martinov T, Fife BT. Type 1 diabetes pathogenesis and the role of inhibitory receptors in islet tolerance. Ann N Y Acad Sci. 2020;1461:73-103.
8. Noble JA. Immunogenetics of type 1 diabetes: A comprehensive review. J Autoimmun. 2015; 64:101-112.
9. Rewers M, Ludvigsson J. Environmental risk factors for type 1 diabetes. Lancet. 2016;387(10035):2340-2348.
10. Visalli N, Sebastiani L, Adorisio E, et al. Environmental risk factors for type 1 diabetes in Rome and province. Arch Dis Child. 2003;88(8):695-698.
11. Sadauskaite-Kuehne V, Ludvigsson J, Padaiga Z, Jasinskiene E, Samuelsson U. Longer breastfeeding is an independent protective factor against development of type 1 diabetes mellitus in childhood. Diabetes Metab Res Rev. 2004;20(2):150-157.
12. Wahlberg J, Fredriksson J, Nikolic E, Vaarala O, Ludvigsson J. Environmental factors related to the induction of beta-cell autoantibodies in 1-yr-old healthy children. Pediatr Diabetes 2005;6:199–205. 109.
13. Wahlberg J, Vaarala O, Ludvigsson J. Dietary risk factors for the emergence of type 1 diabetes-related autoantibodies in 21/2 yearold Swedish children. Br J Nutr 2006;95:603–8
14. Vaarala O, Knip M, Paronen J, Hamalainen AM, Muona P, Vaatainen M, Ilonen J, Simell O, Akerblom HK. Cow's milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. Diabetes 1999;48: 1389–94.

15. Virtanen SM, Laara E, Hypponen E, Reijonen H, Rasanen L, Aro A, Knip M, Ilonen J, Akerblom HK. Cow's milk consumption, HLA-DQB1 genotype, and type 1 diabetes: a nested case-control study of siblings of children with diabetes. Childhood diabetes in Finland study group. Diabetes 2000;49:912–7
16. Marshall AL, Chetwynd A, Morris JA, Placzek M, Smith C, Olabi A, Thistlethwaite D. Type 1 diabetes mellitus in childhood: a matched case control study in Lancashire and Cumbria, UK. Diabet Med 2004;21:1035–40
17. Mathieu C, van Etten E, Decallonne B, Guilietti A, Gysemans C, Bouillon R, Overbergh L. Vitamin D and 1,25-dihydroxyvitamin D3 as modulators in the immune system. J Steroid Biochem Mol Biol 2004;89–90:449–52. 132.
18. Gysemans CA, Cardozo AK, Callewaert H, Giulietti A, Hulshagen L, Bouillon R, Eizirik DL, Mathieu C. 1,25- Dihydroxyvitamin D3 modulates expression of chemokines and cytokines in pancreatic islets: implications for prevention of diabetes in nonobese diabetic mice. Endocrinology 2005;146: 956–64.
19. Stene LC, Joner G. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset T1D: a large, population-based, case-control study. Am J Clin Nutr 2003;78: 1128–34.
20. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 2001;358:1500–3
21. Martinov T, Fife BT. Type 1 diabetes pathogenesis and the role of inhibitory receptors in islet tolerance. Ann N Y Acad Sci. 2020;1461(1):73-103.
22. In't Veld P. Insulitis in human type 1 diabetes: The quest for an elusive lesion. Islets. 2011;3(4):131-138.
23. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. Lancet. 2014;383(9911):69-82.
24. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013;309(23):2473-2479.
25. de Ferranti SD, de Boer IH, Fonseca V, Fox CS, Golden SH, Lavie CJ, Magge SN, Marx N, McGuire DK, Orchard TJ, Zinman B, Eckel RH. Type 1 diabetes mellitus and cardiovascular disease: a scientific statement from the American Heart Association and American Diabetes Association. Circulation. 2014;130(13):1110-30.

26. Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, Jacobsen LM, Schatz DA, Lernmark Å. Type 1 diabetes mellitus. Nat Rev Dis Primers. 2017 ;3:17016.
27. Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT. Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. Lancet Diabetes Endocrinol. 2018;6(2):122-129.
28. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 33, S62–S69 (2010).
29. WHO | Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. WHO https://www.who.int/diabetes/publications/diagnosis_diabetes2006/en/.
30. Klingensmith GJ, Pyle L, Arslanian S, Copeland KC, Cuttler L, Kaufman F, Laffel L, Marcovina S, Tollefsen SE, Weinstock RS, Linder B; TODAY Study Group. The presence of GAD and IA-2 antibodies in youth with a type 2 diabetes phenotype: results from the TODAY study. Diabetes Care. 2010;33(9):1970-1975.
31. The epidemiology of obesity - Metabolism - Clinical and Experimental. https://www.metabolismjournal.com/article/S0026-0495(18)30194-X/fulltext.
32. Tridgell DM, Spiekerman C, Wang RS, Greenbaum CJ. Interaction of onset and duration of diabetes on the percent of GAD and IA-2 antibody-positive subjects in the type 1 diabetes genetics consortium database. Diabetes Care. 2011;34(4):988-993
33. Davis AK, DuBose SN, Haller MJ, Miller KM, DiMeglio LA, Bethin KE, Goland RS, Greenberg EM, Liljenquist DR, Ahmann AJ, Marcovina SM, Peters AL, Beck RW, Greenbaum CJ; T1D Exchange Clinic Network. Prevalence of detectable C-Peptide according to age at diagnosis and duration of type 1 diabetes. Diabetes Care. 2015;38(3):476-481.
34. Banting FG, Best CH. Pancreatic extracts. 1922. J Lab Clin Med. 1990;115(2):254-272.
35. Richter B, Neises G. 'Human' insulin versus animal insulin in people with diabetes mellitus. Cochrane Database Syst Rev. 2002;(3):CD003816.
36. Evans M, Schumm-Draeger PM, Vora J, King AB. A review of modern insulin analogue pharmacokinetic and pharmacodynamic profiles in type 2 diabetes: improvements and limitations. Diabetes Obes Metab. 2011;13:677–684.
37. Cohen O, Filetti S, Castañeda J, Maranghi M, Glandt M. When intensive insulin therapy (MDI) fails in patients with type 2 diabetes: switching to GLP-1 receptor agonist versus insulin pump. Diabetes Care. 2016;39: S180–S186.
38. Yeh HC, Brown TT, Maruthur N, Ranasinghe P, Berger Z, Suh YD, Wilson LM, Haberl EB, Brick J, Bass EB, Golden SH. Comparative effectiveness and safety of methods of

insulin delivery and glucose monitoring for diabetes mellitus: a systematic review and meta-analysis. Ann Intern Med. 2012;157(5):336-347.

39. Franek E, Haluzík M, Canecki Varžić S, Sargin M, Macura S, Zacho J, Christiansen JS. Twice-daily insulin degludec/ insulin aspart provides superior fasting plasma glucose control and a reduced rate of hypoglycaemia compared with biphasic insulin aspart 30 in insulin-naïve adults with type 2 diabetes. Diabet Med. 2016;33:497–505.
40. Jacobson AM, Braffett BH, Cleary PA, Gubitosi-Klug RA, Larkin ME; DCCT/ EDIC Research Group. The long-term effects of type 1 diabetes treatment and complications on health-related quality of life: a 23-year follow-up of the diabetes control and complications/epidemiology of diabetes interventions and complications cohort. Diabetes Care. 2013;36:3131–3138.
41. Priya G, Kalra S. A review of insulin resistance in type 1 diabetes: is there a place for adjunctive metformin? Diabetes Ther. 2018;9:349–361
42. Vora J, Cohen N, Evans M, Hockey A, Speight J, Whately-Smith C. Intensifying insulin regimen after basal insulin optimization in adults with type 2 diabetes: a 24-week, randomized, open-label trial comparing insulin glargine plus insulin glulisine with biphasic insulin aspart (LanScape). Diabetes Obes Metab. 2015;17:1133–1141.
43. Shah RB, Patel M, Maahs DM, Shah VN. Insulin delivery methods: past, present and future. Int J Pharm Investig. 2016;6:1–9.
44. Abramson A, Caffarel-Salvador E, Khang M, Dellal D, Silverstein D, Gao Y, Frederiksen MR, Vegge A, Hubálek F, Water JJ, Friderichsen AV, Fels J, Kirk RK, Cleveland C, Collins J, Tamang S, Hayward A, Landh T, Buckley ST, Roxhed N, Rahbek U, Langer R, Traverso G.An ingestible self-orienting system for oral delivery of macromolecules. Science. 2019;363:611–615.
45. Seaquist ER, Anderson J, Childs B, Cryer P, Dagogo-Jack S, Fish L, Heller SR, Rodriguez H, Rosenzweig J, Vigersky R. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. Diabetes Care. 2013;36:1384-95.
46. Ly TT, Anderson M, McNamara KA, Davis EA, Jones TW. Neurocognitive outcomes in young adults with early-onset type 1 diabetes: a prospective follow-up study. Diabetes Care. 2011;34(10):2192-2197.
47. Cengiz E, Xing D, Wong JC, Wolfsdorf JI, Haymond MW, Rewers A, Shanmugham S, Tamborlane WV, Willi SM, Seiple DL, Miller KM, DuBose SN, Beck RW; T1D Exchange Clinic Network. Severe hypoglycemia and diabetic ketoacidosis among youth with type 1 diabetes in the T1D Exchange clinic registry. Pediatr Diabetes. 2013;14:447-54.

48. Li W, Huang E, Gao S. Type 1 Diabetes Mellitus and Cognitive Impairments: A Systematic Review. J Alzheimers Dis. 2017;57(1):29-36.
49. Karges B, Rosenbauer J, Holterhus PM, Beyer P, Seithe H, Vogel C, Böckmann A, Peters D, Müther S, Neu A, Holl RW; DPV Initiative. Hospital admission for diabetic ketoacidosis or severe hypoglycemia in 31,330 young patients with type 1 diabetes. Eur J Endocrinol. 2015;173:341-50.
50. Larsen J, Brekke M, Sandvik L, Arnesen H, Hanssen KF, Dahl-Jorgensen K. Silent coronary atheromatosis in type 1 diabetic patients and its relation to long-term glycemic control. Diabetes. 2002;51(8):2637-2641.
51. Livingstone SJ, Levin D, Looker HC, Lindsay RS, Wild SH, Joss N, Leese G, Leslie P, McCrimmon RJ, Metcalfe W, McKnight JA, Morris AD, Pearson DW, Petrie JR, Philip S, Sattar NA, Traynor JP, Colhoun HM; Scottish Diabetes Research Network epidemiology group; Scottish Renal Registry. Estimated life expectancy in a Scottish cohort with type 1 diabetes, 2008-2010. JAMA. 2015;313:37-44.
52. Nathan DM; DCCT/EDIC Research Group. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. Diabetes Care. 2014;37(1):9-16.
53. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med. 2005;353:2643-53.
54. Adler AI, Erqou S, Lima TA, Robinson AH. Association between glycated haemoglobin and the risk of lower extremity amputation in patients with diabetes mellitus-review and meta-analysis. Diabetologia. 2010;53(5):840-849.
55. Carter RE, Lackland DT, Cleary PA, Yim E, Lopes-Virella MF, Gilbert GE, Orchard TJ; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive treatment of diabetes is associated with a reduced rate of peripheral arterial calcification in the diabetes control and complications trial. Diabetes Care. 2007;30:2646-8.
56. Donaghue KC, Marcovecchio ML, Wadwa RP, Chew EY, Wong TY, Calliari LE, Zabeen B, Salem MA, Craig ME. ISPAD Clinical Practice Consensus Guidelines 2018: Microvascular and macrovascular complications in children and adolescents. Pediatr Diabetes. 2018;19 Suppl 27(Suppl 27):262-274.

57. Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. Diabetes. 1983;32(suppl 2):64–78.
58. Mogensen CE, Keane WF, Bennett PH, Jerums G, Parving HH, Passa P, Steffes MW, Striker GE, Viberti GC. Prevention of diabetic renal disease with special reference to microalbuminuria. Lancet. 1995;346(8982):1080-4.
59. Gaborit B, Julla JB, Besbes S, Proust M, Vincentelli C, Alos B, Ancel P, Alzaid F, Garcia R, Mailly P, Sabatier F, Righini M, Gascon P, Matonti F, Houssays M, Goumidi L, Vignaud L, Guillonneau X, Erginay A, Dupas B, Marie-Louise J, Autié M, Vidal-Trecan T, Riveline JP, Venteclef N, Massin P, Muller L, Dutour A, Gautier JF, Germain S. Glucagon-like Peptide 1 Receptor Agonists, Diabetic Retinopathy and Angiogenesis: The AngioSafe Type 2 Diabetes Study. J Clin Endocrinol Metab. 2020;105(4):dgz069.
60. Duh EJ, Sun JK, Stitt AW. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. JCI Insight. 2017; 2:e93751.
61. Lee R, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. Eye Vis.2015;2:17. 10.1186/s40662-015-0026-2.
62. Yuuki T, Kanda T, Kimura Y, Kotajima N, Tamura J, Kobayashi I, Kishi S. Inflammatory cytokines in vitreous fluid and serum of patients with diabetic vitreoretinopathy. J Diabetes Complications.2001; 15:257–9.
63. Rubsam A, Parikh S, Fort PE. Role of inflammation in diabetic retinopathy. Int J Mol Sci. 2018;19:942.
64. Masuda T, Shimazawa M, Hara H. Retinal diseases associated with oxidative stress and the effects of a free radical scavenger (Edaravone). Oxid Med Cell Longev. 2017;2017:9208489.
65. Otto-Buczkowska E, Kazibutowska Z, Sołtyk J, Machnica L. Neuropathy and type 1 diabetes mellitus. Pediatr Endocrinol Diabetes Metab. 2008;14(2):109-116.
66. Donaghue KC, Chiarelli F, Trotta D, Allgrove J, Dahl-Jorgensen K. Microvascular and macrovascular complications associated with diabetes in children and adolescents. Pediatr Diabetes. 2009;10:195-203.
67. Julie Rosner; Tijana Samardzic; Manbeer S. Sarao. Physiology, Female ReproductionIn: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan.2021 Oct 9.
68. Clarke IJ, Campbell R, Smith JT, Prevot V,Wray S. Neuroendocrine control of reproduction.2011; In Handbook of Neuroendocrinology, pp 197–236.
69. Diaz A, Laufer MR, Breech LL. Menstruation in girls and adolescents: using the menstrual cycle as a vital sign. Pediatrics. 2006;118:2245–2250

70. Fritz MA, Speroff L. Clinical gynecologic endocrinology and infertility, 8th edn. South Holland, Netherlands: Wolters Kluwer; 2011
71. Harlow SD, Ephross SA. Epidemiology of menstruation and its relevance to women's health. Epidemiol Rev. 1995;17:265–286;
72. Mumford SL, Kim K. Subtle changes in menstrual cycle function-Pieces of the puzzle. Paediatr Perinat Epidemiol. 2018;32:235–236.
73. Farage MA, Neill S, MacLean AB. Physiological changes associated with the menstrual cycle: a review. Obstet. Gynecol. Surv. 2009 ;64:58–72.
74. Girdler SS, Light KC. Reproductive hormones and stages of life in women: moderators of mood and cardiovascular health. In: Steptoe A, editor. Handbook of Behavioral Medicine. Paris, France: Springer; 2010. pp. 585–602
75. Manfredi-Lozano M, Roa J, Tena-Sempere M. Connecting metabolism and gonadal function: Novel central neuropeptide pathways involved in the metabolic control of puberty and fertility. Front Neuroendocrinol. 2018;48:37-49.
76. Sliwowska JH, Fergani C, Gawałek M, Skowronska B, Fichna P, Lehman MN. Insulin: its role in the central control of reproduction. Physiol Behav. 2014;133:197-206.
77. Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH. The GPR54 gene as a regulator of puberty. N Engl J Med. 2003; 349 :1614–1627.
78. Dupont J, Scaramuzzi RJ. Insulin signalling and glucose transport in the ovary and ovarian function during the ovarian cycle. Biochem J. 2016; 473:1483–1501.
79. Schweiger BM, Snell-Bergeon JK, Roman R, McFann K, Klingensmith GJ. Menarche delay and menstrual irregularities persist in adolescents with type 1 diabetes. Reprod Biol Endocrinol. 2011;9:61.
80. Gaete X, Vivanco M, Eyzaguirre FC, López P, Rhumie HK, Unanue N, Codner E. Menstrual cycle irregularities and their relationship with HbA1c and insulin dose in adolescents with type 1 diabetes mellitus. Fertil Steril. 2010;94:1822-6.
81. Codner E, Cassorla F. Puberty and ovarian function in girls with type 1 diabetes mellitus. Horm Res 2009; 71:12.
82. Bergqvist N. The gonadal function in female diabetics. Acta Endocrinol Suppl (Copenh).1954; 19: 1–20.
83. Joslin E, Root H, White P. The growth, development and prognosis of diabetic children. JAMA 1925; 85: 420–22

84. Arrais RF, Dib SA. The hypothalamus–pituitary–ovary axis and type 1 diabetes mellitus: a mini review. Hum Reprod.2006; 21: 327–37.
85. Shindel AW, Lue TF. Sexual Dysfunction in Diabetes. In: Feingold KR, Anawalt B, Boyce A, et al., eds. Endotext. South Dartmouth (MA): MDText.com, Inc.; June 8, 2021
86. Enzlin P, Rosen R, Wiegel M, Brown J, Wessells H, Gatcomb P, Rutledge B, Chan KL, Cleary PA; DCCT/EDIC Research Group. Sexual dysfunction in women with type 1 diabetes: long-term findings from the DCCT/ EDIC study cohort. Diabetes Care. 2009;32:780-5.
87. Esposito K, Maiorino MI, Bellastella G, Giugliano F, Romano M, Giugliano D. Determinants of female sexual dysfunction in type 2 diabetes. Int J Impot Res. 2010;22:179–184.
88. Pontiroli AE, Cortelazzi D, Morabito A. Female sexual dysfunction and diabetes: a systematic review and meta-analysis. J Sex Med. 2013;10:1044-1051.
89. Zamponi V, Mazzilli R, Bitterman O, Olana S, Iorio C, Festa C, Giuliani C, Mazzilli F, Napoli A. Association between type 1 diabetes and female sexual dysfunction. BMC Womens Health. 2020;20:73.
90. Rahmanian E, Salari N, Mohammadi M, Jalali R. Evaluation of sexual dysfunction and female sexual dysfunction indicators in women with type 2 diabetes: a systematic review and meta-analysis. Diabetol Metab Syndr. 2019;11:73.
91. Meeking, D.R.; Fosbury, J.A.; Cummings, M.H. Sexual dysfunction and sexual health concerns in women with diabetes. Pract. Diabetes.2013;30:327–331.
92. Eyada M, Atwa M. Sexual function in female patients with unstable angina or non-ST-elevation myocardial infarction. J Sex Med. 2007;4:1373–1380
93. Doumas M, Tsiodras S, Tsakiris A, et al. Female sexual dysfunction in essential hypertension: a common problem being uncovered. J Hypertens. 2006;24:2387–2392
94. Lewis RW, Fugl-Meyer KS, Corona G, Hayes RD, Laumann EO, Moreira ED Jr, Rellini AH, Segraves T. Definitions/epidemiology/risk factors for sexual dysfunction. J Sex Med. 2010;7:1598-607.
95. Dennerstein L, Randolph J, Taffe J, Dudley E, Burger H. Hormones, mood, sexuality, and the menopausal transition. Fertil Steril. 2002;77:S42–S48
96. Krychman M, Millheiser LS. Sexual health issues in women with cancer. J Sex Med. 2013;10:5–15
97. Maiorino MI, Bellastella G, Esposito K. Diabetes and sexual dysfunction: current perspectives. Diabetes Metab Syndr Obes. 2014;7:95-105.

98. Berman JR, Berman LA, Toler SM, Gill J, Haughie S; Sildenafil Study Group. Safety and efficacy of sildenafil citrate for the treatment of female sexual arousal disorder: a double-blind, placebo controlled study. J Urol. 2003;170:2333–2338.
99. Min K, Munarriz R, Kim NN, Goldstein I, Traish A. Effects of ovariectomy and estrogen and androgen treatment on sildenafilmediated changes in female genital blood flow and vaginal lubrication in the animal model. Am J Obstet Gynecol. 2002;187:1370–1376
100. Bargiota A, Dimitropoulos K, Tzortzis V, Koukoulis GN. Sexual dysfunction in diabetic women. Hormones (Athens). 2011;10: 196–206.
101. Meeking DR, Fosbury JA, Cummings MH. Sexual dysfunction and sexual health concerns in women with diabetes. Practical Diabetes. 2013;30:327–331.
102. Feldhaus-Dahir M. The causes and prevalence of hypoactive sexual desire disorder: part I. Urol Nurs. 2009;29:259–260, 263.
103. Bhasin S, Enzlin P, Coviello A, Basson R. Sexual dysfunction in men and women with endocrine disorders. Lancet. 2007;369: 597–611.
104. Rockliffe-Fidler C, Kiemle G. Sexual function in diabetic women: a psy- chological perspective. Sex Relation Ther. 2003;18:143–159.
105. Ogbera AO, Chinenye S, Akinlade A, Eregie A, Awobusuyi J. Frequency and correlates of sexual dysfunction in women with diabetes mellitus. J Sex Med. 2009;6:3401–3406.
106. Rosen R, Brown C, Heiman J, Leiblum S, Meston C, Shabsigh R, Ferguson D, D'Agostino R Jr. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther. 2000;26:191-208.
107. Derogatis LR, Rosen R, Leiblum S, Burnett A, Heiman J. The Female Sexual Distress Scale (FSDS): initial validation of a standardized scale for assessment of sexually related personal distress in women. J Sex Marital Ther. 2002;28:317-30)
108. Meston CM, Derogatis LR. Validated instruments for assessing female sexual function. J Sex Marital Ther. 2002;28 Suppl 1:155-164.
109. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275:964-7.
110. Hristov M, Erl W, Weber PC. Endothelial progenitor cells: mobilization, differentiation, and homing. Arterioscler Thromb Vasc Biol. 2003;23:1185-9.
111. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA, Rafii S. Expression of VEGFR-2 and AC133 by circulating human CD34 + cells identifies a population of functional endothelial precursors. Blood. 2000;95:952–958,

112. Murohara T. Therapeutic vasculogenesis using human cord blood-derived endothelial progenitors. Trends Cardiovasc Med. 2001;11:303–307.
113. Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, Deliliers GL. Differentiation and expansion of endothelial cells from human bone marrow CD133+ cells. Br J Haematol. 2001;115:186–194.
114. Fadini GP, Sartore S, Schiavon M, Albiero M, Baesso I, Cabrelle A, Agostini C, Avogaro A. Diabetes impairs progenitor cell mobilisation after hindlimb ischaemia-reperfusion injury in rats. Diabetologia. 2006 ;49:3075-84.
115. Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation. 2002;106:2781-6.
116. Fadini GP, Miorin M, Facco M, Bonamico S, Baesso I, Grego F, Menegolo M, de Kreutzenberg SV, Tiengo A, Agostini C, Avogaro A. Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. J Am Coll Cardiol. 2005;45:1449-57.
117. Fadini GP, Sartore S, Agostini C, Avogaro A. Significance of endothelial progenitor cells in subjects with diabetes. Diabetes Care. 2007;30(5):1305-1313.
118. Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. Diabetes. 2004;53:195-9.
119. Longo M, Scappaticcio L, Bellastella G, Pernice V, Cirillo P, Maio A, Castaldo F, Giugliano D, Esposito K, Maiorino MI; METRO Study Group. Alterations in the Levels of Circulating and Endothelial Progenitor Cells Levels in Young Adults with Type 1 Diabetes: A 2-Year Follow-Up from the Observational METRO Study. Diabetes Metab Syndr Obes. 2020;13:777-784
120. Werner N, Nickenig G. Influence of car- diovascular risk factors on endothelial progenitor cells: limitations for therapy? Arterioscler Thromb Vasc Biol.2006;26: 257–266
121. Besler C, Doerries C, Giannotti G, Lüscher TF, Landmesser U. Pharmacological approaches to improve endothelial repair mechanisms. Expert Rev Cardiovasc Ther. 2008;6:1071–1082
122. De Falco E, Avitabile D, Totta P, Straino S, Spallotta F, Cencioni C, Torella AR, Rizzi R, Porcelli D, Zacheo A, Di Vito L, Pompilio G, Napolitano M, Melillo G, Capogrossi MC,

Pesce M. Altered SDF-1-mediated differentiation of bone marrow-derived endothelial progenitor cells in diabetes mellitus. J Cell Mol Med. 2009;13:3405-14.

123. Zaruba MM, Theiss HD, Vallaster M, Mehl U, Brunner S, David R, Fischer R, Krieg L, Hirsch E, Huber B, Nathan P, Israel L, Imhof A, Herbach N, Assmann G, Wanke R, Mueller-Hoecker J, Steinbeck G, Franz WM. Synergy between CD26/DPP-IV inhibition and G-CSF improves cardiac function after acute myocardial infarction. Cell Stem Cell. 2009;4:313-23
124. Mayer-Davis EJ, Lawrence JM, Dabelea D, et al. Incidence trends of type 1 and type 2 diabetes among youths, 2002–2012. N Engl J Med 2017; 376: 1419–29.
125. Lind M, Svensson AM, Kosiborod M, et al. Glycemic control and excess mortality in type 1 diabetes. N Engl J Med 2014; 371: 1972–82.
126. Rahman S, Rahman T, Ismail AA, Rashid AR. Diabetes-associated macrovasculopathy: pathophysiology and pathogenesis. Diabetes Obes Metab. 2007;9(6):767–780.
127. Vergès B. Cardiovascular disease in type 1 diabetes: a review of epidemiological data and underlying mechanisms. *Diabetes Metab.* 2020;46(6):442–9,
128. Rawshani A, Rawshani A, Franzén S, Eliasson B, Svensson AM, Miftaraj M, et al. Mortality and cardiovascular disease in type 1 and type 2 diabetes. *N Engl J Med*. 2017;376:1407–18.
129. Koivisto VA, Stevens LK, Mattock M, et al. Cardiovascular disease and its risk factors in IDDM in Europe. EURODIAB IDDM complications study group. *Diabetes Care* 1996; 19: 689–97.
130. Anand SS, Islam S, Rosengren A, et al. Risk factors for myocardial infarction in women and men: insights from the INTERHEART study. *Eur Heart J*. 2008;29:932–940.
131. Maiorino MI, Bellastella G, Giugliano D, Esposito K. From inflammation to sexual dysfunctions: a journey through diabetes, obesity, and metabolic syndrome. J Endocrinol Invest 2018;41:1249–1258.
132. Robb AO, Mills NL, Smith IB, Short A, Tura-Ceide O, Barclay GR, Blomberg A, Critchley HO, Newby DE, Denison FC. Influence of menstrual cycle on circulating endothelial progenitor cells. Hum Reprod. 2009;24:619-25.
133. Foresta C, De Toni L, Di Mambro A, Ferlin A, Perilli L, Bertuzzi I, Galan A, Zuccarello D. Role of estrogen receptors in menstrual cycle-related neoangiogenesis and their influence on endothelial progenitor cell physiology. Fertil Steril. 2010;93:220-8.
134. McCabe MP, Sharlip ID, Atalla E, Balon R, Fisher AD, Laumann E, Lee SW, Lewis R, Segraves RT. Definitions of Sexual Dysfunctions in Women and Men: A Consensus

Statement From the Fourth International Consultation on Sexual Medicine 2015. J Sex Med. 2016;13:135-43.

135. Longo M, Cirillo P, Scappaticcio L, Tomasuolo M, Pernice V, Caruso P, Bellastella G, Maiorino MI, Esposito K. Female Sexual Function in Young Women With Type 1 Diabetes and Additional Autoimmune Diseases. *J Sex Med*. 2021;18:219-223.)
136. Fadini GP, de Kreutzenberg S, Albiero M, et al. Gender differences in endothelial progenitor cells and cardiovascular risk profile: the role of female estrogens. *Arterioscler Thromb Vasc Biol*. 2008;28:997-1004.
137. Lemieux C, Cloutier I, Tanguay JF. Menstrual cycle influences endothelial progenitor cell regulation: a link to gender differences in vascular protection?. *Int J Cardiol*. 2009;136:200-210.
138. Dantas AP, Fortes ZB, de Carvalho MH. Vascular disease in diabetic women: Why do they miss the female protection?. Exp Diabetes Res. 2012;2012:570598.
139. Maiorino MI, Bellastella G, Casciano O, Petrizzo M, Gicchino M, Caputo M, Sarnataro A, Giugliano D, Esposito K. Gender-differences in glycemic control and diabetes related factors in young adults with type 1 diabetes: results from the METRO study. Endocrine. 2018;61:240-247.
140. Enzlin P, Mathieu C, Vanderschueren D, Demyttenaere K: Diabetes mellitus and female sexuality: a review of 25 years' research. Diabet Med.1998;15:809–815.
141. Enzlin P, Mathieu C, Van den Bruel A, Bosteels J, Vanderschueren D, Demyttenaere K. Sexual dysfunction in women with type 1 diabetes: a controlled study. Diabetes Care. 2002;25:672-677.