Age at Natural Menopause and Its Determinants in Women with Type 1 Diabetes

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Submitted to the Graduate Faculty of the

Department of Epidemiology

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2020

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

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October 21, 2020

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University of Pittsburgh, 2020

Abstract

Earlier age at menopause is associated with increased risks of cardiovascular disease, osteoporosis, fracture, and mortality. Existing findings regarding whether women with type 1 diabetes experience earlier menopause than women without diabetes are conflicting. Moreover, data on determinants of an earlier age at menopause in women with type 1 diabetes are scarce and to date, no study has assessed the impact of the timing of developing diabetes complications on age at menopause in these women. This dissertation aimed to fill these research gaps by utilizing data from two well-characterized cohorts.

In paper 1, we demonstrated that women with type 1 diabetes have a shorter reproductive period compared with non-diabetic women, exhibiting delayed menarche and earlier natural menopause. These findings were restricted to women who were diagnosed with type 1 diabetes before reaching menarche. In paper 2, we observed that higher average levels of insulin dose and albumin excretion rate over time were significantly associated with an earlier age at natural menopause in type 1 diabetes after multivariable adjustments, including for HbA1c. In paper 3, we found that menopause appears to occur 2.06 years earlier in women with microalbuminuria diagnosed before age 30 compared with women without microalbuminuria. A similar pattern was observed for overt nephropathy and coronary artery disease, although results did not reach statistical significance.

Given the enormous impact on health associated with early menopause, our comprehensive work has significant public health and clinical implications as it 1) identified the subgroup of

women with type 1 diabetes who have a high likelihood of experiencing early age at natural menopause so that efforts to unearth the biologic rationale and target potential prevention practices would be better focused, 2) raised significant questions relating to a potentially deleterious effect of high exogenous insulin doses, in addition to that of kidney disease, which may constitute a useful source in clinical reproductive counseling for women with type 1 diabetes, and 3) suggested that premature ovarian aging may be one phenotype of diabetes vascular complications which increased awareness of diabetes complications and further emphasized the importance of preventing vascular damage in type 1 diabetes.

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Preface

I would like to give my appreciation and gratitude to wonderful people who helped me throughout my PhD studies. Without their support, comments, suggestions, and criticisms, I would not be able to accomplish my dissertation research and complete my PhD program.

To Dr. Tina Costacou, my dissertation director, for her kind help and responsible guidance for my entire study during my PhD program. Without her, this research project would not be possible. She listened to me and gave me valuable advice. She made me feel like I had a friend who cares for me so much.

To my dissertation committees, for giving me invaluable advice, comments, and ideas to my research project, which always help me find solution to my dilemmas.

To my dear parents, for supporting my study in US and giving me such selfless and unconditional love.

And lastly, to those who are not mentioned above but give me help and support, especially my friends and classmates.

1.0 INTRODUCTION

1.1 TYPE 1 DIABETES

1.1.1 Incidence and Prevalence

Type 1 diabetes is a disorder, which results from the autoimmune destruction of pancreatic β-cells and the subsequent insulin deficiency [1,2]. Although traditionally type 1 diabetes was considered as childhood/juvenile onset, the disorder can occur at any age, with two peaks at age of 5 years and at puberty and a rapid decline thereafter [3-6]. Actually, half of the type 1 diabetes cases occur in adulthood [7] and half of the adulthood-onset type 1 diabetes cases may be initially misdiagnosed as type 2 diabetes [8,9]. Childhood-onset type 1 diabetes usually presents with overt hyperglycemia, a classic set of symptoms (i.e., polydipsia, polyphagia, polyuria) or even diabetic ketoacidosis, whereas the adulthood-onset cases might not present with these classic symptoms [10].

1.1.1.1 Incidence at a global level

At a global level, the incidence of type 1 diabetes varies considerably over geographic regions owing to both genetic and environmental influences [9,11]. Generally, northwestern Europe has the highest incidence of type 1 diabetes, with >60 cases per 100,000 people per year for children aged 0–14 years in Finland [4,12]. Sardinia, a large Italian island in the Mediterranean Sea, has the second highest incidence, with >40 cases per 100,000 people per year [13,14]. In contrast, Venezuela, Pakistan and China have the lowest incidence (<0.1 cases per 100,000 people per year) [13]. Although a variation of over 600-fold is shown in the incidence of type 1 diabetes globally

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(from 60 in Finland to 0.1 in Venezuela, per 100,000 people), the incidence of type 1 diabetes has been rising all over the world for several decades, with a global average annual increase of 2.8% based on the report from the DIAMOND project group [13] and around 3% based on the Diabetes Atlas report from the International Diabetes Federation [15].

1.1.1.2 Incidence in Europe

In Europe, the EURODIAB study which was designed to assess trends in the incidence of type 1 diabetes, reported that the average annual increase in incidence in Europe was 3.9% (95% CI 3.6-4.2), with ranging from 0.6% in Spain to 9.3% in Poland [16]. In addition, the increases in incidence were not consistent across age groups, with the highest (5.8%) increase in the 0-4 year age group, a 4.3% increase in the 5-9 year age groups, and a 2.9% increase in the 10-14 year age group[16]. These EURODIAB findings indicated that the incidence trends have a left shift to the youngest children. If these trends continue, the prevalent cases of type 1 diabetes under age 15 is predicted to increase by 70% and rise from 94,000 cases in 2005 to 160,000 cases in Europe by 2020 [16].

1.1.1.3 Incidence in the United States

In the United States, the SEARCH study, a multicenter observational study was initiated in 2002 with the aim to identify the incidence and prevalence of diabetes among youth (<20 years old) [5]. Findings from this study also suggest an increase in the incidence of type 1 diabetes from 19.5 cases in 2002-2003 to 21.7 cases per 100,000 youth per year in 2011-2012, with an average annual increase of 1.8% after adjusting for age, sex and race [5]. The age group of 10-14 years persistently had the highest incidence compared with other age groups over time [5]. In addition, type 1 diabetes was reported to be more common in boys [5] and this gender difference in incidence

was also observed in Sweden [5,6,17]. Although the incidence of type 1 diabetes is highest in non-Hispanic whites, the annual rates of increase in incidence were reported to be much greater in other ethnic groups, being 4.2% in Hispanic, 3.7% in Asian or Pacific Islander, 2.2% in Black and only 1.2% in non-Hispanic whites [5]. These changes in incidence trends in the 2002-2012 period will result in a substantial increase in the number of youths with type 1 diabetes in minority race/ethnicity in the following decades [5,18].

1.1.1.4 Prevalence

The SEARCH study has also shown a prevalence of 2.12/1,000 in 2010 for type 1 diabetes, a number expected to nearly triple to 5.2/1,000 by 2050, among individuals under 20 years of age in the United States [18]. Therefore, type 1 diabetes will continue to be one of the most common chronic diseases in children in the following decades [19-21]. According to data from the National Health and Nutrition Examination Survey (NHANES) from 1999-2010, the overall prevalence of type 1 diabetes across all age groups in the United States, is estimated to range between 2.6 and 3.4 per 1,000 people [22]. Although type 1 diabetes onset usually occurs in childhood, most prevalent cases (84%) in the United States are adults [23].

1.1.2 Pathophysiology and Natural History

1.1.2.1 The immunopathogenesis of type 1 diabetes

Type 1 diabetes (previously called insulin-dependent or juvenile-onset diabetes) is caused by the auto-immune destruction of the pancreatic insulin-producing β-cells and is characterized by endogenous insulin deficiency and exogenous insulin administration dependency for survival. The pathogenesis of type 1 diabetes is driven by the joint effects of the innate and adaptive immune

systems (Figure 1.1). Antigen-presenting cells (APCs) initiate this immunopathogenesis process by presenting pancreatic β-cell autoantigens to CD4+ T lymphocytes in the pancreatic lymph nodes, thus leading to the activation of CD8+ T lymphocytes [9]. These activated CD8+ T lymphocytes migrate to islets, bind to β-cells, which are expressing immunogenic autoantigens on their major histocompatibility complex (MHC) class I surface molecules, and then lyse β-cells. The destruction of β-cells is also exacerbated by innate immune cells (e.g., natural killer cells) through releasing cytokines and chemokines [9]. The above islet inflammatory infiltration process, along with the loss of insulin-producing β-cells function and mass, is called insulitis, and is considered as a hallmark of type 1 diabetes pathogenesis [24]. Meanwhile, the B-lymphocytes located in the pancreatic lymph nodes begin to produce and release autoantibodies against specific β-cell proteins (e.g., insulin, zinc transporter 8, glutamic acid decarboxylase, and islet antigen 2), under the stimulation of activated CD4+ T lymphocytes [25]. These autoantibodies are detectable and measurable in peripheral blood and are thus used as biomarkers aiding in the differentiation between type 1 and type 2 diabetes.
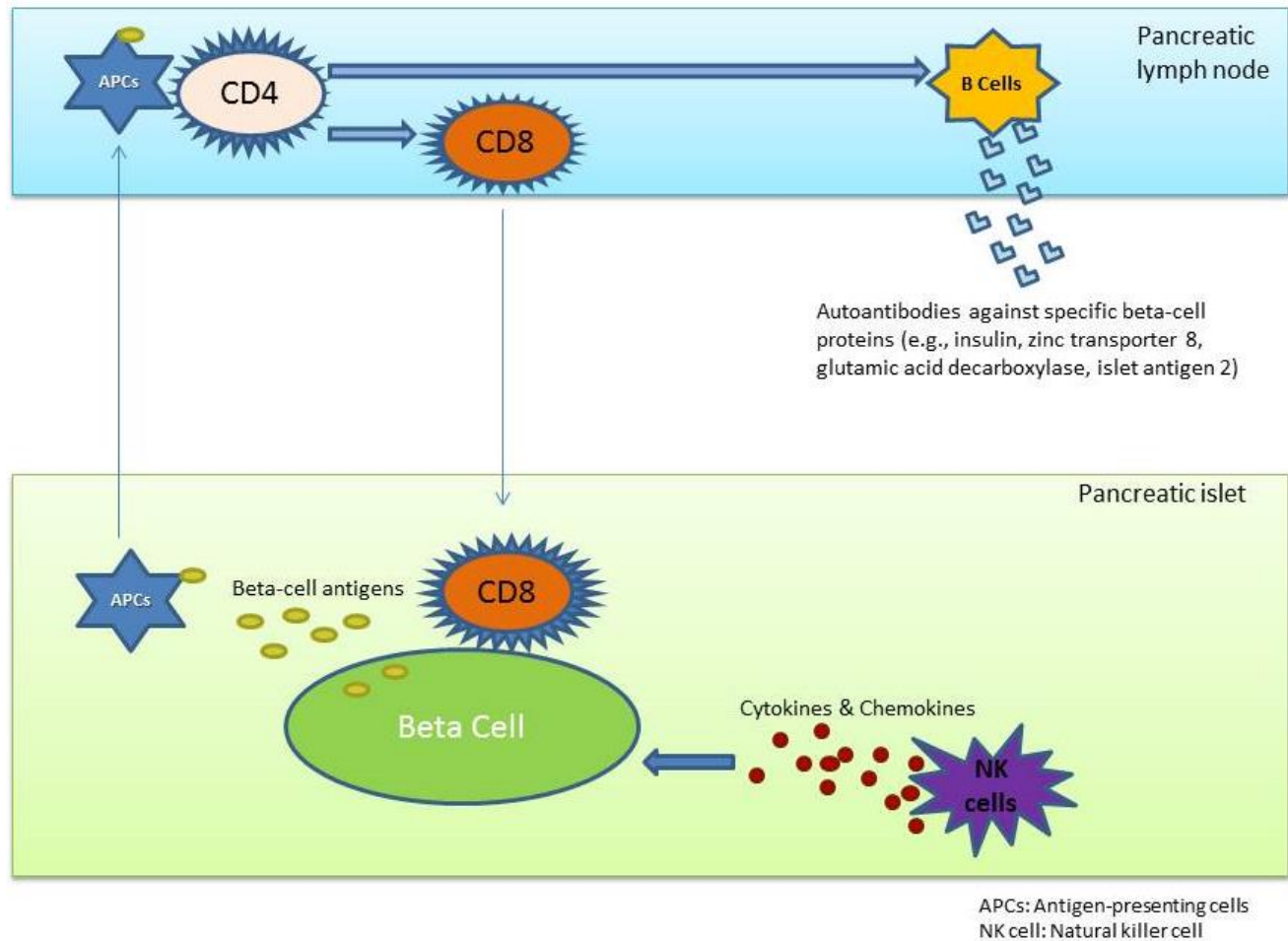
Figure 1.1 The Immunopathogenesis of Type 1 Diabetes

1.1.2.2 Autoantibodies and natural history of type 1 diabetes

As many as 90% of newly diagnosed type 1 diabetes cases have detectable β-cell autoantibodies in blood [25]. However, not all children with autoantibodies will progress to type 1 diabetes [26]. Approximately only 10% of children with a single autoantibody will progress, whereas over 85% of children with two or more autoantibodies will progress to this disorder by the age of 18 years [9,27]. Due to the high risk of developing type 1 diabetes in children with multiple autoantibodies, the presence of two or more autoantibodies with normal blood glucose level is defined as stage 1 in the natural history of type 1 diabetes (preclinical stage) [28,29]. The subsequent

stage, 2, is defined as dysglycemia, and stage 3 is defined as a clinical diagnosis of type 1 diabetes [28,29]. The last stage is long-standing type 1 diabetes [29]. The more β-cell autoantibody types present, the more rapid the progression from the preclinical stage to the overt type 1 diabetes stage [30].

1.1.2.3 Self-destruction of β-cells

In addition to being targeted and destroyed by the autoimmune responses, the self-destruction of β-cells (β-cell suicide) may also contribute to insulin deficiency and the occurrence of type 1 diabetes [9,31]. One possible mechanism behind the β-cell suicide hypothesis is the hyperexpression of the HLA class 1, which attracts more cytotoxic T cells to interact with β-cells and then accelerate the loss of β-cells in the pancreas [32]. Another potential mechanism is the elevated endoplasmic reticulum stress of β-cells, leading to errors in mRNA splicing and protein translation and folding [33,34]. These abnormal protein products will be considered as immunogenic antigens and exacerbate autoimmune responses in islets [35]. Moreover, evidence also indicates that the abnormalities in vascularity and the extracellular matrix in people with type 1 diabetes are associated with partial islet destruction as well [36,37].

1.1.2.4 Partial recovery of β-cells function after receiving diabetes management

After receiving diabetes management and along with amelioration of hyperglycemia, some β-cells are able to recover from autoimmune attacks and partially restore their insulin-producing function; this is called the honeymoon period [9]. However, the persistence of the autoimmune response eventually leads to a decline in the residual β-cells over time. Interestingly, the pancreatic β-cells are not completely destroyed even in patients with long-term type 1 diabetes [38-43]. This finding led to research projects focusing on β-cell immune evasion and β-cell regeneration, as the Diabetes Control and Complications Trial (DCCT) has indicated that residual β-cell activity was

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associated with reduced risk of retinopathy and nephropathy and was also very important in avoiding hypoglycemia [44,45].

1.1.3 Genetic Risk Factors

Genetic susceptibility plays a key role in both initiating β-cell autoimmunity (e.g., the presence of autoantibodies) and in the progression from the preclinical stage to overt type 1 diabetes. The cumulative risk of type 1 diabetes was up to 65% among monozygotic twins who were initially discordant for type 1 diabetes [46]. Moreover, the risk of type 1 diabetes varies slightly among individuals whose siblings (8%), father (5%) or (3%) mother have type 1 diabetes [30]. Two HLA class 2 haplotypes, HLA-DR3-DQ2, and HLA-DR4-DQ8 are well-known to increase the risk of type 1 diabetes, as they are both related to the presence of β-cell autoantibodies. Children with HLA-DR3-DQ2 are more likely to have glutamic acid decarboxylase autoantibodies, whereas children with HLA-DR4-DQ8 are more likely to have insulin autoantibodies present first [47-49]. Over 50% of disease heritability can be attributed to these two haplotypes [50]. These two haplotypes are also greatly prevalent among children with type 1 diabetes in Scandinavia (one or both, approximately 90%) [30,51,52]. In addition, PTPN22 gene, a SNP in the lymphoid tyrosine phosphatase (LYP) on chromosome 1p13, correlates strongly with the incidence of type 1 diabetes [53]. In contrast, there are also some haplotypes, which are associated with reduced risk of type 1 diabetes, including HLA-DR15-DQ6 [50]. The protective mechanisms of these haplotypes remain unclear.

1.1.4 Environmental Risk Factors

Although genetic predisposition is considered as the primary risk factor, environmental triggers are essential for both the initiation of islet autoimmunity and the progression from autoimmunity to overt type 1 diabetes. Such environmental triggers include viral infections, the microbiome, vaccines, and dietary factors. One noteworthy finding is that the incidence of type 1 diabetes differs by six-fold among people who have similar genetic susceptibility but live in different social environments, an observation that highlights the substantial effects of environmental and lifestyle factors in the development of this disorder [54]. Additional support for a role of environmental triggers comes from studies indicating that migrants tend to have the same risk of type 1 diabetes as natives of their new place of residence [55,56]. Moreover, the rapid and global increase in the incidence of type 1 diabetes cannot solely be explained by genetic predisposition changes over decades.

1.1.4.1 Viral infection

Many studies have been conducted to investigate the role of viral infections in the pathogenesis of type 1 diabetes [57-59], whereas bacterial infections have rarely been explored so far. The enteroviruses are the most commonly discussed since evidence has shown that they are associated with type 1 diabetes in both animal models [60] and human studies [61]. Not only have enteroviruses been detected in the islets of individuals who were recently diagnosed with type 1 diabetes [59,62], but also the enteroviral capsid protein VP1 was shown to be more prevalent in the pancreatic β-cells of children with type 1 diabetes compared with age-matched non-diabetic controls [63,64]. Moreover, some studies have indicated that maternal enteroviral infections during pregnancy are associated with increased risk of islet autoimmunity in both mothers and their

offspring [65-67].   In addition, rotavirus infection, the most common cause of childhood gastroenteritis, may trigger or exacerbate islet autoimmunity in genetically susceptible children as it contains peptide sequences highly similar to T-cell epitopes in the islet autoantigens glutamic acid decarboxylase (GAD) and tyrosine phosphatase IA-2 (IA-2), suggesting T-cells to rotavirus could trigger islet autoimmunity by molecular mimicry [68]. A recent study showed a lower incidence rate of type 1 diabetes in the US after rotavirus vaccination which further confirmed the role of rotavirus in the etiology of type 1 diabetes [69]. Microbiome changes may also be one of the possible triggers of type 1 diabetes as a marked drop in microbial diversity was observed during progression from autoimmunity to overt type 1 diabetes [70,71]. In contrast, a meta-analysis of 23 studies has shown that routine vaccinations were not associated with childhood-onset type 1 diabetes [72].

1.1.4.2 Dietary

Dietary (e.g., breastfeeding, infant formula, cereals, vitamin D) factors have attracted great interest in recent decades as perhaps more readily modifiable factors, compared to genetic predisposition and the microbiome, contributing to the pathogenesis of type 1 diabetes. Nevertheless, the role of both breastfeeding and infant formula in type 1 diabetes development remain unclear. In the early 80s, the hypothesis that an insufficient length of breastfeeding in genetically susceptible infants may lead to type 1 diabetes later in life was formulated based on data from case-control studies reporting a shorter duration of breastfeeding among children with type 1 diabetes compared with both their healthy siblings and the general population [73]. In 2003, the German BABYDIAB study reported that breastfeeding duration during the first year of life did not significantly influence the risk of autoantibody development by age 5 among children of parents with type 1 diabetes [74]. More recent data from a prospective population-based study (the

ABIS study) in Sweden in 2007 indicated that short duration breastfeeding (less than 4 months) is associated with increased risk for β-cell autoantibodies at five years of age in children from the general population [75]. The different findings in BABYDIAB and ABIS studies may be due to differences in the study population and the exposure definitions. The BABYDIAB study was among children of parents with type 1 diabetes and the exposures were breastfeeding ≤ 3 months and ≥ 6 months (compared with the referent 3-6 month respectively), whereas the ABIS study was among children from the general population and the exposure was breastfeeding < 4 months (compared with ≥ 4months and up to 5 years).

The possible protective effect of longer duration of breastfeeding against the development of type 1 diabetes may be attributed to the benefits of breast milk itself as it contains cytokines, growth factors, and many other immunomodulatory factors which are beneficial for the maturation of intestinal mucosal and the normal function of the gut immune system [76]. It may also be explained by the delayed introduction of cow's milk in infants with longer duration of breastfeeding, as studies have suggested that exposure to bovine insulin in cow's milk may modify the immunization to insulin in infants at genetic risk for type 1 diabetes [77,78]. In the 1990s, many studies suggested that cow's milk may trigger the development of type 1 diabetes [79-81], giving rise to the hypothesis that casein hydrolysate formula, which is free of intact bovine insulin, may reduce type 1 diabetes risk compared to conventional cow's milk. In the past decade, however, conflicting findings regarding the effects of casein hydrolysate formula vs. conventional cow's milk formula among infants at risk for type 1 diabetes were reported. Thus, although the pilot study of a trial, based on a mere 230 participants from Finland, suggested that casein hydrolysate formula may protect against autoimmunity compared with cow's-milk–based formula [82], a protective effect was not observed in the larger phase 3 trial [83]. The phase 3 trial included a larger and more heterogeneous

study population (comprising 2,159 participants from Finland, Canada, and the United States), and therefore had greater statistical power and external validity.

The timing of exposure to solid foods has also been implicated in type 1 diabetes development. The DAISY study, which enrolled 1,183 children with increased genetic risk of type 1 diabetes and prospectively followed them from birth, found that introducing gluten-free or gluten-containing cereals to infants' daily diet too early (prior to age 3 months) or too late (after age 7 months) increased the risk of islet autoimmunity [84]. The BABYDIAB study similarly reported that islet autoantibody risk was significantly increased in children who received food supplementation with gluten-containing foods before age 3 months [74]. Exposure timing to other solid foods was reported in a later paper from the DAISY study and suggested that both early and late exposure to solid foods, especially early exposure to fruit and late exposure to rice/oat, also increases islet autoimmunity risk in genetic susceptible children [85]. Interestingly, practicing breastfeeding while introducing solid foods reduced risk in DAISY [85]. In addition, the Finnish DIPP study found that first exposure to eggs before age 8 months was associated with an increased risk of islet autoimmunity, but only during the first 3 years of life, whereas the early introduction of root vegetables was related to an increased risk during the whole follow-up period among children genetically susceptible to Type 1 diabetes [86].

1.1.4.3 Vitamin D

Interestingly, in a large case-control study nested within a cohort of 35,940 women who gave birth in Norway, higher serum vitamin D levels during late pregnancy were associated with reduced odds of type 1 diabetes in the offspring [87], whereas this association was not observed in a study from Finland, in which vitamin D was measured in early pregnancy among women with vitamin D deficiency [88]. A recent birth-cohort study (DIPP) from Finland of infants at genetic type

1 diabetes risk concurred that maternal vitamin D intake, including from food or supplements during pregnancy, was not related to reduced risk of islet autoimmunity or type 1 diabetes [89]. However, the findings about the effect of vitamin D supplementation in childhood on the development of type 1 diabetes are conflicting. A meta-analysis reviewed four case-control studies and found that vitamin D supplementation in early childhood may offer protection against the development of type 1 diabetes in later life [90], whereas the DAISY study suggested that later childhood exposure to vitamin D did not reduce the risk of islet autoimmunity and type 1 diabetes among genetically susceptible children [91]. These conflicting findings may be due to differences in the populations studied (children at high genetic susceptibility or not), differences in the timing of exposure (Vitamin D supplementation in early or later childhood) or recall bias in case-control studies.

In summary, despite continuing research interests in dietary factors, there is no consistent and firm evidence to allow conclusions on their effects in the development of type 1 diabetes. Additional research studies are required to understand the role of genetic and environmental factors and their interactions in the type 1 diabetes pathogenesis.

1.1.5 Complications and Mortality

1.1.5.1 Macrovascular complications

Individuals with type 1 diabetes have an increased risk of numerous macrovascular and microvascular complications. A study based on 7,400 patients with 15 years duration of type 1 diabetes from the large UK General Practice Research Database (GPRD), found that the incidence of acute coronary heart disease (CHD) was 3.5/1,000 person years in men and 2.9/1,000 person years in women [92]. Our Pittsburgh Epidemiology of Diabetes Complications (EDC) study has

indicated that the incidence of major coronary artery disease was 0.98% per year among people having a type 1 diabetes duration of 20-30 years [93]. As discussed in a scientific statement from the American Heart Association and American Diabetes Association [94], the age-adjusted risk of cardiovascular complications is approximately ten-fold higher in people with type 1 diabetes compared with the general population [95-97], and women are disproportionately affected compared with men [94,98]. In addition, cardiovascular events occur earlier in people with type 1 diabetes than in the general population [94]. Cardiovascular disease-related mortality was shown to be increased 30-fold in people with type 1 diabetes compared with the general population in Allegheny County, PA [99], thus making it the leading cause of death in people with type 1 diabetes [100].

1.1.5.2 Microvascular complications

The Pittsburgh Epidemiology of Diabetes Complications (EDC) study has provided evidence for an increased burden also of microvascular complications among people with type 1 diabetes. Investigators from EDC showed that the cumulative incidence of proliferative retinopathy (PR), confirmed distal symmetric polyneuropathy (CDSP), and overt nephropathy (ON) were over 50%, 40%, and 30%, respectively, by 25 years of type 1 diabetes duration [93]. Much attention has also been paid to renal complications, as in addition to cardiovascular disease, renal disease is a main driver of the excess risk of mortality in type 1 diabetes [100]. Our EDC study has shown that, by 50 years of type 1 diabetes, microalbuminuria, macroalbuminuria, and end-stage renal disease (ESRD) affected 88%, 72%, and 60% respectively of the EDC cohort, and these cumulative incidences did not differ by sex [101]. Unfortunately, the incidence of increased albuminuria appeared similar across diagnosis cohorts in this study, suggesting no improvement in the rates of disease with a more recent onset. However, a decline in the incidence of ESRD has been observed with a more recent onset of type 1 diabetes in a number of studies [101-104]. This

decline may be partially attributed to the increase in the usage of angiotensin-converting-enzyme (ACE) inhibitors/angiotensin-receptor blocker (ARB) in people with type 1 diabetes, which have been shown to have a protective effect against the progression of kidney disease in clinical trials [105-107]. The EDC and FinnDiane Studies both reported that in the absence of renal disease, mortality in people with type 1 diabetes was similar to mortality in the general population [108,109], emphasizing the importance of prevention of even early stages of kidney disease in type 1 diabetes.

Few clinical trials have been conducted among individuals with type 1 diabetes. The Diabetes Control and Complications Trial (DCCT) however confirmed the effectiveness of intensive diabetes therapy in type 1 diabetes for the prevention of complications. Indeed, DCCT results definitely showed that intensive diabetes treatment substantially reduced the incidence of PR, macroalbuminuria, and neuropathy by 47%, 54%, and 60% respectively at the end of a mean of 6.5 years follow-up, compared to the conventional treatment [110]. The subsequent observational follow-up to the DCCT study, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, further demonstrated that the benefit of intensive treatment in terms of reducing complication risk continued after the end of the trial. At EDIC year 8, the DCCT intensive treatment group experienced a 64% risk reduction in retinopathy progression, and this reduction persisted (53% lower risk) after 10 years of EDIC [111]. The magnitude of the benefit provided by intensive treatment was substantial also for macroalbuminuria, with an 84% reduction at EDIC year 8 [112]. The difference in risk of CDSP between the intensive treatment and conventional group narrowed over time, but a 30% reduction in the risk of CDSP was still observed at EDIC year 13/14 [113]. In addition, a 42% reduction in cardiovascular events was observed with intensive treatment at EDIC year 11 [114]. The risk of cardiovascular disease was reduced by 21% per 10% lower HbA1c during the DCCT [114]. After 30 years of diabetes, the cumulative incidences of PR,

ON, and cardiovascular disease remained lower in the former DCCT intensive treatment group (21%, 9%, and 9%, respectively), compared with the conventional treatment group (50%, 25%, and 14%, respectively) [115]. The DCCT and EDIC results thus highlight the long-term benefits of intensive treatment and the significance of optimizing glucose and other risk factor control as early as possible in patients with type 1 diabetes [111].

1.1.5.3 Mortality

Due to the improvement in the management of type 1 diabetes and its complications, the mortality of type 1 diabetes has been declining in the past decades [116,117]. In Sweden, all-cause mortality declined by 29% and cardiovascular disease-related deaths declined by 42% in individuals with type 1 diabetes from 1998 to 2014 [118]. Remarkable improvements in the life expectancy of individuals with type 1 diabetes have also been observed in the EDC study, as those diagnosed in 1965-1980 were shown to have a 15-year increase in life expectancy compared to those diagnosed in 1950-1964 [119]. However, all-cause mortality in type 1 diabetes remains twofold to fourfold higher than in the general population [120,121], with a substantial loss of life expectancy (11 years for men and 13 years for women) [120]. A systematic review of 26 studies confirmed that women with type 1 diabetes have an approximately 40% greater excess risk of all-cause mortality compared to men with type 1 diabetes [98].

1.1.6 Financial Burden

The American Diabetes Association (ADA) reported that the total estimated cost of diabetes in 2017 was $327 billion in the United States in 2017, with $237 billion direct medical costs and $90 billion in productivity lost [122]. Although the total economic burden for type 1

diabetes is lower than for type 2 diabetes in the United States ($14.9 vs. $159.5 billion, per year), the costs per case of diabetes is much greater in type 1 diabetes compared to type 2 diabetes ($14,856 vs. $9,677, per case per year) [123]. Type 1 diabetes presents a substantial financial burden due to the usual onset in early life, the lifetime duration, and the high risks of subsequent acute and chronic complications. The management of type 1 diabetes and its complications requires daily insulin usage and regular monitoring which are the main drivers of direct medical costs. In addition, the childhood-onset type 1 diabetes may cause a long-term adverse impact on patients' social life, which results in large indirect costs. Compared with age-matched non-diabetic population, people with childhood-onset type 1 diabetes have an average of 3.3 more absent days per year from school, 5.5 more absent days per year from work, and 7.6 more bed days per year [124,125]. As such, they are more likely to experience disadvantages in employment and have a lower income in adulthood [124,125].

1.1.7 Brief Cohort Profile of Diabetes Studies

1.1.7.1 The Pittsburgh Epidemiology of Diabetes Complication (EDC) study

The Pittsburgh Epidemiology of Diabetes Complication (EDC) study, which started in 1985, aims to investigate the natural history of childhood-onset type 1 diabetes and risk factors related to the development of diabetes macrovascular and microvascular complications [126]. The eligibility criteria of the EDC Study were subjects who 1) have been diagnosed with type 1 diabetes prior to 17 years old, or seen within one year of diagnosis, at Children's Hospital of Pittsburgh between 1950 and 1980 and 2) were living within 100 miles of Pittsburgh and 3) had completed a prior survey. at baseline. Among 1,124 potentially eligible individuals, 145 had died before the study started (and are included in some analyses) and 788 individuals participated, with 658

subjects providing in both survey and clinical exam assessment and 130 subjects providing survey information only, at the baseline assessment in 1986-1988 (Figure 1.2). The mean baseline age of the 658 subjects was 28 years (range 8-48 years) and the duration of type 1 diabetes was 19 years (range 8-37 years).

These 658 participants (325 female and 333 male) were subsequently surveyed or reexamined biennially for up to 30 years (Table 1.1). The reproductive questionnaires, which aim to assess female participants' reproductive health, including menarche, menstruation, menopause, sex hormone use, and pregnancy outcomes, were introduced at cycle 12.

Table 1.1 Timeline of the Pittsburgh Epidemiology of Diabetes Complication (EDC) Study

Year	Cycle	Survey	Clinical Exams	Reproductive Questionnaires
1986-1988	C1	X	X	
-1990	C2	X	X	
-1992	C3	X	X	
-1994	C4	X	X	
-1996	C5	X	X	
-1998	C6 (10-YEARS)	X	X	
-2000	C7	X		
-2002 -2004	C8 - C9 Combined	X		
-2006	C10 (18-YEARS)	X	X	
-2008	C11	X		
-2010	C12	X		X
-2012	C13	X		X
-2014	C14 (25-YEARS)	X	X	X
-2017	C15	X		X
-2019	C16 (30-YEARS)	X	X	X
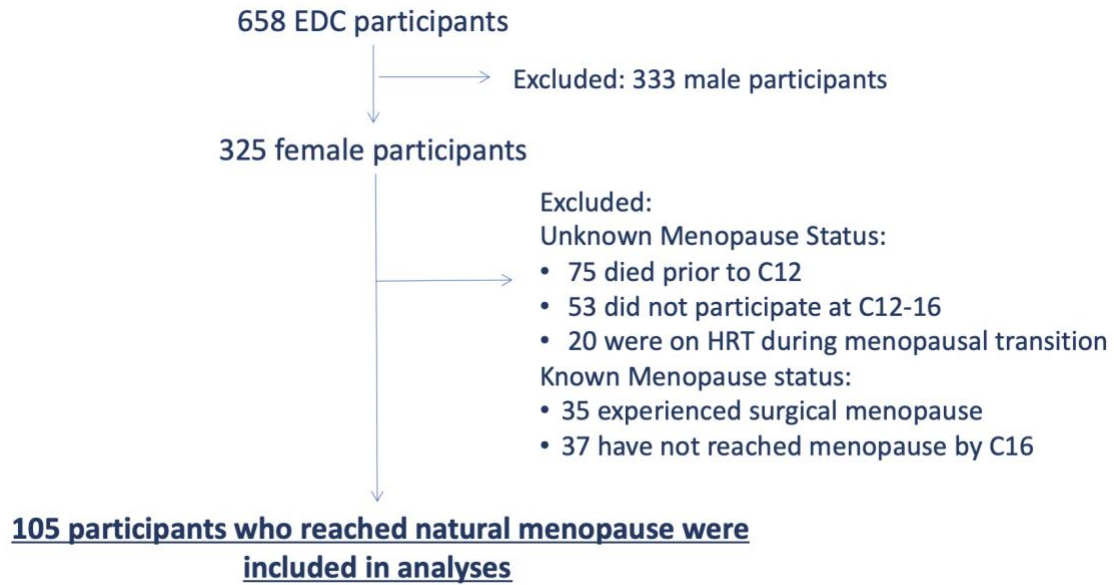
Figure 1.2 Flow Chart of the Epidemiology of Diabetes Complication (EDC) study

1.1.7.2 Other diabetes studies

Other studies discussed in previous sections are briefly described in Table 1.2, grouping by the study population.

Table 1.2 A Summary of Diabetes Studies

Name	Start year	Country	Design	Research question	Population	Size
General population-based cohorts						
DIAMOND: The World Health Organization (WHO) Multinational Project for Childhood Diabetes (WHO DIAMOND Project) [127]	1990	100 centers in 60 countries worldwide	Population-based study	To investigate and characterize global incidence, mortality and health care of T1D		
ABIS: The All Babies In Southeast Sweden (ABIS) study [128]	1997	Sweden	Population-based cohort study	To identify the importance of environmental factors in autoimmune diseases (e.g., type 1 diabetes) and how genetic and environmental factors may interact in such diseases	Newborn babies from Southeast Sweden born between 1 October 1997 and 1 October 1999	17,055
Cohorts of children with genetic susceptibility						
BABYDIAB [129]	1989	Germany	Cohort	To determine when autoimmunity commences, which are the initial targets of autoimmunity, what is the risk for disease, and what factors are associated with increased risk for autoimmunity and diabetes development in childhood	Newborns of parents with type 1 diabetes	1,353
DAISY: The Diabetes Autoimmunity Study in the Young (DAISY) [130]	1993	US	Cohort	To examine the characteristics of individuals developing autoimmunity at age 8 years or later, compared with children with early-onset autoimmunity	Children (<7 yrs) with first-degree relatives having type 1 diabetes or with increased risk of type 1 diabetes based on HLA genotype screening at birth	2,547
DIPP: The Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Project [131]	1994	Finland	Cohort	To understand the pathogenesis of type 1 diabetes (T1D), predict the disease, and find preventive treatment	Newborn baby carrying a DR-DQ genotype associated with increased risk for T1D	16,193
TEDDY: The Environmental Determinants of Diabetes in the Young (TEDDY) [132]	2003	Six clinical centers worldwide (Finland, Germany, Sweden and three in North America)	Cohort	To identify infectious agents, dietary factors, or other environmental agents, including psychosocial factors which trigger T1DM in genetically susceptible individuals or which protect against the disease	Newborns (<4 months) with first-degree relatives having type 1 diabetes or with increased risk of type 1 diabetes based on HLA genotype screening at birth	7,801
Cohorts of individuals with type 1 diabetes						
EDC: The Pittsburgh Epidemiology of Diabetes Complications (EDC) study [126]	1986	US	Cohort	To investigate the natural history of childhood-onset type 1 diabetes and risk factors related to the development of diabetic macrovascular and microvascular complications	Subjects who were diagnosed T1D prior to 17 years old, or seen within one year of diagnosis, at Children's Hospital of Pittsburgh between 1950 and 1980 and 2) are living within 100 miles from Pittsburgh	658

Study	Year	Location	Type	Purpose	Inclusion	N
EURODIAB: The Eurodiab type 1 complications study [133]	1989	16 European Countries	Cohort	To exam the prevalence of microvascular and acute diabetic complications, and their relation to duration of diabetes and glycaemic control in T1D	Subjects were diagnosed diabetes before the age of 36 years with a continuous need for insulin within 1 year of diagnosis	3,250
FinnDiane: The Finnish Diabetic Nephropathy (FinnDiane) Study [134]	1997	Finland	Cohort	To reveal the risk factors and mechanisms of diabetic complications in individuals with type 1 diabetes, with a specific focus on nephropathy	Adults were diagnosed diabetes before 35 years of age, permanent insulin treatment started within 1 year of diagnosis, and serum C-peptide concentrations <0.20 nmol/l	5,272
SEARCH: The SEARCH for Diabetes in Youth [135]	Registry 2000, cohort 2012	US (5 sites)	A registry and a cohort study	1. An active registry: to assess prevalence, annual incidence, and trends by age, race/ethnicity, sex, and diabetes type. 2. Alongitudinal cohort: to assess the natural history and risk factors for acute and chronic diabetes-related complications as well as the quality of care and quality of life of persons with diabetes from diagnosis into young adulthood	US youth diagnosed with diabetes at age <20 years	Registry: Approximately 3.5 million children <20 years of age

Clinical trial and follow-up observational cohort: DCCT/EDIC

Study	Year	Location	Type	Purpose	Inclusion	N
DCCT: Diabetes Control and Complications Trial https://edic.bsc.gwu.edu/web/edic/dcct-study	1983	US	Clinical trial	To examine the effects of intensive compared with conventional diabetes treatment on the development and progression of early microvascular, neurologic and other complications	The DCCT was comprised of 1,441 research subjects with T1DM who were recruited between 1983 and 1989	1,441
EDIC: Epidemiology of Diabetes Interventions and Complications https://edic.bsc.gwu.edu/	1994	US	EDIC is a multi-center, longitudinal, observational study	The primary goal of the EDIC study is to determine the long-term effects of prior DCCT treatment assignment on diabetes complications, based on an intention-to-treat analysis. Data collection focuses on nephropathy and macrovascular complications. Annual or biennial measurements allows the following analyses: 1) continuation of intention-to-treat analyses to determine long-term effects of prior separation of glycemic levels; 2) risk factors for macrovascular outcomes; 3) correlation of progression of micro- and macrovascular outcomes	In 1994, 96% of the surviving cohort of DCCT agreed to participate in the EDIC study	1,375

1.2 FEMALE REPRODUCTION IN TYPE 1 DIABETES: MECHANISM

1.2.1 Overview of the Female Reproductive Axis

The initiation of puberty and hypothalamus-pituitary-ovary (HPO) axis is induced by the action of kisspeptin released by kisspeptin neurons and regulated by Kiss 1 gene, which is also considered as an important regulator in the maintenance of a functional reproductive axis [136,137]. As an upstream regulatory element of GnRH (gonadotropin-releasing hormone), kisspeptin regulates the pulsatile secretion of hypothalamus GnRH and GnRH subsequently drives the action of the downstream element by simulating the pituitary to release gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [138]. These gonadotropins are the main driving force for ovary development, ovulation and secretion of sex steroids, including estrogens, progestogens, androgens and inhibin [139]. The regular ovulation cycle is the key element in maintaining a regular menstrual cycle by the effects of periodicity estrogens and progestogens on the endometrium. In addition, gonadotropins and sex steroids both exert negative loop feedback control of GnRH releasie and self-control their own secretion (autocrine feedback) (Figure 1.3) [140].
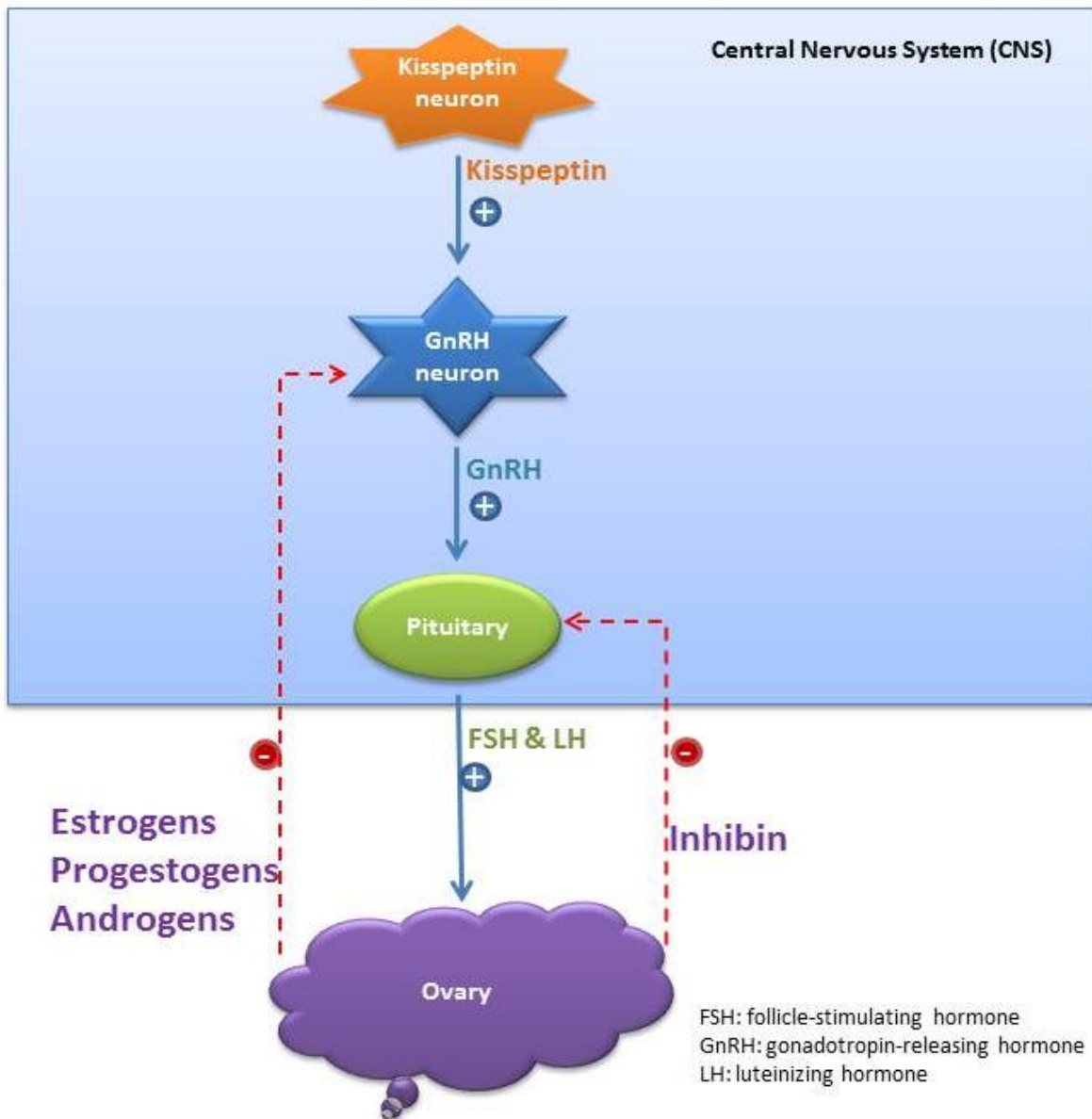
Figure 1.3 The Hypothalamus-Pituitary-Ovary (HPO) Axis

1.2.2 The Role of Insulin in the Female Reproductive System

Not only do sex steroids themselves participate in regulating the function of the HPO axis,
but other metabolic factors, such as insulin, also play key roles in tuning the HPO axis. The

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classical metabolic organs targeted by insulin action are the liver, muscle, and adipose tissue. However, widely distributed insulin receptors (IRs) were also observed throughout the central nervous system (CNS) [141] and all components of the ovary, including the granulosa, thecal, stromal, and follicular [142,143]. In mice with a neuron-specific destruction of the IR gene, female mice showed impaired ovarian follicle maturation because of dysregulation of LH resulting from hypothalamic dysregulation [141], whereas the restoration of IRs function in the brain normalized the female mice reproductive function in IRs gene knockout mice through genetic reconstitution experiments [144]. In hyperinsulinemia clamp studies in mice, increased circulating insulin was associated with a substantial rise in LH secretion [145]. Moreover, studies have suggested the direct central effects of insulin on the neuroendocrine by using primary hypothalamic cell cultures and a GnRH neuronal cell line [146,147]. These findings show that insulin can regulate female reproduction through its effects on GnRH neurons in CNS, the upstream elements of the HPO axis.

At the ovarian level, the downstream element of the HPO axis, insulin exerts influences by its gonadotropin-like function [148] or through enhancing steroidogenesis responses to gonadotropins [149-152]. As such, many in vitro studies have shown that insulin stimulates the ovarian production of estrogens, progestogens, and androgens [153,154]. In addition, insulin was positively correlated with ovarian volume in patients with polycystic ovary syndrome (PCOS) [155]. In contrast, insulin has been shown to decrease the synthesis of sex hormone-binding globulin (SHBG) in both in vivo [156] and in vitro studies [157]. This suppression effect on SHBG may result in an increased level of free sex steroids. Moreover, a low level of SHBG, potentially resulting from compensatory hyperinsulinemia in insulin resistance, may be used to predict the risk of type 2 diabetes [158].

In summary, insulin can regulate the female reproductive function through both its impact on GnRH neurons in the CNS and its gonadotropin-like effect on the ovaries.

1.2.3 Pathophysiology of Reproductive Dysfunction in Type 1 Diabetes

The pathophysiology of reproductive dysfunction in women with type 1 diabetes is complicated and intricate, but it can be appropriately viewed as three mechanisms, which interact with each other, comprising 1) endogenous insulin deficiency, 2) exogenous hyperinsulinemia, and 3) hyperglycemia (Figure 1.4) [139].
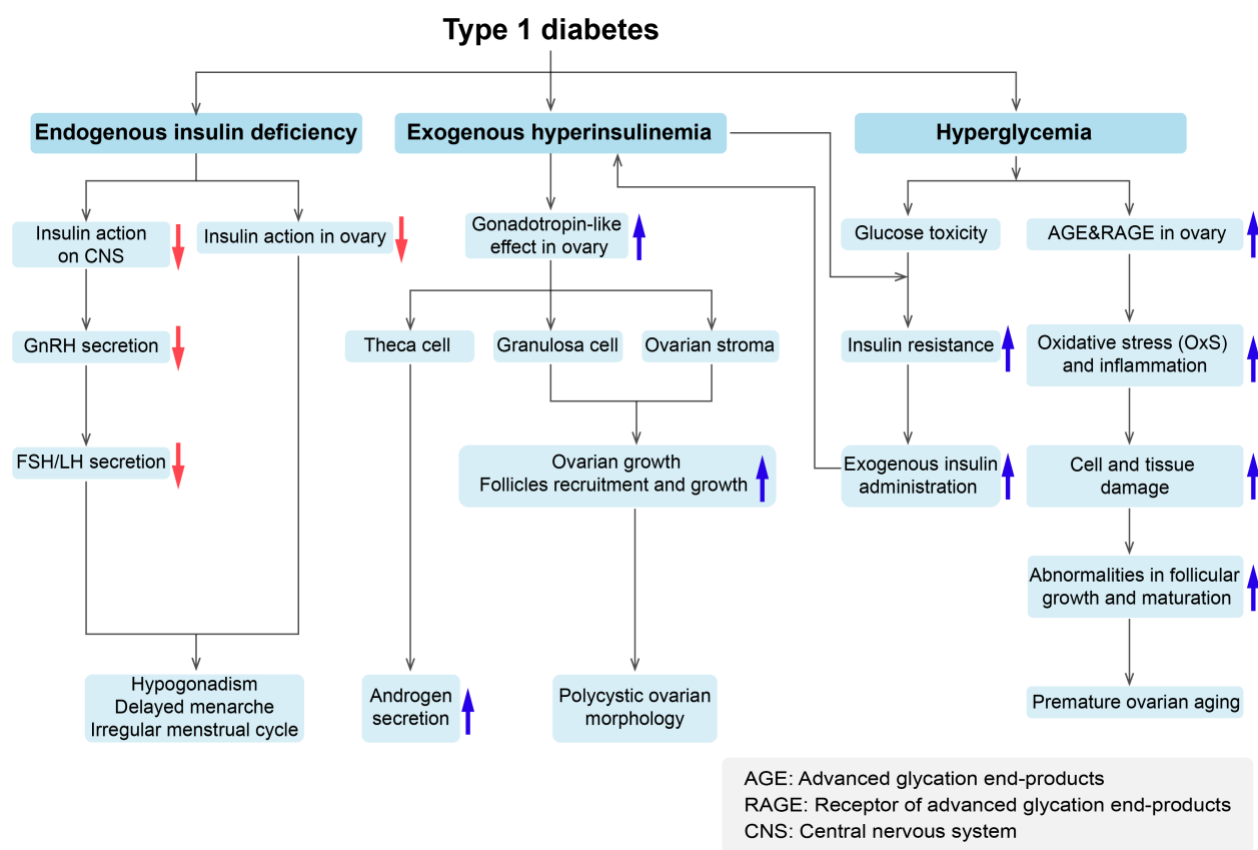
Figure 1.4 The Pathophysiology of Reproductive Dysfunction in Women with Type 1 Diabetes

1.2.3.1 Endogenous insulin deficiency

Due to the important role of insulin in maintaining the normal function of the female HPO axis, the endogenous insulin deficiency in women with type 1 diabetes was, not surprisingly,

correlated with increased risk of hypogonadism, delayed menarche, and irregular ovulation and menstrual cycles. The effect of endogenous insulin deficiency on hypogonadism, which is characterized by a low basal level of FSH/LH and sex steroids, has been confirmed in human and animal studies [139]. In women with type 1 diabetes, the pituitary function may decline with increasing duration of diabetes [159]. In mice with streptozotocin-induced diabetes, hypogonadism was observed subsequently, along with the decreased level of LH, FSH, and estradiol [160]. However, hypogonadism was reversed after continuous administration of insulin, which successfully counteracted most of the effects of diabetes on the gonadal axis [160,161]. Moreover, the finding that the mice with brain IRs gene knockout developed hypogonadism [141], has further suggested that diabetic hypogonadism results from a deficient insulin action on CNS. In women with type 1 diabetes and delayed menarche and/or irregular ovulation and menstrual cycles, in addition to the lack of insulin action at the central nervous system level, alterations at the ovarian level may also contribute to the above clinical symptoms. Thus, the lack of direct insulin action in the ovary was thought to contribute to the observed abnormal oocyte maturation and development and granulosa cell apoptosis in mice models [162,163].

1.2.3.2 Exogenous hyperinsulinemia

In recent decades, along with the widespread use of intensive insulin treatment, excessive exogenous insulin use has been identified as the culprit of new reproductive problems [164]. In the physiological state, endogenous insulin produced by the pancreas, goes through first-pass metabolism through the liver [140]. After this process, 50% to 70% of endogenous insulin is eliminated before it goes to the peripheral circulation [140]. Unlike endogenous insulin, however, exogenous insulin is usually administrated via a subcutaneous injection in a non-physiological fashion. As such, exogenous insulin does not go through hepatic first-pass metabolism and

clearance, and thus peripheral tissues are exposed to excessive insulin levels [165]. As discussed previously, IRs are present in all components of the ovary. Therefore, exogenous hyperinsulinemia can stimulate the increased secretion of androgens and testosterone, as confirmed by an in vitro study showing increased secretion of androgens from the theca cells in ovarian tissues incubated with insulin [153]. Excessive insulin can also act as a co-gonadotropin (e.g., FSH) to drive ovarian growth, cyst formation, and recruitment and growth of follicles [164], resulting in the development of PCOS. A systematic review concluded that PCOS affects 31% of adult women with type 1 diabetes when using Androgen Excess Society criteria for diagnosis, and 40% when using Rotterdam PCOS criteria [166] whereas the prevalence of PCOS in the US general population is 4-12% [167]. The prevalence of polycystic ovarian morphology was approximately 50% in women with type 1 diabetes [166]. Meanwhile, the prevalence of hirsutism in adult women with type 1 diabetes (30.6%) was approximately four-fold higher [168] in comparison with nondiabetic women (7.1%) from similar ethnic and genetic backgrounds [169]. Apart from excessive exogenous insulin, the high prevalence of PCOS in women with type 1 diabetes can also be caused by insulin resistance, resulting from hyperglycemia and subsequent glucose toxicity [170] and hyperinsulinemia [171]. The fact that the insulin resistance observed in some patients with type 1 diabetes at poor glycemic control can be ameliorated by tightening glycemic control, indicates that hyperglycemia induced glucose toxicity may be a factor partially responsible for the development of insulin resistance [172]. Research in diabetic animal models further suggests that the mechanism of glucose toxicity involved in the development of insulin resistance is associated with the downregulation of the glucose-transport system [172]. In addition, a recent human subjects study shown that the iatrogenic hyperinsulinemia is the predominant driver for insulin resistance in type 1 diabetes [171]. Overall, insulin resistance caused by hyperglycemia and exogenous hyperinsulinemia increases

the demand for exogenous insulin, thus further exacerbating the effect of excessive exogenous insulin on the ovaries, and accelerating the development of PCOS in women with type 1 diabetes.

1.2.3.3 Hyperglycemia

Meanwhile, elevated concentrations of advanced glycation end-products (AGEs) caused by hyperglycemia is a potential mechanism for premature ovarian aging in women with type 1 diabetes. AGEs are produced by a series of non-enzymatic reactions forming an irreversible covalent cross-link between reducing sugars, such as glucose, and amino groups, such as proteins, lipids, and nucleic acids. This process is known as the Maillard reaction [173]. The formation and accumulation of AGE progresses with aging and occurs at an accelerated rate in people with diabetes [174,175]. Several studies have indicated that the serum concentration of AGE in children with type 1 diabetes is significantly higher than in age-matched non-diabetic control children [176,177]. The two mechanisms by which AGE exert their damaging effects on cells and tissues, comprise the pathological covalent cross-link formations in proteins and the reaction with receptors of AGE (RAGE). The pathological cross-link formation in proteins leads to stiffness of the protein matrix and subsequent tissue dysfunction [178,179], as well as an impediment in tissue remodeling because the advanced glycated proteins are usually stable, long-lived, and have increased resistance to removal by proteolytic reactions [180]. A histological study described the damage caused by AGE cross-link formation at the molecular level and linked this damage to diabetic microvascular and macrovascular impairments [181]. In humans, it was also suggested that biomarkers of AGE cross-link in skin collagen were independently associated with retinopathy and early nephropathy in patients with type 1 diabetes, further confirming the role of AGE in the development of diabetes complications [182]. Meanwhile, RAGE is widely distributed in cells, including endothelial cells, monocytes, macrophages, pericytes, and podocytes [183]. The interaction

between AGE and RAGE triggers/accelerates oxidative stress (OxS) and inflammation, thus leading to cell and tissue damage [184,185]. The above processes also initiate intracellular signaling which is thought to disrupt normal cellular function [186]. An animal model study showed that using a RAGE blocker completely suppressed the enhanced formation of vascular lesions in diabetic/atherosclerotic mice [187]. All these mentioned mechanisms and clinical research suggest that elevated AGE in people with diabetes play a key role in vascular and other tissue damage.

One study group detected RAGE and AGE-modified proteins expressed in human ovarian tissue [188], and noted a stronger localization of AGE and RAGE in granulosa cells in women with PCOS compared with controls [189]. These studies are important as they are suggesting the involvement of AGE/RAGE in ovarian dysfunction [190]. In addition to the ovarian vascular damage caused by AGE cross-link formations in proteins, the damaging effect of AGE on ovarian tissue may also be mediated through oxidative stress and inflammation during the AGE formation and AGE/RAGE interaction process. [191] Thus, increased oxidative stress has been associated with significant adverse effects on women's reproductive function, including ovarian vascular endothelium damage and abnormalities in follicular growth, oocyte maturation, corpus luteum formation, and embryonic growth [192]. Although no study has directly investigated whether elevated AGE concentrations cause ovarian dysfunction in women with type 1 diabetes, their damaging effect on other tissues in diabetes and the adverse effects of RAGE and AGE-modified proteins on ovarian aging in the general population, makes it logical to postulate that AGE/RAGE contribute to premature ovarian aging in women with type 1 diabetes.

1.3 FEMALE REPRODUCTION IN TYPE 1 DIABETES: EPIDEMIOLOGY

1.3.1 Age at Menarche in Women with Type 1 Diabetes

In our recent study, we noted that the onset of menses in women with childhood-onset type 1 diabetes is delayed [193]. Indeed, menarche occurred ~0.6 years later in our cohort of women with type 1 diabetes compared with non-Hispanic white women in the general NHANES population born during the same period (1940-1979) [193,194]. Menarche delay is a common finding in women with type 1 diabetes [195-200], especially when the type 1 diabetes onset precedes menarche onset [196,199,200], although not all studies agree (Table 1.3) [201,202]. The Familial Autoimmune and Diabetes research group has shown that the menarche in women with type 1 diabetes was approximately delayed 1 year compared to nondiabetic sisters and unrelated controls, being 13.5, 12.5 and 12.6 years, respectively [197].

The possible reason for a delayed menarche in women with type 1 diabetes relates to the deficiency of insulin action in the Hypothalamus-Pituitary-Ovary (HPO) axis as discussed previously. Two previous studies with type 1 diabetes found that higher HbA1c concentrations and lower insulin doses were associated with an older age at menarche, with a 0.84 month greater delay per 1% increase in HbA1c among young German women [203,204] and a 1.3 month greater delay among U.S. women [198]. Evidence from animal studies also point to impaired reproductive function, including hypogonadism and impaired ovarian follicle maturation [205] with insulin deficiency, defects which are ameliorated with insulin administration [145]. Nevertheless, several studies indicated that glycemic control was not associated with age at menarche in type 1 diabetes [199,201,202]. Indeed, two studies have provided evidence to suggest that intensive diabetes treatment and good glycemic control are not sufficient to resolve the delayed menarche timing in women

with type 1 diabetes [206,207]. These studies suggest that in addition to the delayed activation of gonadotropin secretion caused by insulin deficiency in puberty [195], there are other factors leading to delayed menarche in women with type 1 diabetes. The factors may relate to the appearance of hyperandrogenism in pubertal girls with type 1 diabetes [208], which inhibits the normal function of the HPO axis. In addition, elevated advanced glycation end products [177], sex hormone binding globulin (SHBG) levels [209] and ovarian antibodies [210] may also play a role in delayed menarche in this population subgroup.

Another topic relating to the delayed menarche in type 1 diabetes is its effects on the risk of microvascular complications development later in life. Our analyses suggested that delayed menarche is significantly associated with the development of overt nephropathy (ON) in women with type 1 diabetes [193], which concurred with findings from the FinnDiane study [200]. However, contrary to FinnDiane results of an association between delayed menarche and the presence of laser-treated retinopathy [200], we failed to detect consistent significant associations between delayed menarche and either PR or CDSP [193].

The mechanism for an association between age at menarche and diabetic microvascular disease has not been investigated extensively. It could be speculated that delayed menarche reflects more severe latent microvascular damage caused by poor glycemic control earlier in life as it is widely known that hyperglycaemia constitutes a strong risk factor for diabetic nephropathy. A further possibility is that the delay of being exposed to higher estrogen, which accompanies delayed menarche, leads to greater risk of kidney disease as many studies have shown a renoprotective effect of estrogen in both the general and the diabetes population [211-213]. It is also possible, although strong relevant data are currently lacking, that the onset of menses and kidney disease have common genetic underpinnings. Further studies in a larger population are warranted

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to investigate the role of sex hormones in the development of ON in women with type 1 diabetes and delayed menarche.

Table 1.3 A Summary of Studies Related to Age at Menarche in Type 1 Diabetes

Author	Year	Country	Age at Menarche (AAM, yrs)		Findings
			T1D (n)	**Controls** (n)	
Clarson et al. [202]	1985	Canada	13.2 ± 1.2 (122)	-	T1D onset before puberty; Participants displayed normal pubertal development; HbA1 levels was not a major determinant of pubertal development in T1D
Kjaer et al. [214]	1992	Denmark	14.2 ± 1.5 (245)	13.4 ± 1.4* (253)	T1D onset before the age of 10 yrs
Salerno et al. [201]	1997	Italy	12.8 ± 1.4 (30)	12.5 ± 1.0 (50)	Children with T1D have normal onset of puberty
Strotmeyer et al. [197]	2003	USA	13.5 ± 1.9 (143)	12.6 ± 1.4* (158)	T1D onset before puberty
Codner et al. [215]	2004	Chile	13.0 ± 0.2 (100)	12.5 ± 1.1* (576)	BMI, but not HbA1c levels, was a determinant of pubertal development in T1D
Danielson et al. [198]	2004	USA	12.8 ± 1.33 (188)	12.5 * (NHANES)	AAM was delayed 1.3 months with each 1% increase in HbA1c
Elamin et al. [216]	2006	Sudan	15.1 ± 1.25 (35)	13.3 * (Population data)	T1D onset at the age of 7-13 yrs; AAM was related to HbA1c and duration of T1D
Rohrer et al. [203,204]	2007	Germany	13.22 ± 1.31 (643)	12.7 ± 1.09* (Population data)	T1D onset at the age of 7-17 yrs; AAM was delayed 0.07 years with each 1% increase in HbA1c
Snell-Bergeon et al. [217]	2008	USA	13.1 ± 1.8 (293)	12.8 ± 1.5* (319)	T1D onset before the age of 30 yrs;
Lombardo et al. [218]	2008	Italy	12.77 ± 1.13 (73)	12.0 ± 1.10* (280)	T1D onset before menarche;

Study	Year	Country	AAM (n)	AAM control (n)	Notes
Picardi et al. [219]	2009	Italy	12.6 ± 1.5 (162)	12.3 ± 1.4* (214)	T1D onset before menarche; Menarche in T1D is still delayed despite good metabolic control
Deltsidou et al. [196]	2009	Greece	12.2 ± 1.4 (100)	11.7 ± 1.2* (225)	T1D onset before the age of 10 yrs;
Schweiger et al. [199]	2010	USA	12.81 ± 0.09 (185)	12.27 ± 0.038* (NHANES)	T1D onset before menarche;
Zachurzok et al. [220]	2013	Poland	13.1 ± 1.4 (47)	12.0 ± 1.2* (19)	-
Gomes et al. [221]	2015	Brazil	12.7 ± 1.7 (1527)	-	-
Harjutsalo et al. [200]	2016	Finland	13.82 ± 1.65 (600) [a]; 12.89 ± 1.43 (704) [b]		a: T1D onset before menarche; b: T1D onset after menarche; There was a decreasing trend in the AAM in recent birth cohorts; Delayed menarche was associated with an increased risk of diabetic nephropathy and retinopathy;
Yi et al. [193]	2019	USA	13.0 (315)	-	AAM was significantly associated with the prevalence and cumulative incidence of overt nephropathy

*p<0.05;

AAM, age at menarche; NHANES, National Health and Nutrition Examination Surveys; T1D, type 1 diabetes.

1.3.2 Age at Natural Menopause in Women with Type 1 Diabetes

Natural menopause is the cessation of ovarian function and the end of women's reproductive life, resulting from oocyte depletion. Although menopause is a universal event among women, the timing of natural menopause onset varies widely by race/ethnicity, demographic factors, and lifestyle [222]. The estimated average age at natural menopause (ANM) in Western countries is 51 years [223], with a wide range from 40 to 60 years [224]. The study on ANM has both clinical and public health significance as menopause transition brings substantial physiological and metabolic changes in the human body and ANM is considered as a marker of body aging and health [222]. Firstly, early natural menopause is related to increased all-cause mortality [225] and also mortality from cardiovascular disease [226,227], atherosclerosis [228], and stroke [229], with a 2% increase in age-adjusted mortality per year decline in ANM [230]. In addition, early natural menopause is associated with increased risks of cardiovascular disease [227], osteoporosis [231], and fracture [232]. However, in 1990s, a pooled analysis of 3 European case-control studies found an increased risk of ovarian cancer in women with later natural menopause compared with women who had ANM at age 44 or earlier [233], whereas a review which analyzed data from six studies from the U.S has indicated little evidence to the relationship of ANM and the risk of ovarian cancer [234]. A large prospective cohort study (10,591 participants) from the Netherlands has suggested that women with ANM at 44 years or younger had a substantial (34%) lower risk of breast cancer compared with women with ANM over 54 years, with an 2.6% decline per year reduction in ANM [235]. Although ANM has already been studied extensively in the general population, little is known about the ANM and its implications in women with type 1 diabetes.

The Familial Autoimmune and Diabetes (FAD) study was the first to report that women with type 1 diabetes reached menopause at a younger age compared with their nondiabetic sisters or unrelated control subjects (41.6, 49.9, and 48.0 years respectively) (Table 1.4) [236]. However, this finding was based on small sample size (n=15, 21, and 15 respectively), and the average age at the assessment of menopausal status was 42 years old and therefore only a small proportion of study participants had researched menopause at the time of assessment [236]. The European Prospective Investigation into Cancer and Nutrition (EPIC) study also suggested that early-onset diabetes (onset before the age of 20 years) was associated with earlier menopause onset (HR=1.43; 95% CI=1.02-2.01), compared with nondiabetic controls [237]. However, the EPIC study could not distinguish the effect of type 1 and type 2 diabetes or determine the sequence of diabetes and menopause onset, as the data were cross-sectionally collected [237]. On the contrary, mean ANM did not differ between women with type 1 diabetes and controls in the more recent Ovarian Aging in type 1 Diabetes mellitus (OVADIA) study, although the response rate was only ~50% [238]. Moreover, in a study from Finland, the median ANM in women with type 1 diabetes (52.5 years) did not significantly differ than that of the general Finish population (51 years) [239]. The conflicting findings regarding whether ANM differs in women with type 1 diabetes may be attributed to the different age at onset of diabetes, as in OVADIA study, a large proportion of type 1 diabetes cases were not childhood-onset (age at onset: 28±14.2 years) and had shorter diabetes duration compared to participants with diabetes in the FAD and EPIC studies. Moreover, only a small proportion of participants had reached menopause in FAD study, and thus the ANM of women with T1D in FAD study may not reflect the actual ANM for the entire T1D cohort, potentially exaggerating the effect of T1D on ANM [236].Furthermore, the Finnish study used the population level data rather than the

individual level data for non-diabetic controls, and thus investigators were unable to control for confounding factors in their analysis [239].

In the general population, it is well known that smoking accelerates the natural menopause onset [240,241], whereas body mass index (BMI) and high parity were associated with later natural menopause onset [223,242]. Nevertheless, little is known about the effects of these traditional risk factors, such as smoking and BMI, or diabetes specific factors, such as glycemic control and diabetes complications, on the ANM in women with type 1 diabetes. The Finnish study first reported that end-stage renal disease and proliferative retinopathy were associated with earlier natural menopause in women with type 1 diabetes, although it was unclear whether the microvascular complications occurred prior to the onset of natural menopause, due to the natural flaw of the cross-sectional design [239]. Kim et al. examined the impact of intensive treatment, HbA1c, and microvascular complications upon menopause among women with type 1 diabetes in the Diabetes Control and Complications Trial (DCCT) and its observational follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study [243]. The study results suggested that intensive versus conventional therapy, HbA1c level, and microvascular complications were not associated with menopause, whereas greater insulin dose was associated with later menopause [243]. The possible explanation for the non-significant findings could be that the DCCT/EDIC participants have better glycemic control than participants in observational studies or patients in the real world, thus that the HbA1c levels do not affect the ovarian reserve to the extent that natural menopause is accelerated. Similar to glycemic control, the microvascular complications are less common in the DCCT/EDIC study. More complications cases (exposed) are needed to test the effect of complications (exposure) upon the menopause (outcome).

Table 1.4 A Summary of Studies Related to Age at Natural Menopause in Type 1 Diabetes

Author	Year	Country	Age at Natural Menopause (ANM, yrs)		Findings
			T1D **(n)**	**Controls** **(n)**	
Dorman et al. [236]	2001	USA	41.6 (15)	49.9 (21 sisters); * 48.0 (15 unrelated controls) *	Small sample size, may not be generalizable.
Sjoberg et al. [239]	2011	Finland	52.5 (476)	51 (Finish population)	- Population level data were available for non-diabetic controls, which prohibited adjustment for potential confounders - Half of the study participants were diagnosed with T1D after menarche
Kim et al. [243]	2014	USA	49.9 (Intensive therapy) 49.0 (Conventional therapy)	-	Did not have a control group who did not have diabetes
Brand et al. [237]	2015	Europe	-	-	They cannot distinguish the effect of T1D and T2D or determine the sequence of diabetes and menopause onset
Yarde et al. [238]	2015	Dutch	49.89 ± 4.7 (140)	49.8 ± 4.1 (5426)	- The response rate was only ~50% - Large proportion of women were diagnosed with T1D after menarche (mean age at diabetes onset: 28±14.2 years)

*p<0.05;

ANM, age at natural menopause; HR, hazard ratio; T1D, type 1 diabetes

1.3.3 Ovarian Reserve in Women with Type 1 Diabetes

Given the perception that the effect of type 1 diabetes or its complications does not decrease ovarian reserve to the extent that natural menopause is accelerated, some studies have assessed their effects on intermediate outcomes, including anti-Mullerian hormone (AMH), follicle stimulating hormone (FSH), and antral follicle count (AFC). AMH is produced solely by the granulosa cells of the small growing ovarian follicles. It is a serum endocrine biomarker for ovarian reserve and reflects the quantity of the ovarian follicle pool. Serum AMH levels begin to decline 10 years prior to the final menstrual period and declined to below the assay detection limits 5 years prior to the final menstrual period [244].

Soto et al. [245] found that AMH levels were lower in women with type 1 diabetes compared with non-diabetic controls in later reproductive age (4.1 versus 9.5 pmol/l, p = 0.006), suggesting an earlier depletion of ovarian follicle pool [245]. On the contrary, Yarde et al. found that the relative difference in AMH was not significant (0.92, 95% CI: 0.68–1.23) between women with type 1 diabetes compared with non-diabetic controls, and no significant association between vascular health and ovarian aging (AMH and AFC) was detected in women with type 1 diabetes [246]. In addition, Kim et al. repeatedly assessed AMH among DCCT/EDIC study participants and founded that AMH levels were similar in women with and without type 1 diabetes after 35 years of age, although AMH levels were indeed lower in women with type 1 diabetes before 35 years of age, suggesting that type 1 diabetes may only be associated with a lower number of arrested follicles in the earlier reproductive years [247]. Moreover, among DCCT/EDIC female participants, none of intensive therapy (p = 0.39), diabetes duration (p = 0.86), time-weighted HA1c (p = 0.34), and time-weighted insulin dose (p = 0.73), was associated with AMH levels [248]. The conflicting

findings regarding AMH levels in women with type 1 diabetes may be attributed to the different age groups studied and analytic strategy adopted in each study, as in the studies by Soto et al. and Kim et al., analyses were stratified by age, whereas Yarde et al. did not conduct stratified analyses.

1.3.4 Pregnancy Outcome in Women with Type 1 Diabetes

Although Type 1 diabetes is thought to account for 5–10% of those with a diabetes diagnosis overall [249], it has a prevalence rate of 0.33% among pregnant women [250]. Moreover, the numbers of pregnancies complicated by pre-existing type 1 diabetes have increased significantly over decades, by 44 % increase in Scotland [251] and 50% increase in Canada [252]. Pregnancies in those with pre-existing type 1 diabetes have a higher risk of adverse obstetric and perinatal outcomes, including congenital malformations, pregnancy losses, fetal large for gestational age (LGA), preeclampsia, and preterm delivery than in the general population [253-260]. Based on a large, nationwide, population-based study, the congenital malformation rate (5.0%) was approximately two times higher, the perinatal mortality rate (3.1%) and stillbirth rate (2.1%) was over four times higher, and the preterm delivery rate (41.7%) was up to seven times higher in pregnancies with type 1 diabetes, compared to pregnancies in the general population [254]. An eightfold increased risk of fetal LGA in pregnancies complicated with type 1 diabetes was also reported [255].

Many observational studies suggested a significant dose-response relationship between Hemoglobin A1c (HbA1c) level during pregnancy and the risk of preterm delivery [261-264], pre-eclampsia [262,264], LGA [261,262,264] and a composite of adverse pregnancy outcomes [262,263,265]. Murphy et al. reported a 15% reduction in stillbirth rates in UK [266] and Feig et al. reported a 23% reduction in congenital malformations rate in Canada [252], due to the improvement of glycemic control in pregnancies with pre-existing type 1 diabetes in recent decades. However, this marked association

of glycemic control and pregnancy outcomes was not observed in clinical trials. The Diabetes Control and Complications Trials (DCCT) indicated that long-term intensive preconception therapy did not make a difference in the pregnancy outcome compared to conventional preconception therapy in type 1 diabetes women [267]. The evidence for an association between good glycemic control during organogenesis and decreased congenital malformation risk in women with type 1 diabetes was limited as well [268]. In addition, a Cochrane systematic review of three clinical trials [269-271] concluded that tight glycemic control during pregnancy was not associated with decreased risk of adverse pregnancy outcome in pregnancies complicated with pre-existing type 1 diabetes [272]. These conflicting findings may be attributed to different time periods when HbA1c was measured across studies (e.g., during preconception, organogenesis, second or third trimester of pregnancy) and the limited sample size in clinical trials.

Overall, the rates of adverse pregnancy outcomes among women with pre-existing type 1 diabetes continued to be higher than non-diabetic population [251,252,266]. It is still far away to reach the 1989 St Vincent Declaration's five-year goal of improving pregnancy outcomes for pregnancies with type 1 diabetes, and thus approximating to those for pregnancies without type 1 diabetes [256].

1.4 SUMMARY

1.4.1 Epidemiology of Type 1 Diabetes

Although the incidence of type 1 diabetes varies considerably over geographic regions, it has been rising all over the world, with a global average annual increase of 3% [15]. In the United States, the incidence of type 1 diabetes among youth (<20 years old) is 21.7 per 100,000 youth per year [5], whereas the prevalence was 2.12 per 1,000 youth in 2010 and is expected to triple to 5.2 per 1,000 by 2050 [18]. Therefore, type 1 diabetes will continue to be one of the most common chronic diseases in children in the following decades [19-21].

However, type 1 diabetes is a health challenge not only due to the rise in its incidence and prevalence but also owing to the burden of diabetes-associated macrovascular and microvascular complications, which adversely impact the finances and quality of life of patients and their families. The age-adjusted risk of cardiovascular disease is approximately ten-fold higher [95-97] and cardiovascular disease-related mortality is 30-fold higher in people with type 1 diabetes compared with the general population [99]. Our EDC study showed that the cumulative incidence of proliferative retinopathy (PR), confirmed distal symmetric polyneuropathy (CDSP), and overt nephropathy (ON) were over 50%, 40%, and 30%, respectively, by 25 years of type 1 diabetes duration [93].

1.4.2 Female Reproductive Disorders in Type 1 Diabetes

Women with type 1 diabetes have additional reproductive health burden because insulin deficiency and hyperglycemia disrupt the normal function of female reproductive system [160,161].

Many studies suggested that women with type 1 diabetes have delayed menarche [195-200] and a higher likelihood of adverse pregnancy outcomes [254,255] compared with non-diabetic controls. Nevertheless, data relating to menopause timing in women with type 1 diabetes are scarce and findings are conflicting. As it is well known that early natural menopause is related to increased risk of cardiovascular disease [227], osteoporosis [231], fracture [232] and increased all-cause [225] and cardiovascular disease mortality [226,227], atherosclerosis [228], and stroke [229], definitive conclusions on menopause timing among women with type 1 diabetes, who are at increased cardiovascular risk, would be important.

1.4.3 Aim 1: To Assess Whether Age at Natural Menopause Differs in Women with Type 1 Diabetes and Non-Diabetic Controls

The Familial Autoimmune and Diabetes (FAD) study was the first to report that women with type 1 diabetes reached menopause at a younger age compared with their nondiabetic sisters or unrelated control subjects [236]. However, this finding was based on small sample size (n=15, 21, and 15 respectively) [236] which largely affects the reliability of study's result. The European Prospective Investigation into Cancer and Nutrition (EPIC) study also suggested that early-onset diabetes was associated with earlier menopause onset (HR=1.43), compared with nondiabetic controls [237]. However, the EPIC study could not distinguish the effect of type 1 and type 2 diabetes or determine the sequence of diabetes and menopause onset, as the data were cross-sectionally collected [237]. On the contrary, mean age at natural menopause did not differ between women with type 1 diabetes and controls in the more recent Ovarian Aging in type 1 Diabetes mellitus (OVADIA) study, although the response rate was only ~50% [238]. In a study from Finland, the

median ANM in women with type 1 diabetes did not significantly differ than that of the general Finish population [239], although it did not adjust for other confounding factors.

Given the limitations and conflicting results of existing studies, we propose to assess whether age at natural menopause differs in women with type 1 diabetes and non-diabetic controls by using data from the Pittsburgh Epidemiology of Diabetes Complication (EDC) study and the Study of Women's Health Across the Nation (SWAN). The EDC is a prospective cohort of individuals with childhood-onset type 1 diabetes living in the greater Pittsburgh area. Of 658 participants (96% non-Hispanic white, 4% Black), 325 are women. A first exam occurred in 1986-88 and the cohort was followed with subsequent biennial surveys throughout the follow-up and biennial exams for 10 years and again at the 18, 25 and 30 year of follow-up. SWAN is a multi-site longitudinal epidemiologic study designed to examine the health of midlife women during their transitioning to menopause period. SWAN female participants from the Pittsburgh site would comprise an excellent comparison group to the Pittsburgh EDC for assessing age at menopause in non-diabetic women.

1.4.4 Aim 2 & 3: To Assess Whether Changes in Glycemic Control and Other Risk Factor Levels Over Time and Whether Complications Are Associated with Age at Natural Menopause in Women with Type 1 Diabetes

Likewise, little is known about the risk factors relating to age at natural menopause in women with type 1 diabetes, such as glycemic control and diabetes complications. The Finnish study first reported that end-stage renal disease and proliferative retinopathy were associated with earlier natural menopause in women with type 1 diabetes, but it was unclear whether the microvascular complications occurred prior to the onset of natural menopause, due to the natural

flaw of the cross-sectional design [239]. Kim et al. examined the impact of intensive treatment, HbA1c, and microvascular complications upon menopause among women with type 1 diabetes in the Diabetes Control and Complications Trial (DCCT) and its observational follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study [243]. However, the DCCT/EDIC participants have better glycemic control than participants in observational studies or patients in the real world, thus limiting the generalizability of their finding in the general type 1 diabetes population.

Since above research gaps persist in the knowledge of age at natural menopause and its risk factors in women with type 1 diabetes**, the aims of this dissertation are to fill these research gaps by assessing whether changes in glycemic control and other risk factor levels over time relate to age at natural menopause in type 1 diabetes (Aim 2) and whether micro- and macro-vascular complications is associated with age at natural menopause (Aim 3) in women with type 1 diabetes.** We will utilize data from the EDC study to do analysis for aim 2 and 3. The 30-year EDC cohort study provides rich longitudinal data on metabolic factors, including BMI, blood pressure, HbA1c, and lipid profiles, and accurate measurements of diabetic complications of women with type 1 diabetes.

2.0 MANUSCRIPT 1: WOMEN WITH TYPE 1 DIABETES (T1D) EXPERIENCE A SHORTER REPRODUCTIVE PERIOD COMPARED WITH NONDIABETIC WOMEN: THE PITTSBURGH EPIDEMIOLOGY OF DIABETES COMPLICATIONS (EDC) STUDY AND THE STUDY OF WOMEN'S HEALTH ACROSS THE NATION (SWAN)

Objective: Evidence suggests that insulin deficiency and hyperglycemia may disrupt the female reproductive system's normal function, leading to delayed menarche and premature ovarian aging. We thus compared the length of the reproductive period of women with type 1 diabetes (T1D) to women without diabetes.

Methods: Women with childhood-onset T1D (diagnosed in 1950-80) from the prospective Epidemiology of Diabetes Complications (EDC) study and non-diabetic women from the Pittsburgh site of the Study of Women's Health Across the Nation (SWAN) were studied. Exclusion criteria comprised not having reached natural menopause, hysterectomy/oophorectomy before menopause, and sex hormone therapy during the menopausal transition. Reproductive history was self-reported. The historical and Women's Ischemia Syndrome Evaluation hormonal algorithms were also used to assess menopause status.

Results: Women in the T1D cohort (n=105) were younger, more likely to be white, never smokers, with lower BMI and higher HDL-C levels (all p-values<0.05) compared with women without diabetes (n=341). After covariate adjustment, T1D women were also older at menarche (1.0-year delay, p<0.0001) but younger at natural menopause (-1.8 years, p<0.0001). Women with T1D thus experienced 2.9 fewer reproductive years compared to those without diabetes

(p<0.0001). These findings were restricted to women who were diagnosed with T1D before reaching menarche (n=78).

Conclusions: Women with T1D onset before menarche have a shorter reproductive period compared with non-diabetic women, exhibiting delayed menarche and earlier natural menopause. Factors which may be related to a shorter reproductive period in T1D should be investigated.

2.1 INTRODUCTION

Insulin plays a key role in regulating female reproductive function through its effects on both gonadotropin-releasing hormone (GnRH) neurons in the central nervous system [141] and the granulosa, thecal, and stromal components in the ovarian system [142,143]. The impact of disruption in insulin regulation and hyperglycemia on the length of the female reproductive period has been reported separately by assessment of the two components of reproductive years: menarche and menopause. Existing epidemiological studies have shown that insulin deficiency and hyperglycemia disrupt the normal function of the female reproductive system, leading to delayed menarche in women with type 1 diabetes [195-200], especially when the onset of type 1 diabetes precedes the onset of menarche [196,199,200].

The evidence regarding the effect of type 1 diabetes on age at natural menopause, however, is limited and conflicting [236-239]. Natural menopause is the cessation of ovarian function and the end of a woman's reproductive life, resulting from oocyte depletion. Studying the age at natural menopause has both clinical and public health significance, as the menopause transition brings substantial physiological and metabolic changes in the human body and age at menopause is considered to be a marker of body aging and health [222]. First, early natural menopause is related to

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increased risk of both all-cause [225] and cardiovascular disease mortality [226,227], with a 2% increase in age-adjusted mortality per year decline in age at natural menopause [230]. In addition, early natural menopause is associated with increased risk of atherosclerosis [228], stroke [229], cardiovascular disease [227], osteoporosis [231], and fracture [232].

The Familial Autoimmune and Diabetes (FAD) study was the first to report that women with type 1 diabetes reached natural menopause at a younger age compared with their nondiabetic sisters or unrelated control subjects (41.6, 49.9, and 48.0 years respectively) [236]. However, this finding was based on a small number of women having reached menopause (n=15, 21, and 15 respectively), as the average age in this study was only 42 years [236]. The European Prospective Investigation into Cancer and Nutrition (EPIC) study also suggested that early-onset diabetes (onset between 10-20 years) was associated with earlier menopause onset (HR=1.43, 95% CI 1.02-2.01) [237]. However, the EPIC study could not distinguish the effect of type 1 and type 2 diabetes [237]. On the contrary, mean age at natural menopause did not differ between women with type 1 diabetes and controls in the more recent Ovarian Aging in type 1 Diabetes mellitus (OVADIA) study, although the participant response rate was only ~50% [238]. Moreover, in a study from Finland, the median age at natural menopause in women with type 1 diabetes (52.5 years) did not significantly differ from that of the general Finnish population (51 years) [239]. The Finnish study used population level data rather than individual level data for non-diabetic controls, and, thus, investigators were unable to control for confounding factors in their analysis [239].

Thus, to date, research gaps and conflicting findings persist in terms of the age at natural menopause in type 1 diabetes and no study has directly assessed the length of the reproductive period in women with this chronic disorder. Therefore, the aim of this study was to fill these research gaps by assessing the length of the reproductive years of women with type 1 diabetes

from the Pittsburgh Epidemiology of Diabetes Complication (EDC) study and of women without diabetes from the Pittsburgh site of the Study of Women's Health Across the Nation (SWAN). We hypothesized that women with type 1 diabetes experience a shorter reproductive period compared with non-diabetic women.

2.2 RESEARCH DESIGN AND METHODS

2.2.1 Study Population

Eligible women with type 1 diabetes were from the Pittsburgh Epidemiology of Diabetes Complication (EDC) study. The Pittsburgh EDC Study recruited childhood-onset (<17 years) type 1 diabetes patients diagnosed, or seen within one year of diagnosis, at Children's Hospital of Pittsburgh between 1950 and 1980. Among 658 individuals who completed a baseline assessment in 1986-1988, the mean baseline age and diabetes duration were 28 years (range 8-48 years) and 19 years (range 8-37 years), respectively. These 658 participants (325 female and 333 male) were subsequently surveyed and reexamined biennially for up to 10 years. Subsequently, biennial surveys continued up to 30 years whereas additional exams occurred at 18, 25 and 30 years of follow-up. A more detailed EDC Study description can be found elsewhere [126].

Although all the biennial surveys administered to female EDC participants included a question on the age at which menopause began, a detailed women's reproductive health questionnaire, which allowed more accurate assessment of the age at natural menopause, was first administered during the 22-year follow-up (2009-2011). Testing for plasma follicle stimulating hormone (FSH) and estradiol to help with menopausal status determination was also initiated at

that time. Of 325 female participants, 128 did not complete the detailed women's reproductive health questionnaire and sex hormone tests due to loss to follow-up (n=53) or death (n=75) prior to the 22-year assessment. Of the remaining 197, 37 had not yet reached menopause and thus did not have a corresponding age at menopause by the last available follow-up, 35 had a hysterectomy/oophorectomy before menopause, and 20 received sex hormone therapy during the menopausal transition and were thus excluded from analyses. Thus, the analytic sample comprised 105 women (Figure 1.2). Sensitivity analyses were further performed including 18 EDC women who either died (n=12) prior to the 22-year assessment or did not partake in exams after 20 years (n=6) and thus had no hormone data available but had previously self-reported age at natural menopause.

Women without diabetes were from the Pittsburgh site of the Study of Women's Health Across the Nation (SWAN) and formed the comparison group. SWAN is a multi-site, longitudinal, epidemiologic study designed to examine the health of women during their menopausal transitional period and was initiated in 1996-1997. A detailed study design has been previously published [273]. Briefly, the inclusion criteria comprised women who 1) were 42-52 years of age, 2) had experienced a menstrual period within the past 3 months, and 3) had at least one intact ovary. The SWAN Pittsburgh site included 463 women at baseline (1998), with a mean age of 45.7 years (range 44.1-47.8 years) who were followed-up annually for up to 20 years. The study design required that approximately 1/3 of the SWAN Pittsburgh cohort were black and 2/3 white. Of these 463 SWAN Pittsburgh site participants, women who developed diabetes before or during the annual follow-ups (n=74) or received hysterectomy and/or oophorectomy before menopause (n=48) were excluded from analysis. A given SWAN participant was defined as having diabetes if she met any of the following criteria: 1) using anti-diabetic medication at any visit, 2) having a

fasting glucose >=126 mg/dL (while not on steroids) on 50% of at least 3 attended visits or 2 consecutive visits, or 3) having any two visits with self-reported diabetes and at least one visit with fasting glucose >=126 mg/dL (while not on steroids). Therefore, a total of 341 SWAN participants were included in the data analyses (Figure 2.1).

2.2.2 Covariate Assessment

Since EDC participants were younger at study entry compared with participants of the SWAN cohort, covariate data for women with type 1 diabetes were taken from the follow-up visit preceding menopause in which chronological age was closest to the mean baseline age (i.e., 46.0 years) in SWAN. This time point constituted the "baseline" assessment for the present analyses.

In the EDC study, survey questionnaires regarding demographic characteristics, medical history, and reproductive history were sent to participants before each clinical examination visit. During the clinical visits, body mass index (BMI) was recorded as weight in kilograms (kg) divided by height in meters squared (m^2). The waist to hip ratio (WHR) was recorded as the circumference of the waist divided by that of the hips. A random zero sphygmomanometer was used to measure blood pressure after a 5-minute rest according to the Hypertension Detection and Follow-up Program protocol [274]. Hypertension was defined as blood pressure ≥140/90 mm Hg or use of antihypertensive medications. High-density lipoprotein cholesterol (HDL-C) was measured by means of a precipitation technique (heparin and manganese chloride) with a modification of the Lipid Research Clinics method [126,275]. Total cholesterol and triglycerides were measured enzymatically [126,275]. Non-HDL cholesterol was computed by subtracting HDL-C from total cholesterol.

In the SWAN study, demographic characteristics, medical history, and reproductive history were derived from questionnaires administered at the baseline assessment. BMI, WHR, and hypertension were measured/defined as for the EDC study. As in EDC, HDL-C was isolated by using heparin-2M manganese chloride[276] while total cholesterol and triglycerides were analyzed by enzymatic methods (on a Hitachi 747 analyzer -Boehringer Mannheim Diagnostics, Indianapolis, Indiana)[276]. Non-HDL cholesterol was computed by subtracting HDL-C from total cholesterol.

2.2.3 Menopause Status Assessment

In the EDC study, reproductive history information was self-reported. Menopausal status was defined as follows: women <45 years reporting regular menstrual cycles were classified as pre-menopausal, whereas those >55 years with no menstrual periods for 12 months were classified as post-menopausal. Plasma FSH and estradiol were measured in women falling outside this classification, in which case the Women's Ischemia Syndrome Evaluation (WISE) hormonal and historical algorithms [277] were used to assess menopausal status. Briefly, women who 1) were >50 years, 2) had no menstrual periods for more than 6 months, and 3) had FSH >30 IU/L were classified as post-menopausal. The complete WISE algorithm for determination of menopausal status can be found elsewhere [277]. Age at natural menopause was defined as the chronological age at the time menopause was determined to have occurred (Figure 2.2).

In the SWAN study, annual assessments of menstrual bleeding patterns was used to define the following menopause transition stages: premenopausal (no change in menses regularity), early perimenopausal (menses within the prior 3 months but change in length of bleed or interbleed interval), late perimenopausal (3-11 months without menses), and natural postmenopausal (≥12

months without menses not due to surgery) [276]. For women defined as postmenopausal, their age

at menopause was calculated by subtracting date of birth from date of participants' last menstrual

period [278]. For those women with missing natural menopause age (164 out of 341), a multiple

imputation model (10 runs) was used to estimate their age at natural menopause by utilizing the

following variables: chorological age, race, BMI, smoking status, educational level, employment

status, vasomotor symptoms, bleeding patterns, sex hormone levels, history of hormone use, and

prevalent disease (diabetes, cardiovascular disease, osteoporosis etc.).

In the EDC study and SWAN, the length of reproductive years was determined by

subtracting the age at menarche from the age at natural menopause.

2.2.4 Statistical Analyses

Univariable analyses, including two sample t-test for normally distributed continuous

variables, the Wilcoxon test for non-normally distributed continuous variables, and the Chi-

Squared test or Fisher's exact test for categorical variables, were performed to assess differences

in baseline characteristics between EDC and SWAN female participants. General linear models

and multiple imputation analyses were used to assess whether mean age at natural menopause

differed between EDC women and SWAN women after adjusting for covariates. Multiple

imputation analysis was used to account for uncertainty in the imputation of SWAN menopause

age data by using PROC MIANALYZE in SAS to combine the 10 sets of imputation results.

Adjustments were made for age, race, BMI, smoking status, hypertension, HDL-C, non-HDL, and

the number of pregnancies when assessing age at natural menopause and the length of reproductive

years. Adjustments were made for race only when assessing age at menarche as other covariates

were measured after menarche. Sensitivity analyses were performed 1) excluding the SWAN

imputed data (164 out of 341) or 2) including 18 EDC women who provided self-reported age at natural menopause without sex hormone data before visit 12 thus increased the EDC sample size to 123. To address potential bias from excluding 37 EDC women who had not yet reached menopause and whose age at menopause may be later than those partaking in the analyses, we conducted sensitivity analyses assigning the mean age at natural menopause in SWAN to these 37 women. SAS version 9.4 (SAS Institute, Cary, NC) was used for all analyses.

2.3 RESULTS

Although covariate data for female EDC study participants were selected from a follow-up visit so that chronological age was more similar to that of non-diabetic women at SWAN baseline, women in the T1D cohort were statistically significantly younger (42.8±7.3 vs. 46.0±2.5 years, p=0.0003). Their mean diabetes duration was 33.5±8.0 years and their age at diabetes onset was 9.3±3.9 years. Differences between women with and without type 1 diabetes were also observed in terms of race/ethnicity, with the vast majority (96.2%) of women in EDC being non-Hispanic white and only 3.8% being black, whereas the proportion of non-Hispanic white women in SWAN was statistically significantly lower by study design (68.3% non-Hispanic white vs. 31.7% black, p<0.0001). In addition, compared to women without diabetes, those with type 1 diabetes were less likely to smoke (never smoker: 67.3% vs. 48.1%, p=0.002), and had lower BMI (25.2 vs. 26.5 kg/m², p=0.002), diastolic blood pressure (65.0 vs. 71.0 mmHg, p<0.0001), total cholesterol (181.0 vs. 193.5 mg/dl, p=0.0003), LDL cholesterol (100.9 vs. 117.0 mg/dl, p<0.0001) and triglyceride concentrations (74.0 vs. 86.0 mg/dl, p=0.002), but higher HDL-C levels (61.0 vs. 53.0 mg/dl, p<0.0001). No other statistically significant differences were observed (Table 2.1).

Compared with women without diabetes, those with type 1 diabetes had a 0.6-year delay in menarche (13.2±1.7 vs. 12.6±1.5 years, p=0.0002). The delay in menarche was restricted to women who were diagnosed with type 1 diabetes before reaching menarche, (n=80), among whom the age at menarche was 13.6±1.7 years (p<0.0001 compared with non-diabetic women). The age at menarche did not statistically significantly differ between women with type 1 diabetes whose diabetes onset occurred after menarche (12.2±1.2 years, n=25) and SWAN study participants (p=0.173) (Table 2.1).

At the last available follow-up, the mean age of women with type 1 diabetes was 58.7±6.1 years and of non-diabetic women was 61.0±7.4 years (p<0.0001). Women with type 1 diabetes were also less likely to have ever used oral contraceptives (63.5% vs. 76.8%, p=0.007) and to have been pregnant (72.4% vs. 90.0%, p<0.0001). Among those who reported at least one pregnancy (n=76, 307), the mean number of pregnancies (2.4±1.3 vs. 3.2±1.5, p<0.0001) and the mean number of live births (1.4±0.8 vs. 2.3±1.0, p<0.0001) were both lower in women with type 1 diabetes compared with women without diabetes (Table 2.2).

The unadjusted age at natural menopause was 2.6 years younger in women with type 1 diabetes compared to women without diabetes (49.5±4.1 vs. 52.1±3.3 years, p<0.0001) and this finding was statistically significant both among women who were diagnosed with type 1 diabetes before (p<0.0001 compared with non-diabetic women) and after (p=0.037 compared with non-diabetic women) menarche. Similarly, women with type 1 diabetes had 3.3 years shorter unadjusted length of reproductive period compared to non-diabetic women (36.2±4.4 vs. 39.5±3.0 years, p<0.0001), irrespective of whether type 1 diabetes onset preceded (p<0.0001) or followed (p=0.020) menarche (Table 2.2). Similar results were obtained when analyses were restricted to SWAN women with observed natural menopause data only (n=178) or when the 18 EDC women

who self-reported age at beginning of menopause were included in the analytic sample (n=123) (Table 2.2).

In multivariable analyses, the delay in age at menarche was still 0.6 years (p=0.0008) after adjusting for race. The difference in age at natural menopause between these two cohorts was reduced from 2.6 years (p<0.0001, unadjusted) to 1.8 years (p<0.0001, after adjusting for age, race, BMI, smoking status, hypertension, HDL-C level, having ever taken oral contraceptives, and number of pregnancies). After adjustment, type 1 diabetes was associated with 2.3 fewer reproductive years (p<0.0001). When multivariable models were stratified by menarche onset before or after type 1 diabetes onset, differences remained statistically significant only among women whose onset of type 1 diabetes occurred prior to menarche. Age at menarche (p=0.141), age at natural menopause (p=0.080) and the length of women's reproductive period (p=0.276) were not statistically significantly different between women whose menarche onset preceded type 1 diabetes development and non-diabetic women in these multivariable analyses (Table 2.3).

Similar results were apparent when analysis included SWAN women with observed natural menopause data only (n=176) or including 18 EDC women who provided self-reported age at natural menopause without sex hormone data (n=121) (Table 2.3). Similarly, results did not change significantly when we assigned the mean age at natural menopause in SWAN to the 37 EDC women who had not yet reached menopause (0.4 years delay in menarche, 1.8 years earlier in natural menopause, and 2.9 fewer reproductive years in women with type 1 diabetes).

2.4 DISCUSSION

In the current study, we found that the length of the reproductive period of women with type 1 diabetes onset prior to menarche was 2.9 years shorter (1-year delay in menarche and 1.8 years early in menopause) compared to women without diabetes, a difference that persisted after adjusting for age, race/ethnicity, BMI, smoking status, hypertension, lipid concentrations, number of pregnancies, and oral contraceptive use. However, no association between type 1 diabetes and age at menarche, age at natural menopause, and length of reproductive years was found when type 1 diabetes developed after menarche although the small sample size (n=25) may undermine the statistical power to detect a difference. Another possible explanation about the findings which were restricted to women whose type 1 diabetes onset occurred prior to menarche may be that type 1 diabetes occurring before menarche would lead to a greater disruption of the female reproductive system compared with type 1 diabetes occurring after menarche.

Previously, data from the large, international, EPIC study suggested that women with diabetes diagnosed before the age of 20 years had an earlier menopause compared with non-diabetic women, whereas women with diabetes diagnosed after the age of 50 years had a later menopause [237]. However, the EPIC study could not distinguish the effect of type 1 and type 2 diabetes and the sequence of diabetes development and menopause onset in women with a late age at diabetes [237]. The magnitude of the difference in age at natural menopause between female participants with type 1 diabetes and non-diabetic controls in the present study (1.8 years) was far smaller than observed in the FDA study (~ 6.4 years) [236]. The reasons for this difference may relate to the very small sample size of women with type 1 diabetes in the FDA study (n=15), as well as the selection of the controls. A total of 96 healthy women and their mothers and female siblings were examined and served as controls in the FDA study, but the authors did not take into account

for clustering between the diabetes cases and controls in their analysis [236]. Furthermore, selection of controls from the same household may compromise the representativeness of the control sample, although it takes into account any genetic influences[236]. On the contrary, the Finnish study failed to detect a difference in age at natural menopause by type 1 diabetes, although only population level data were available for non-diabetic controls, which prohibited adjustment for potential confounders [239]. In addition, in the Finnish study, the median age at diagnosis of type 1 diabetes (12.8 years) was very close to the median age at menarche (13 years) which means that half of the study participants were diagnosed with type 1 diabetes after menarche and investigators did not assess whether the timing of type 1 diabetes onset relative to menarche affected study findings [239]. It is thus possible that a small difference in the age at menopause was not made apparent in the entire population studied. Similarly, the OVADIA study failed to detect a difference as well, which could also be attributed to the proportion of women who were diagnosed with type 1 diabetes after menarche (mean age at diabetes onset: 28±14.2 years) as both childhood and adult-onset type 1 diabetes patients were included in the OVADIA study analysis [238].

A possible mechanism underlying the observed shorter length of the reproductive years of women with type 1 diabetes may relate to the disruption of the hypothalamus-pituitary-ovary (HPO) axis function and premature ovarian aging caused by endogenous insulin deficiency, hyperglycemia and exogenous hyperinsulinemia. It is well-known that insulin receptors (IRs) are widely distributed in the central nervous system (CNS) [141] and the ovaries [142,143]. Thus, insulin plays a key role in maintaining the normal function of the female's HPO axis, not only through its impact on the upstream component – neurons in the CNS [141] but also on the downstream component - granulosa, thecal, and stromal in the ovaries [142,143]. In mice with a neuron-specific destruction of the insulin receptor gene, *INSR*, female mice showed impaired ovarian follicle

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maturation because of dysregulation of luteinizing hormone (LH) resulting from hypothalamic dysregulation [141]. In addition, restoration of the function of the IRs in the brain through genetic reconstitution experiments normalized the reproductive function of female *INSR* knockout mice [144]. Moreover, studies have suggested the presence of direct central effects of insulin on the neuroendocrine system by using primary hypothalamic cell cultures and a GnRH neuronal cell line [146,147]. At the ovarian level, the downstream element of the HPO axis, insulin exerts influences by its gonadotropin-like function [148] or through enhancing steroidogenesis responses to gonadotropins [149-152].

Due to the important role of insulin in maintaining the normal function of the female HPO axis, the disruption in insulin regulation in women with type 1 diabetes may underlie our findings of delayed menarche and premature ovarian aging compared with general population. Meanwhile, elevated concentrations of advanced glycation end-products (AGE) caused by hyperglycemia comprise another potential mechanism for premature ovarian aging in women with type 1 diabetes. Existing studies have suggested that the interaction between AGE and receptor for advanced glycation end-products (RAGE) triggers/accelerates oxidative stress (OxS) and inflammation, contributing to cell, tissue, and vascular damage [184,185], and leading to the development of diabetes complications [181,182]. Although no study has directly investigated whether elevated AGE concentrations cause ovarian dysfunction in women with type 1 diabetes, it is logical to postulate that AGE/RAGE also affect the normal function of the ovaries, leading to premature ovarian aging in women with type 1 diabetes, based on their damaging effect on cells and tissues. In addition, in type 1 diabetes blood insulin levels are often increased in the systemic circulation as exogenous insulin does not go through an initial liver passage and thus avoids significant early clearance.

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Increased exposure of the ovary tissue to insulin might lead to excess follicle recruitment and thus accelerated depletion of the ovarian reserve due to the gonadotropic function of insulin [279].

A limitation of the present study is the different ascertainment of menopause status in the EDC study and SWAN. However, a previous study has suggested that the two algorithms (WISE and SWAN) agreed for 73% of the SWAN women for menopausal status determination, with especially high concordance for classifying postmenopausal status (30.5% of women classified as postmenopausal by SWAN algorithms and 32.7% of women classified as postmenopausal by WISE algorithm) [280]. Another limitation of the present analysis was the unavailability of data on reproductive health (especially hormone levels) prior to the 12th follow-up visit of the EDC study. Thus, selection or survival bias may have been introduced given that women who did not participate in assessments post 2009 due to death, dropout, or other reasons, were excluded from analyses. However, early loss due to death or ill health would also be more likely to be associated with an earlier age at menopause, again confirming the main finding. Sensitivity analyses addressing some of these limitations confirm the main finding of a younger age at natural menopause in type 1 diabetes.

The present study identified the subgroup of women with type 1 diabetes who have a high likelihood of experiencing early age at natural menopause so that efforts to unearth the biologic rationale and target potential prevention practices would be better focused. Given the high likelihood of experiencing early menopause in type 1 diabetes, and the enormous impact on health associated with early menopause, further studies are needed to determine modifiable factors that contribute to early menopause to improve reproductive health in women with type 1 diabetes.

Table 2.1 Characteristics of Women with (EDC study) or without (SWAN study) Type 1 Diabetes

Characteristics at baseline	EDC (n=105)	SWAN (n=341)	p-value
Age (years, n=105, 341)	42.8 (7.3)	46.0 (2.5)	0.0003
Age at diabetes onset (years, n=105, -)	9.3 (3.9)	-	-
Age at menarche (years, n=105, 338)	13.2 (1.7)	12.6 (1.5)	0.0002
T1D onset before menarche (n=80, 338)	13.6 (1.7)	12.6 (1.5)	<0.0001
T1D onset after menarche (n=25, 338)	12.2 (1.2)	12.6 (1.5)	0.173
Non-Hispanic white women only (n=101, 230)	13.2 (1.7)	12.6 (1.4)	0.002
Data adding 18 EDC women (n=123, 338) [#]	13.2 (1.7)	12.6 (1.5)	0.0001
Diabetes duration (years, n=105)	33.5 (8.0)	-	-
Race (%, n=105, 341)			
Non-Hispanic White	96.2 (101)	68.3 (233)	<0.0001
Black	3.8 (4)	31.7 (108)	
Marital status (%, n=104, 341)			
Single, never married	13.5 (14)	10.3 (35)	0.516
Currently married or cohabitating	69.2 (72)	66.3 (226)	
Divorced	12.5 (13)	16.1 (55)	
Separated, Widowed, or others	4.8 (5)	7.3 (25)	
Education (%, n=104, 341)			
High school or less	31.7 (33)	24.3 (83)	0.250
Some college or received bachelor's degree	51.0 (53)	53.1 (181)	
Graduate education beyond bachelor's degree	17.3 (18)	22.6 (77)	
BMI (kg/m^2, n=105, 338)	25.2 (22.6, 28.1)	26.5 (23.7, 30.9)	0.002
Waist to hip ratio (n=94, 334)	0.81 (0.76, 0.86)	0.79 (0.75, 0.84)	0.099
Smoking status (%, n=104, 339)			
Never smoker	67.3 (70)	48.1 (163)	0.002
Past smoker	16.4 (17)	28.9 (98)	
Current smoker	16.4 (17)	23.0 (78)	
Systolic blood pressure (mmHg, n=105, 341)	114.0 (102.0, 124.0)	111.0 (104.0, 123.0)	0.708
Diastolic blood pressure (mmHg, n=105, 341)	65.0 (58.0, 71.0)	71.0 (66.0, 79.0)	<0.0001

Characteristics at baseline	EDC (n=105)	SWAN (n=341)	p-value
Blood pressure medication use (%, n=105, 341)	16.2 (17)	8.8 (30)	0.031
Lipid medication use (%, n=105, 341)	19.05 (20)	0.29 (1)	<0.0001 *
Total cholesterol (mg/dl, n=105, 340)	181.0 (162.0, 197.0)	193.5 (173.5, 219.0)	0.0003
HDL cholesterol (mg/dl, n=105, 340)	61.0 (50.6, 73.0)	53.0 (46.0, 62.0)	<0.0001
LDL cholesterol (mg/dl, n=84, 329)	100.9 (87.0, 127.4)	117.0 (101.0, 140.0)	<0.0001
Triglycerides (mg/dl, n=86, 330)	74.0 (51.0, 100.0)	86.0 (65.0, 125.0)	0.002

Data are means (SD), median (interquartile range) or percent (n)

Sensitivity analysis included 18 EDC women who provided self-reported age at natural menopause without sex hormone data prior to the 22-year assessment

Table 2.2 Characteristics of Women with (EDC study) or without (SWAN study) Type 1 Diabetes at the Last

Available Follow-up

Characteristics	EDC (n=105)	SWAN (n=341)	p-value
Age (years, n=105, 341)	58.7 (6.1)	61.0 (7.4)	<0.0001
Diabetes duration (years, n=105)	49.5 (6.8)	-	-
Percent ever on oral contraceptives (%, n=104, 341)	63.5 (66)	76.8 (262)	0.007
Percent ever pregnant (%, n=105, 341)	72.4 (76)	90.0 (307)	<0.0001
Number of pregnancies (n=76, 307)	2.4 (1.3)	3.2 (1.5)	<0.0001
Number of live births (n=76, 300)	1.4 (0.8)	2.3 (1.0)	<0.0001
Age at natural menopause (n=105, 341)	49.5 (4.1)	52.1 (3.3)	<0.0001
T1D onset before menarche (n=80, 341)	49.3 (4.0)	52.1 (3.3)	<0.0001
T1D onset after menarche (n=25, 341)	50.2 (4.4)	52.1 (3.3)	0.037
Observed data only (n=105, 178)	49.5 (4.1)	52.1 (2.8)	<0.0001
Non-Hispanic white women only (n=101, 231)	49.6 (3.9)	52.2 (3.2)	<0.0001
Data adding 18 EDC women (n=123, 341) [#]	48.9 (4.7)	52.1 (3.3)	<0.0001
Reproductive years (n=105, 338)	36.2 (4.4)	39.5 (3.0)	<0.0001
T1D onset before menarche (n=80, 338)	35.7 (4.2)	39.5 (3.0)	<0.0001
T1D onset after menarche (n=25, 338)	38.0 (4.6)	39.5 (3.0)	0.020
Observed data only (n=105, 177)	36.2 (4.4)	39.6 (3.2)	<0.0001
Non-Hispanic white women only (n=101, 230)	36.3 (4.3)	39.6 (2.9)	<0.0001
Data adding 18 EDC women (n=123, 338) [#]	35.7 (4.9)	39.5 (3.0)	<0.0001

Data are means (SD) or percent (n)

[#] Sensitivity analysis included 18 EDC women who provided self-reported age at natural menopause without sex hormone data prior to the 22-year assessment.

Table 2.3 Effect of Type 1 Diabetes on the Length of Reproductive Period

Characteristics	Type 1 diabetes (Beta coefficient, 95% CI)	p-value
Age at menarche^ (n=105, 338)	0.6 (0.3,1.0)	0.0008
T1D onset before menarche^ (n=80, 338)	1.0 (0.6,1.4)	<0.0001
T1D onset after menarche^ (n=25, 338)	-0.5 (-1.1, 0.2)	0.141
Data adding 18 EDC women^ (n=123, 338) [#]	0.6 (0.2, 1.0)	0.0049
Age at natural menopause* (n=103, 336)	-1.8 (-2.5, -1.0)	<0.0001
T1D onset before menarche* (n=78, 336)	-1.8 (-2.7, -1.0)	<0.0001
T1D onset after menarche* (n=25, 336)	-1.0 (-2.1, 0.1)	0.080
Observed data only* (n=103, 176)	-2.0 (-2.9, -1.0)	<0.0001
Data adding 18 EDC women* (n=121, 336) [#]	-1.8 (-2.5, -1.0)	<0.0001
Reproductive years* (n=103, 336)	-2.3 (-3.1, -1.5)	<0.0001
T1D onset before menarche* (n=78, 336)	-2.9 (-3.8, -2.0)	<0.0001
T1D onset after menarche* (n=25, 336)	-0.7 (-1.9, 0.6)	0.276
Observed data only* (n=103, 176)	-2.5 (-3.6, -1.5)	<0.0001
Data adding 18 EDC women* (n=121, 336) [#]	-2.4 (-3.2, -1.5)	<0.0001

Models were constructed among study participants with complete covariate data

^Model allowed for race

*Model allowed for age, race, BMI, smoking status, blood pressure, HDL-C, non-HDL-C, number of pregnancies, and having ever taken oral contraceptives

[#]Sensitivity analysis included 18 EDC women who provided self-reported age at natural menopause without sex hormone data prior to the 22-year assessment.

463 participants at
SWAN Pittsburgh site

Excluded:
- 74 Developed diabetes
  before or during follow-ups
- 48 experienced surgical
  menopause

341 participants were
included in analyses
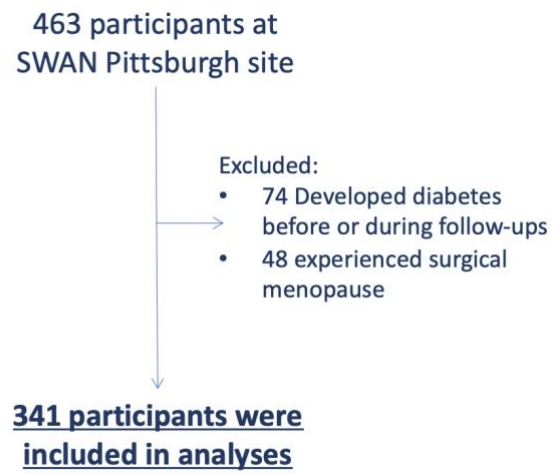
Figure 2.1 Flow Chart of the Pittsburgh Site of the Study of Women's Health Across the Nation (SWAN)

Age

<45 yrs   45-55 yrs   >55 yrs

Menstrual cycles

No menstrual periods >12 months

Regular   Irregular   No   Yes

Pre-menopausal   Blood test: FSH and E2   Post-menopausal

Regular periods
or
LMP<3 months

Irregular periods
or
LMP≥3 months

FSH<10   FSH≥10

Modified from Women's Ischemia Syndrome Evaluation (WISE) hormonal and historical algorithms

Johnson BD, et al. Determination of menopausal status in women: the NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE) Study. J Womens Health (Larchmt). Oct 2004;13(8):872-887.

FSH<20   FSH≥20

LMP≤6 months and E2<200   LMP>6 months or E2≥200

FSH> 30 and Age> 50 and LMP> 6 months

No   All others   Yes

Pre-menopausal   Peri-menopausal   Pre-menopausal   Peri-menopausal   Post-menopausal

Note:
- E2: estradiol, FSH: follicle-stimulating hormone, LMP: last menstrual period
- In EDC, we excluded women who received hysterectomy or bilateral oophorectomy before menopause or who are currently on hormone replacement therapy during menopause transition
- In EDC, we did not make a distinction between peri- and pre-menopause. These two categories were grouped together.

Figure 2.2 Determination of Menopausal Status in the EDC Study

3.0 MANUSCRIPT 2: PREDICTORS OF AGE AT NATURAL MENOPAUSE IN WOMEN WITH TYPE 1 DIABETES: THE PITTSBURGH EPIDEMIOLOGY OF DIABETES COMPLICATIONS (EDC) STUDY

Objective: Women with type 1 diabetes are thought to experience menopause earlier than women without diabetes, although not all studies agree. We assessed metabolic predictors of age at natural menopause in women with type 1 diabetes.

Methods: Female participants of the Epidemiology of Diabetes Complications (EDC) Study of childhood-onset (<17 years) type 1 diabetes who experienced natural menopause and who never received hormone therapy during their menopausal transition were included in the analysis (n=105; mean baseline age, 29.5 and diabetes duration, 20.2 years). Self-reported reproductive history and the Women's Ischemia Syndrome Evaluation hormonal algorithms were used to assess menopause status. Linear regression models were used to assess whether time-weighted metabolic factors (e.g., BMI, lipids, HbA1c, insulin dose, and albumin excretion rate (AER)) were associated with age at natural menopause.

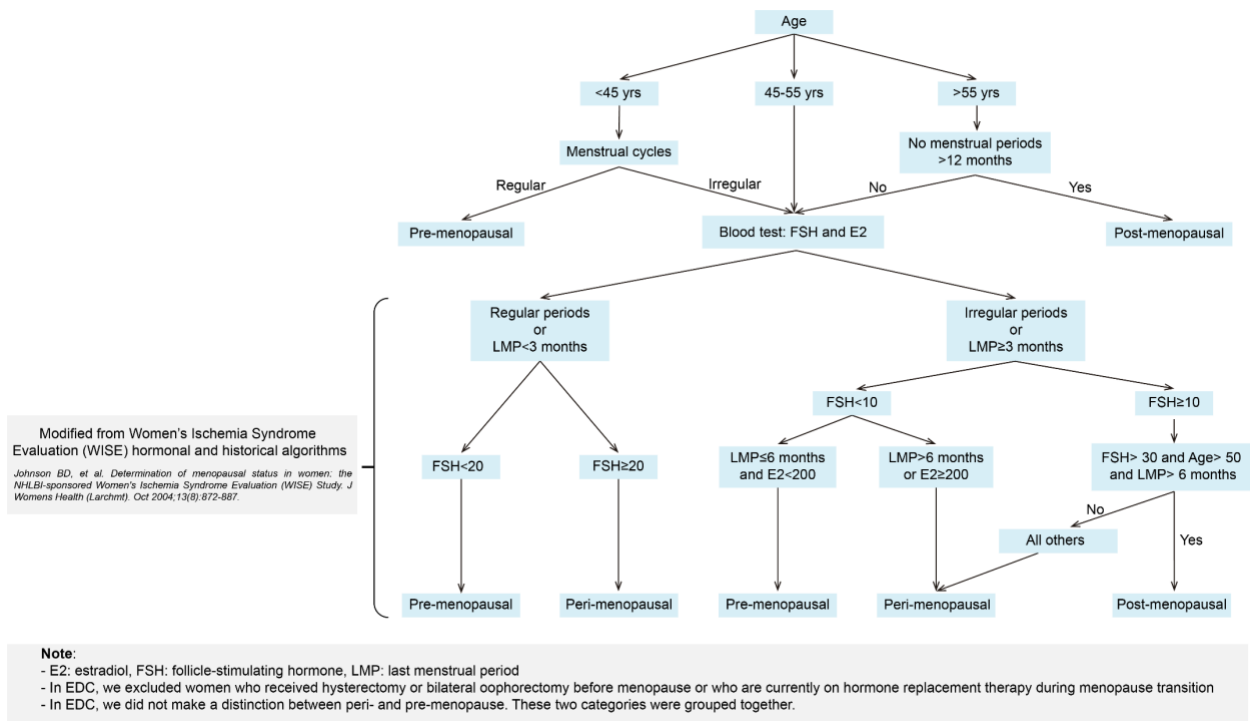**Results:** In univariate models, insulin dose (p=0.04) and AER (p=0.02) were inversely associated with age at natural menopause. No other statistically significant associations were observed. In multivariable models, each 0.1 unit increase in insulin dose per day per kilogram body weight was associated with 0.64 years younger age at natural menopause (p=0.01), while for every 30% increase in AER, age at natural menopause decreases by 0.18 years (p=0.03).

Conclusions: Higher average levels of insulin dose and AER over time were significantly associated with a younger age at natural menopause in women with type 1 diabetes. The biologic

mechanisms underlying the observed associations between exogenous insulin dose and AER on the reproductive health of women with type 1 diabetes should be investigated.

3.1 INTRODUCTION

Natural menopause is the cessation of ovarian function and the end of women's reproductive life, resulting from natural oocyte depletion. Age at natural menopause and its related factors have attracted great research interest due to the substantial impact menopause has on women's health. Earlier age at menopause is associated with increased risks of cardiovascular disease [227], osteoporosis [231], and fracture [232] later in life. Importantly, earlier age at menopause is related to increased all-cause mortality [225] and also mortality from cardiovascular disease [226,227], atherosclerosis [228], and stroke [229], with a 2% increase in age-adjusted mortality per year decline in age at menopause [230].

Due to the important role of insulin in maintaining normal functioning of the female reproductive system, it is logical to postulate that women with type 1 diabetes who have a disruption in insulin regulation might experience earlier natural menopause compared to women without diabetes. Indeed, several studies have provided evidence to support this hypothesis, although not all studies agree [238,239]. The Familial Autoimmune and Diabetes (FAD) study was the first to report that women with type 1 diabetes reached menopause at a younger age compared with their nondiabetic sisters or unrelated control subjects [236]. The European Prospective Investigation into Cancer and Nutrition (EPIC) study also suggested that early-onset diabetes (onset before the age of 20 years) was associated with an earlier onset of menopause, compared with nondiabetic controls [237]. In our recent study, we noted that natural menopause occurred 1.8 years earlier in

women with childhood-onset type 1 diabetes (adjusted mean age at menopause 50.1 years) compared with non-diabetic women (adjusted mean age at menopause 51.9 years) after adjustment for age at baseline, age at menarche, race, BMI, smoking status, blood pressure, HDL, and non-HDL cholesterol, number of pregnancies, and having ever taken oral contraceptives (manuscript submitted for publication).

Nevertheless, data on determinants of an earlier age at menopause in women with type 1 diabetes are scarce. In the general population, it is well known that smoking accelerates natural menopause onset [240,241], whereas high parity is associated with later natural menopause onset [222]. However, little is known about the effects of traditional risk factors, such as smoking and BMI, or diabetes-specific factors, such as glycemic control, insulin dose, and diabetes complications, on the age at natural menopause in women with type 1 diabetes. Previous work from the Diabetes Control and Complications Trial (DCCT) and its observational follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study examined the impact of intensive treatment, HbA1c, and microvascular complications on menopause onset among women with type 1 diabetes [243]. However, in this study, the age at menopause did not differ by treatment group.

Given evidence of an earlier menopause onset in type 1 diabetes, and the enormous health impact induced by early menopause, identifying modifiable factors which contribute to early menopause in type 1 diabetes would have great public health significance. Therefore, our objective was to identify metabolic factors (HbA1c, lipids, blood pressure, insulin dose, etc.) that are independently associated with age at natural menopause in a cohort of women with type 1 diabetes.

3.2 METHODS

3.2.1 Study Population

The Pittsburgh Epidemiology of Diabetes Complications (EDC) Study recruited childhood-onset (<17 years) type 1 diabetes patients diagnosed, or seen within one year of diagnosis, at Children's Hospital of Pittsburgh between 1950 and 1980. All participants (n=658) completed a baseline assessment in 1986-1988. The mean baseline age was 28 years (range 8-48 years) and the duration of type 1 diabetes was 19 years (range 8-37 years). These 658 participants (325 female and 333 male) were subsequently surveyed or reexamined biennially for up to 30 years. The EDC Study has been described in detail elsewhere [126]. All study participants provided written informed consent prior to performing any study procedures. The EDC study protocol was approved by the University of Pittsburgh Institutional Review Board.

For this study, of the 325 female EDC participants, we excluded women who did not complete the sex hormone tests due to missingness (n=53) or death (n=75) and those who had a hysterectomy/oophorectomy before menopause (n=35) or received sex hormone therapy during the menopausal transition (n=20). Additionally, we excluded women who had not yet reached menopause at their last available follow-up (n=37, age 18.3±4.0 years at baseline and 47.5±3.7 years at their last available follow-up). Therefore, a total of 105 female EDC participants who had gone through natural menopause were included in the data analyses (Figure 1.2). Comparisons of women who were or were not included in analyses were presented in Table 3.4.

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3.2.2 Covariate Assessment

Risk factors were assessed at baseline and repeated at 2-, 4-, 6-, 8-, 10-, and 18-years of follow-up. Survey questionnaires regarding demographic characteristics, medical history, and diabetes self-care (e.g., daily insulin dose per kilogram body weight) were sent to each participant before each clinical examination visit. During the clinical visits, body mass index (BMI) was measured as weight in kilograms (kg) divided by height in meters squared (m2). The waist to hip ratio (WHR) was measured as the circumference of the waist divided by that of the hips. Blood pressure was measured according to the HDFP protocol [281]. Hypertension was defined as blood pressure $\geq$140/90 mm Hg or use of antihypertensive medications. The level of stable glycosylated hemoglobin (HbA1) was measured using Ion exchange chromatography (Isolab, Akron, OH) during the first 18 months of the study, whereas automated high-performance liquid chromatography (Diamat, BioRad, Hercules, CA) was used for the remainder of the 10-year follow-up. These two assays were highly correlated (r=0.95). Original HbA1 measures were converted to DCCT standard HbA1c values by using a regression equation derived from duplicate analyses (DCCT HbA1c = [0.83 X EDC HbA1] + 0.14) [282] from baseline through 10 years of follow-up. At the 18-year follow-up, HbA1c was measured using the DCA 2000 analyzer (Bayer Healthcare LLC. Elkhart, IN) and converted to DCCT standard HbA1c values using an equation (DCCT HbA1c = [EDC HbA1c-1.13]/0.81) [282]. Glucose disposal rate was estimated (eGDR) by using a regression equation derived from hyperinsulinemic-euglycemic clamp studies of 24 participants chosen to represent the full spectrum of insulin resistance (eGDR (mg/kg/min)=24.395-(12.971*WHR)-(3.388*Hypertension)-(0.601* HbA1c)) [283].

From baseline through 10 years of follow-up, high-density lipoprotein cholesterol (HDL-C) was measured by means of a precipitation technique (heparin and manganese chloride) with a

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modification of the Lipid Research Clinics method [126,275]. Total cholesterol and triglycerides were measured enzymatically [126,275]. At the 18-year follow-up, serum lipids were measured using the Cholestech LDX (Cholestech Corp., Hayward, CA). Non-HDL cholesterol (non-HDL-C) was computed by subtracting HDL-C from total cholesterol. White blood cell count was measured using a counter S-plus IV. Serum and urinary albumin were measured by immunonephelometry [284], and creatinine was assayed by an Ectachem 400 Analyzer (Eastman Kodak Co., Rochester, NY). Glomerular filtration rate was estimated (eGFR) by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine formula [285]

3.2.3 Menopause Status Assessment

Reproductive history information was self-reported. Natural menopause is defined as cessation of menstruation, which is not induced by surgical procedures or medications, including hysterectomy, oophorectomy, hormone medications etc. Women who had a hysterectomy/oophorectomy before menopause or received sex hormone therapy during the menopausal transition were thus excluded from analysis. Menopausal status was defined as follows: women <45 years reporting regular menstrual cycles were classified as pre-menopausal, whereas those >55 years with no menstrual periods for 12 months were classified as post-menopausal. Plasma follicle stimulating hormone (FSH) and estradiol were measured in women falling outside this classification, in which case the Women's Ischemia Syndrome Evaluation (WISE) hormonal and historical algorithms [277] were used to assess menopausal status, but not making a distinction between peri- and pre-menopausal women (Figure 2.2).

3.2.4 Statistical Analyses

Time-weighted mean values of continuous metabolic factors were constructed based on clinical assessments at baseline and at 2-, 4-, 6-, 8-, 10-, and 18-years of follow-up. They were used for all continuous metabolic factors of interest to represent their average levels over time up to the exam cycle prior to the onset of menopause. The time-weighted mean values were calculated as a sum of the products of average values from two consecutive follow-up visits multiplied by the time interval (years) between the two visits and then divided by the total follow-up time until the examination cycle prior to reaching natural menopause, e.g., time-weighted BMI = (((BMI1 + BMI2)/2)*(time2 - time1)+((BMI2 + BMI3)/2)*(time3 - time2)+((BMI3 + BMI4)/2)*(time4 - time3)+⋯)/((time2 - time1)+(time3 - time2)+(time4 - time3)+⋯). Risk factors evaluated included BMI, blood pressure, total cholesterol, HDL-C, non-HDL-C, LDL-C, triglycerides, HbA1c, eGDR, insulin dose, albumin excretion rate (AER), eGFR, and white blood cell count (WBC). The distribution of AER was skewed, thus natural log transformation was used for this variable. Univariate and multivariable linear regression models (PROC REG) were used to assess whether time-weighted factors were associated with age at natural menopause. In the multivariable models, covariates were selected if $p<0.2$ in univariate models or if they were previously associated with age at menopause (e.g., smoking status, BMI, lipids, contraceptive use, and pregnancy) [222]. To assess the importance of insulin resistance, models were also constructed with eGDR as a covariate. However, since eGDR is derived from WHR, hypertension, and HbA1c, these three variables were excluded in models with eGDR. SAS version 9.4 (SAS Institute, Cary, NC) was used for all analyses.

3.3 RESULTS

Among the 105 EDC women in the present study, mean baseline age was 29.5 years and duration of type 1 diabetes, 20.2 years. All these 105 women had been pre-menopause at the baseline assessment. The vast majority (96.2%) were non-Hispanic white. A summary of the study population's characteristics at the baseline assessment is presented in Table 3.1. By the last available follow-up (after an average of 29.2±7.1 years from baseline), 63.5% of the study participants reported having used oral contraceptives and 72.4 % had been pregnant. Among those who reported at least one pregnancy (n=76), the mean number of pregnancies was 2.4 and the mean number of live births was 1.4. The average age at natural menopause in the study population was 49.5 years. A comparison of women who were or were not included in analyses were presented Table 3.4.

In univariate linear regression models (Table 3.2), insulin dose (β±Standard Error (SE)=-4.87± 2.3, p=0.04) and ln (AER) (-0.62±0.27, p=0.02) were significantly inversely associated with age at natural menopause. No other statistically significant associations were observed. Insulin dose, ln(AER), and HbA1c were thus included in multivariable linear regression models as their p values were <0.2 and smoking status, BMI, lipids, contraceptive use, and pregnancy were also included as they were associated with age at natural menopause based on previous studies.

In multivariable linear regression (Table 3.3, model 1), after adjustment for BMI, smoking status, HDL-C, non-HDL-C, triglycerides, HbA1c, number of pregnancies, having ever taken oral contraceptives and AER, each 0.1 unit increase in daily insulin dose per kilogram body weight was associated with 0.64 years younger age at natural menopause (p=0.01). Moreover, after adjustment for the above-mentioned time-weighted covariates, including daily insulin dose, one natural log microgram per minute increase in AER was associated with 0.67 years earlier age at natural

menopause (p=0.03). For every 30% increase in AER, age at natural menopause decreases by 0.18 years (back transformation: -0.67 * log (1.30) = -0.18). In the multivariable model with eGDR (Table 3.3, model 2), while insulin sensitivity itself (eGDR) was not associated with age at natural menopause (p=0.21), insulin dose (p=0.01) and ln (AER) (p=0.02) both maintained their significant inverse association.

3.4 DISCUSSION

In the present study, we assessed predictors of age at natural menopause among women with long duration type 1 diabetes. We observed that higher average levels of insulin dose and AER over time were significantly associated with an earlier age at natural menopause after multivariable adjustments, including for HbA1c. Interestingly, despite insulin dose being a predictor after accounting for HbA1c levels, insulin sensitivity (eGDR) did not predict age at natural menopause in a separate model. These findings suggest that the adverse effect of insulin dose does not reflect insulin resistance but rather may suggest that the higher exogenous insulin concentrations have a direct, deleterious effect on ovarian aging in women with type 1 diabetes.

Type 1 diabetes is characterized by endogenous insulin deficiency and thus exogenous insulin administration is needed for survival. However, unlike insulin produced by the pancreas, injected exogenous insulin does not go through hepatic first-pass metabolism and clearance; rather, it goes into systemic circulation directly, and thus peripheral tissues, including the ovary, are exposed to excessive insulin levels in type 1 diabetes[165]. Of further relevance are the well-known effects insulin has in potentiating the effect of gonadotropin-releasing hormone (GnRH) on luteinizing hormone (LH) and FSH in the brain [279] and promoting ovarian growth, cyst formation,

follicular recruitment and growth through its gonadotropin-like effect on the ovaries [164]. In addition, insulin can up-regulate LH receptors and insulin-like growth factor (IGF)-1 receptors in the ovary [279]. It is thus likely that increased exposure of the ovary tissue to excessive insulin could bring about excessive cyst formation and follicle recruitment, resulting in a premature depletion of the primordial follicle pool [279] and early age at menopause. In contrast, findings from the DCCT/EDIC study suggested that greater insulin dose was associated with lower menopause risk in women with type 1 diabetes, although age at menopause was not evaluated as an outcome in this study [243]. Furthermore, it is possible, as study investigators note, that the DCCT/EDIC was a chance finding given the relatively high number of comparisons in the study [243].

It has been suggested that increased oxidative stress is associated with significant adverse effects on women's reproductive function, including ovarian vascular endothelium damage and abnormalities in follicular growth, oocyte maturation, corpus luteum formation, and embryonic growth [192]. While there was no direct evidence in type 1 diabetes, HbA1c ($r = -0.51$, $P < 0.001$), and fasting blood glucose ($r = -0.69$, $P < 0.001$) were found to be negatively correlated with antral follicle count (AFC) in women with type 2 diabetes [286]. We thus hypothesized that poor glycemic control would be associated with premature ovarian aging in type 1 diabetes, given the potential ovarian vascular damage caused by advanced glycation end-product (AGE) induced oxidative stress [190,191]. However, the average level of HbA1c over time was not associated with age at natural menopause after multivariable adjustments. Although in the present study the non-significant finding regarding HbA1c may result from the small sample size (n=105) or the small variability in time-weighted HbA1c (8.6 ± 1.0), our findings are consistent with results from the DCCT/EDIC study [243] and a Finnish study [239] which also reported no HbA1c association with age at menopause.

Within the EDC study, AER was also an independent predictor of menopause onset in women with type 1 diabetes, even after adjustment for diabetes-specific confounders (e.g., HbA1c, insulin sensitivity and insulin dose). A general population study previously suggested that women with chronic kidney disease (CKD) tend to experience menopause earlier than women from the general population (47.2 vs. 47.8 years) [287]. Although a direct impact of CKD on the hypothalamic-pituitary-ovary axis is speculative, CKD could lead to cellular senescence and premature aging through the effects of uremic toxins, oxidative stress and persistent inflammation [288], and premature ovarian aging could be one of the aging phenotypes. Our findings therefore provide further insight on the potential effects of kidney disease on ovarian reserve among women with type 1 diabetes. Further evidence of a role of kidney disease on age at menopause comes for the above-mentioned Finnish study, in which end-stage renal disease (ESRD) was associated with early age at menopause, despite only nine ESRD cases in this analysis [239]. On the contrary, no association between nephropathy and menopause was observed in the DCCT/EDIC study [243]. Should further studies confirm that diabetic kidney complications play a role in ovarian function, women with these two risks of early menopause, type 1 diabetes and kidney complications, could be targeted for timely interventions to prevent the health consequences of premature ovarian aging.

Limitations of the present study were the relatively small sample size (n=105) and lacking racial diversity. Another limitation was that selection or survival bias may have been introduced by excluding from analyses women who died or dropped out prior to menopause onset. One strength of the present study was that the analysis was based on longitudinal repeated measurements, which could better reflect the cumulative effect of metabolic factors on menopause compared with using baseline assessments only.

In conclusion, higher insulin dose (after accounting for HbA1c levels or insulin sensitivity) and increased AER independently predicted age at natural menopause among women with type 1 diabetes. While these results require validation in large cohorts of women with type 1 diabetes, our findings raise significant questions relating to a potentially deleterious effect of high exogenous insulin doses, in addition to that of kidney disease, and may (upon replication) constitute a useful source in clinical reproductive counseling for women with type 1 diabetes.

Table 3.1 Characteristics of EDC Female Participants at the Baseline Assessment, 1986-88 (n=105)

Characteristics	
Age at baseline (years)	29.5 ± 6.2
Age at diabetes onset (years)	9.3 ± 3.9
Duration of type 1 diabetes at baseline (years)	20.2 ± 7.0
Race	
Non-Hispanic White	96.2 (101)
Black	3.8 (4)
Marital status	
Single, never married	37.1 (39)
Currently married or living as if married	48.6 (51)
Divorced	7.6 (8)
Separated, Widowed, or others	6.7 (7)
Education (n=103)	
High school or less	35.0 (36)
Some college or received bachelor's degree	56.3 (58)
Graduate education beyond bachelor's degree	8.7 (9)
Smoking status	
Never smoker	67.6 (71)
Past smoker	13.3 (14)
Current smoker	19.1 (20)
BMI (kg/m^2)	22.9 ± 2.9
Waist to hip ratio (n=104)	0.77 ± 0.05
Systolic blood pressure (mmHg)	107.9 ± 11.7
Diastolic blood pressure (mmHg)	68.5 ± 9.6
Hypertension (n=105)	8.6 (9)
Blood pressure medication use (n=103)	6.8 (7)
Total cholesterol (mg/dl)	181.0 (162.0, 197.0)
HDL-C (mg/dl)	61.0 (50.6, 73.0)
LDL-C (mg/dl) (n=100)	100.9 (87.0, 127.4)
Triglycerides (mg/dl) (n=102)	74.0 (51.0, 100.0)
ACE/ARB use (n=104)	1.9 (2)
Lipid medication use (n=104)	0 (0)
HbA1c (%, n=104)	8.4 ± 1.2
HbA1c (mmol/mol, n=104)	68 ± 13.1
eGDR (mg*kg-1*min-1, n=103)	8.9±1.5
Insulin dose (units/day/kg, n=100)	0.71 ± 0.19

Characteristics	
AER (µg/min)	10.4 (6.2, 25.7)
eGFR (mL/min/1.73 m^2)	102.3 ± 27.9
WBC x 10^3/mm^2	6.4 ± 1.9
At the last available follow-up	
Percent ever contraceptive use (n=104)	63.5 (66)
Percent ever pregnant	72.4 (76)
No. of pregnancies (n=76)	2.4 ± 1.3
No. of live births (n=76)	1.4 ± 0.8
Age at last available follow-up (years)	58.7 ± 6.1
Age at natural menopause (years)	49.5 (4.1)

Data are means (SD), median (IQR) or percent (n)

AER, albumin excretion rate; eGDR, estimated glucose disposal rate; eGFR, estimated glomerular filtration rate; WBC, white blood cell count

Table 3.2 Univariate Association Between Time-weighted Values of Metabolic Factors of Interest and Age at Natural Menopause

Time-weighted values	β coefficient (SE)	95% C.I.	P values
BMI (kg/m^2)	0.001 (0.12)	-0.24, 0.25	0.99
SBP (mmHg)	-0.01 (0.04)	-0.09, 0.07	0.85
DBP (mmHg)	-0.04 (0.05)	-0.15, 0.06	0.43
Total cholesterol (mg/dl)	-0.01 (0.01)	-0.04, 0.01	0.36
HDL-C (mg/dl)	-0.01 (0.03)	-0.08, 0.06	0.76
Non-HDL-Cl (mg/dl)	-0.01 (0.01)	-0.04, 0.02	0.45
LDL-C (mg/dl)	-0.01 (0.02)	-0.04, 0.02	0.58
Triglycerides (mg/dl)	-0.002 (0.01)	-0.02, 0.02	0.83
HbA1c (%)	-0.61 (0.40)	-1.41, 0.18	0.13
eGDR (mg*kg-1*min-1)	-0.22 (0.28)	-0.78, 0.33	0.42
Insulin dose (units/kg/day)	-4.87 (2.3)	-9.46, -0.28	0.04*
ln(AER) (μg/min)	-0.62 (0.27)	-1.15, -0.10	0.02*
eGFR (mL/min/1.73 m^2)	-0.02 (0.02)	-0.05, 0.02	0.35
WBC x 10^3/mm^2	0.06 (0.23)	-0.38, 0.51	0.78

Table 3.3 Multivariable Linear Regression Analysis for the Prediction of Age at Natural Menopause

Variables	Model 1		Model 2	
	β coefficient (s.e.)	p-value	β coefficient (s.e.)	p-value
Ever smoked	0.50 (1.20)	0.68	0.41 (1.21)	0.74
Time-weighted BMI (kg/m^2)	-0.12 (0.13)	0.39	-0.07 (0.13)	0.57
Time-weighted HDL-C(mg/dl)	-0.06 (0.04)	0.11	-0.05 (0.04)	0.25
Time-weighted non-HDL-C (mg/dl)	-0.01 (0.02)	0.52	-0.01 (0.02)	0.47
Time-weighted triglycerides (mg/dl)	0.02 (0.02)	0.21	0.01 (0.02)	0.53
Time-weighted HbA1c (%)	-0.76 (0.44)	0.09	NA	NA
Time-weighted eGDR (mg*kg-1*min-1)	NA	NA	-0.43 (0.35)	0.21
Time-weighted insulin dose (units/day/kg)	-6.35 (2.49)	0.01*	-6.3 (2.51)	0.01*
Time-weighted ln(AER) (µg/min)	-0.67 (0.31)	0.03*	-0.72 (0.31)	0.02*
Ever contraceptive use	0.28 (0.85)	0.74	0.07 (0.86)	0.93
No. of pregnancies	-0.41 (0.28)	0.14	-0.39 (0.28)	0.17

Note:

In model 1, predictors were selected if their p values were less than 0.2 in Table 3.2 (e.g., HbA1c, insulin dose, AER) or based on previous literature (e.g., smoking status, BMI, HDL, non-HDL, triglycerides, contraceptive use, and no. of pregnancies).

In model 2, the importance of insulin resistance was assessed thus it was constructed with eGDR as a covariate. However, since eGDR is derived from WHR, hypertension, and HbA1c (eGDR (mg/kg/min) = 24.395- (12.971 * WHR) - (3.388 * Hypertension) - (0.601 * HbA1c)), HbA1c was excluded from models 2.

Table 3.4 Comparison of Women Who Were or Were Not Included in Analyses

Characteristics at baseline	Women who were included in the analyses (n=105)	Women who died prior to reproductive assessment (n=75)	Women who have not reached menopause (n=37)
Age at baseline (years)	29.5 ± 6.2	32.5 ± 7.7	18.3 ± 4.0
Age at diabetes onset (years)	9.3 ± 3.9	8.6 ± 3.9	6.6 ± 3.6
Duration of type 1 diabetes (years)	20.2 ± 7.0	23.9 ± 7.3	11.7 ± 3.5
Year of diabetes diagnosis			
1950-1964	37.1% (39)	60% (45)	0
1965-1980	62.9% (66)	40% (30)	100% (37)
BMI (kg/m^2)	22.9 ± 2.9	23.8 ± 4.0	22.9 ± 2.8
Waist to hip ratio	0.77 ± 0.05	0.80 ± 0.07	0.78 ± 0.05
Systolic blood pressure (mmHg)	107.9 ± 11.7	117.8 ± 16.4	101.9 ± 8.1
Diastolic blood pressure (mmHg)	68.5 ± 9.6	73.4 ± 12.4	67.9 ± 8.2
Total cholesterol (mg/dl)	181.0 (162.0, 197.0)	209.0 (176.0, 234.0)	170.0 (159.0, 207.0)
HDL cholesterol (mg/dl)	61.0 (50.6, 73.0)	54.7 (45.5, 64.9)	55.7 (51.1, 63.9)
LDL cholesterol (mg/dl)	100.9 (87.0, 127.4)	122.6 (104.3, 146.9)	96.8 (87.0, 122.8)
Triglycerides (mg/dl)	74.0 (51.0, 100.0)	111.0 (75.0, 173.0)	81.0 (61.0, 101.5)
HbA1c (%)	8.4 ± 1.2	9.06 ± 1.7	9.2 ± 1.6
Age at last available follow-up (years)	58.7 ± 6.1	59.9 ± 6.8	47.5 ± 3.7

4.0 MANUSCRIPT 3: ASSOCIATION OF AGE AT DIABETES COMPLICATION DIAGNOSIS WITH AGE AT NATURAL MENOPAUSE IN WOMEN WITH TYPE 1 DIABETES: THE PITTSBURGH EPIDEMIOLOGY OF DIABETES COMPLICATIONS (EDC) STUDY

Objective: Vascular damage is thought to have a role in premature ovarian aging. We thus assessed the association between the presence of, and age at onset of, vascular diabetes complications and age at natural menopause in women with type 1 diabetes.

Methods: Female participants of the Epidemiology of Diabetes Complications study with type 1 diabetes who experienced natural menopause and who never received hormone therapy during their menopausal transition were included in the analysis (n=105). Microalbuminuria (MA), overt nephropathy (ON), proliferative retinopathy (PR), confirmed distal symmetric polyneuropathy (CDSP), and coronary artery disease (CAD), were assessed during biennial clinical exanimations for the first 10 years of follow-up and at year 18, 25 and 30. Menopause status was determined via self-report and sex hormone data. For each complication, separate linear regression models were used to assess whether, compared with women without the complication of interest, an earlier age at complication development (i.e., <30 years of age) was associated with an earlier age at natural menopause.

Results: Although results from multivariable linear regression models suggested a similar age at menopause between women with normo-albuminuria and those diagnosed with MA after 30 years of age, menopause occurred 2.06 years earlier ($\beta\pm SE=-2.06\pm1.08$) among women diagnosed with MA before age 30 (p=0.06). No significant association was observed for other complications.

Conclusions: Among women with type 1 diabetes, menopause occurred earlier in those diagnosed with MA before age 30 compared to those with normo-albuminuria, suggesting that vascular dysfunction associated with early microvascular disease may affect ovarian aging.

4.1 INTRODUCTION

An earlier age at menopause has been associated with higher risks of cardiovascular disease and mortality from cardiovascular causes [226,227], atherosclerosis [228], and stroke [229]. The ovaries are highly vascular and have high rates of blood flow [289]; thus, it is logical to postulate that generalized vascular damage may have adverse impact on ovarian aging. Findings from two previous studies were supportive of this hypothesis [290,291]. Investigators from the Framingham Heart Study showed that each 1% increase in premenopausal Framingham risk score which represents the estimate of total coronary heart disease (CHD) risk (%) over the course of 10 years was related to 1.8 years earlier age at menopause [290]. A pooled analysis of over 170,000 women further found that women who experienced CHD or stroke before age 35 years had a two-fold risk of early menopause (<45 years), suggesting a harmful effect of a compromised vasculature following cardiovascular events on ovarian aging [291].

Individuals with type 1 diabetes are at high risk of vascular damage, which is manifested by macro- and microvascular complications. As discussed in a scientific statement from the American Heart Association and the American Diabetes Association [94], the age-adjusted risk of cardiovascular complications is approximately ten-fold higher in people with type 1 diabetes compared with the general population [95-97], and women are disproportionately affected, relative to their nondiabetic counterparts, than men [94,98]. In addition, cardiovascular events occur earlier in

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people with type 1 diabetes than in the general population [94]. For microvascular complications, investigators from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC) showed that the cumulative incidence of proliferative retinopathy (PR), confirmed distal symmetric polyneuropathy (CDSP), and overt nephropathy (ON) were over 50%, 40%, and 30%, respectively, by 25 years of type 1 diabetes duration [93]. Indeed, compared to non-diabetic women, women with a diagnosis of type 1 diabetes were shown to experience an earlier natural menopause [236,237], although not all studies agree [238,239].

Conflicting findings have also been reported regarding the association between vascular complications and age at natural menopause among women with type 1 diabetes. A Finnish study found that proliferative retinopathy (n=17) and end-stage renal disease (n=9) were independently associated with earlier menopause[239]. However, data from the Diabetes Control and Complications Trial (DCCT) and its observational follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, suggested that microvascular complications did not affect the risk of menopause, although their role in the timing of menopause was not evaluated [243]. To date, no study has assessed the impact of timing of complication development on age at menopause. Therefore, the aim of the present study was to assess whether the age at the onset of vascular diabetes complications is predictive of the age at natural menopause in women with type 1 diabetes. We hypothesized that women with an earlier onset of vascular complications experience menopause earlier.

4.2 METHODS

4.2.1 Study Population

The Pittsburgh Epidemiology of Diabetes Complications (EDC) Study is a prospective cohort study of childhood-onset (<17 years) type 1 diabetes. All participants were diagnosed, or seen within one year of diagnosis, at Children's Hospital of Pittsburgh between 1950 and 1980. The EDC study has been described in detail elsewhere [126]. Briefly, there were 658 participants (325 female and 333 male) who completed a baseline assessment in 1986-1988. The mean baseline age was 28 years (range 8-48 years) and the duration of type 1 diabetes was 19 years (range 8-37 years). All participants were subsequently surveyed biennially for up to 30 years and also reexamined at 2-, 4-, 6-, 8-, 10-, 18-, 25- and 30-years of follow-up. The EDC study protocols were approved by the University of Pittsburgh Institutional Review Board and all study participants provided written informed consent prior to performing any study procedures.

For the present analysis, from the 325 female EDC participants, we excluded women who did not have sex hormone data available due to missingness (n=53) or death (n=75), those who had a hysterectomy/oophorectomy before menopause (n=35), and those who received sex hormone therapy during the menopausal transition (n=20). In addition, we excluded women who had not yet reached natural menopause at their last available follow-up (n=37, age 18.3±4.0 years at baseline and 47.5±3.7 years at their last available follow-up). Therefore, a total of 105 female participants who experienced natural menopause during study follow-up were included in the data analyses (Figure 1.2).

4.2.2 Risk Factor Assessment

At baseline assessment, survey questionnaires regarding demographic characteristics, medical history, and diabetes self-care (e.g., daily insulin dose per kilogram body weight) were completed by the participant. During the clinical visits, body mass index (BMI) was measured as weight in kilograms (kg) divided by height in meters squared (m^2). The waist to hip ratio (WHR) was measured as the circumference of the waist divided by that of the hips. Blood pressure was measured according to the hypertension detection and follow-up program (HDFP) protocol [281]. Hypertension was defined as blood pressure ≥140/90 mm Hg or the use of antihypertensive medications. The level of stable glycosylated hemoglobin (HbA1) was measured using ion exchange chromatography (Isolab, Akron, OH) during the first 18 months of the study, whereas automated high-performance liquid chromatography (Diamat, BioRad, Hercules, CA) was used for the remainder of the 10-year follow-up. These two assays were highly correlated (r=0.95). Original HbA1 measures were converted to DCCT standard HbA1c values using a regression equation derived from duplicate analyses (DCCT HbA1c = [0.83 * EDC HbA1] + 0.14) [282]. Glucose disposal rate was estimated (eGDR) by using a regression equation derived from hyperinsulinemic-euglycemic clamp studies of 24 participants chosen to represent the full spectrum of insulin resistance (eGDR (mg/kg/min) = 24.395 - (12.971 * WHR) - (3.388 * Hypertension) - (0.601 * HbA1c)) [283].

High-density lipoprotein cholesterol (HDL-C) was measured by means of a precipitation technique (heparin and manganese chloride) with a modification of the Lipid Research Clinics method [126,275]. Total cholesterol and triglycerides were measured enzymatically [126,275]. Non-HDL cholesterol (non-HDL-C) was computed by subtracting HDL-C from total cholesterol. White blood cell count was measured using a counter S-plus IV. Glomerular filtration rate(eGFR) was

estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine formula [285]. Urinary albumin was measured by immunonephelometry [284].

4.2.3 Diabetes Complication Assessment

At baseline and biennial clinical exanimations for the first 10 years of follow-up and at year 18, 25, and 30, participants underwent examinations for complication assessment, including microvascular complications such as microalbuminuria (MA), overt nephropathy (ON), proliferative retinopathy (PR), and confirmed distal symmetric polyneuropathy (CDSP), and macrovascular complications - coronary artery disease (CAD).

MA was defined as an albumin excretion rate (AER) of 20–200 µg/min (30–300mg/24 h), and ON was defined as an AER >200 µg/min (>300mg/24 h), in at least two out of three validated timed urine collections. Retinopathy was diagnosed by stereoscopic fundus photographs of fields 1, 2, and 4, which were filmed by a Zeiss camera and read by the Fundus Photography Reading Center at the University of Wisconsin–Madison [126,275]. PR was classified according to the modified Arlie House system [126,275]. CDSP was defined as experiencing at least two out of three of the following: symptoms consistent with distal symmetric polyneuropathy; sensory and/or motor signs; and absent/reduced tendon reflexes based on the Diabetes Control and Complications Trial clinical exam protocol [292], in addition to an abnormal age-specific vibratory threshold by the Vibratron II Tester (Physitemp Instruments, Clifton, NJ).

CAD is defined as CAD death, fatal or nonfatal myocardial infarction confirmed either by hospital records or Q-waves on electrocardiogram (Minnesota code 1.1, 1.2), angiographic stenosis ≥50% confirmed by hospital records, revascularization, EDC-diagnosed angina or ischaemic

ECG changes (Minnesota code 1.3, 4.1–4.3, 5.1–5.3, 7.1) which include minor Q-waves, ST depression, T-wave inversion/flattening, or left bundle branch block.

4.2.4 Menopause Status Assessment

Natural menopause was defined as cessation of menstruation not induced by surgical procedures or medications, including hysterectomy, oophorectomy, hormone medications, etc. Women who had a hysterectomy/oophorectomy before menopause or those who received sex hormone therapy during the menopausal transition were thus excluded from analysis. Menopausal status was determined via self-report and sex hormone data as follows: women <45 years reporting regular menstrual cycles were classified as pre-menopausal, whereas those >55 years with no menstrual periods for 12 months were classified as post-menopausal. Plasma follicle-stimulating hormone (FSH) and estradiol were measured in women falling outside this classification, in which case the Women's Ischemia Syndrome Evaluation (WISE) hormonal and historical algorithms [277] were used to assess menopausal status, but not making a distinction between peri- and pre-menopausal women. Age at menopause was defined as the chronological age at the time menopause was determined to have occurred (Figure 2.2).

4.2.5 Statistical Analyses

Spearman correlation analysis was used to determine the correlation between age at complication diagnosis and age at natural menopause among women who had a complication diagnosed before menopause. Women were grouped into three categories based on presence and age at complication diagnosis prior to menopause onset: 1) complication diagnosed <30 years of

age, 2) complication diagnosed >=30 years of age, and 3) no complication. Age 30 was selected as the cutoff because in this childhood-onset type 1 diabetes cohort, it would correspond to approximately 20 years of diabetes duration, and complication development by 20 years duration is considered "early". Linear regression models (PROC GLM) were used to assess the significance of the association between presence/age at complication onset (independent variable) with age at natural menopause (dependent variable). The presence/age at complication onset variable was entered as a class variable with the no complication group as the reference. Multivariable models were further constructed, adjusting for potential confounders, baseline BMI, smoking status, HDL, and HbA1c. Confounders for adjustment were selected based on previous reports of an association with age at menopause and were included in multivariable models regardless of statistical significance [222]. In the present study, age at baseline was statistically significantly correlated with age at complications diagnosis (r=0.35, p=0.03); thus, age at baseline was not included in the multivariable models as a covariate to avoid over adjustment. SAS version 9.4 (SAS Institute, Cary, NC) was used for all analyses.

4.3 RESULTS

A summary of the baseline characteristics of the 105 female participants who reached natural menopause by the end of the 30-year follow-up is presented in Table 4.1. The mean baseline age was 29.5 years, with the mean age at diabetes onset of 9.3 years and duration of type 1 diabetes of 20.2 years. At baseline almost a third (32.3%) had prevalent MA, 16.5% had ON, 25.2% had PR, and 23.7% had CDSP, whereas only 3.8% had CAD. All 105 women were pre-

menopausal at the baseline assessment but reached natural menopause within an average follow-up of 29.2±7.1 years. The mean age at natural menopause was 49.5 years.

For women who had a complication diagnosed before menopause, age at complication diagnosis, age at natural menopause, and their correlation coefficients are presented in Table 4.2. Age at MA diagnosis (r=0.28, p=0.03) and age at CDSP diagnosis (0.36, p=0.01) were positively correlated with age at natural menopause. Women with earlier onset of MA or CDSP had an earlier age at natural menopause. Although no statistically significant correlations were observed for ON (r=0.32, p=0.12) and CAD (r=0.28, p=0.17), the magnitude of their correlations between age at diagnosis and age at menopause is similar to that seen for MA and CDSP. However, low correlation was observed for age at PR diagnosis and age at natural menopause (r=0.10, p=0.44).

Grouping women according to complication presence and diagnosis before or after age 30 (Table 4.3), differences in the age at natural menopause persisted for MA (p=0.02) and CDSP (p=0.02), with the youngest age at natural menopause among women who developed these complications before 30 years of age. Although not statistically significant, a similar pattern was observed for ON (P=0.27) and CAD (P=0.37), whereas age at menopause did not differ at all across categories of PR (P=0.95). Thus, women with ON or CAD diagnosed before 30 years had the youngest age at natural menopause while women with these complications diagnosed after 30 years and women without these complications had similar and older age at natural menopause.

Results from unadjusted and multivariable adjusted linear regression models for the association of presence and age at complications diagnosis with age at natural menopause were shown in Table 4.3. Generally, in unadjusted models, natural menopause occurred at a younger age among women with complications diagnosed before 30 years compared with those not developing the complication, although findings were statistically significant only for MA

($\beta\pm$Standard Error (SE)=-2.4$\pm$1.01, p=0.02)). After multivariable adjustment, this association was slightly attenuated ($\beta\pm$SE=-2.06$\pm$1.08, p=0.06).

In contrast, developing MA, ON, PR, or CAD after 30 years of age did not appear to be associated with an earlier age at menopause compared to those who never developed each respective complication. Interestingly, however, women developing CDSP after age 30 years reached natural menopause 2.19 years later ($\beta\pm$SE=2.19$\pm$0.86, p=0.01) compared with women without CDSP in the unadjusted model. This finding persisted ($\beta\pm$SE=2.95$\pm$0.91, p=0.002) after adjustment for baseline BMI, smoking status, HDL-C, and HbA1c.

4.4 DISCUSSION

In the present study, we assessed the association of diabetes complications status before menopause and age at complication development with age at natural menopause in women with type 1 diabetes, using data from a prospective childhood-onset type 1 diabetes cohort. We observed that women with MA occurring before age 30 years experienced natural menopause earlier than women without MA before menopause, whereas no difference was observed between those diagnosed with MA after age 30 and women with normoalbuminuria. A similar pattern was observed for ON and CAD, although results were not statistically significant due to a small number with these complications. No difference in age at menopause was observed by PR status, whereas women developing CDSP after age 30 reached menopause later compared with women free of CDSP.

Microalbuminuria, defined as an albumin excretion rate of 20–200 µg/min (30–300mg/24 h), is often the first clinical indicator of the presence of diabetic kidney disease [293], as well as a

possible marker for kidney disease progression [294], although not all people with reduced glomerular filtration rate or diabetic kidney disease have or ever had MA [295]. Moreover, MA is a risk marker for cardiovascular events in diabetes [294,296] and also a strong predictor of all-cause mortality in patients with long-term type 1 diabetes [297]. Thus, MA is considered as a marker for generalized vascular dysfunction in patients with diabetes [296]. Despite the significance of MA in reflecting vascular injury, to the best of our knowledge, no study assessed the association of MA and ovarian aging in women with type 1 diabetes. In the present study, we found that women with MA occurring before 30 years had a younger age at natural menopause compared with women without MA before menopause, although this association became of marginal significance after multivariable adjustments (p=0.06). Among women with MA occurring after 30 years, age at natural menopause was similar to that of women with normoalbuminuria (p=0.55). These results suggest that long exposure to microvascular injury may be needed for age at natural menopause to be affected. Another explanation could be that early development of microalbuminuria represents vascular damage which also adversely affects age at natural menopause, i.e., vascular damage leads to both early presentation of complications and early menopause.

Currently, no other data are available on the role of age at MA presentation on age at natural menopause. However, our finding is supported by our previous observation in this population that for every 30% increase in albumin excretion rate over time, age at natural menopause decreased by 0.18 years (unpublished data, submission in progress). Moreover, as MA is known to increase cardiovascular risk [294,296] and data from the Framingham Heart Study showed that cardiovascular risk factors accelerate menopause [290], it is possible that MA affects the age of menopause.

Although not statistically significant, ON and CAD showed a similar pattern as MA in terms of their effects on age at menopause. The smaller number of cases in the present study for

ON (n=26) and CAD (n=25) may not allow for adequate power to detect difference. Studies with a larger sample size of ON and CAD are needed to further confirm their roles in ovarian function in type 1 diabetes. Our results further showed that age at natural menopause was very similar regardless of absence, early or later onset of PR. The small number of cases diagnosed with PR before age 30 may also contribute to lack of association. Another possible explanation would be that the pathology relating to retinopathy may not discriminate in terms of age at menopause.

In contrast to findings for MA, we observed that women who developed CDSP after 30 years of age had an older age at natural menopause compared to women who never developed CDSP during follow-up, suggesting a protective effect of CDSP on ovarian function. While there is no direct evidence supporting this finding, animal studies in rats showed that activation of autonomic nerves (superior ovarian nerve in the suspensory ligament and the ovarian nerve plexus along the ovarian arterioles) to the ovary produces a decrease in ovarian blood flow and inhibition of estradiol and testosterone secretion [298,299]. However, if a protective effect of CDSP truly existed, an incremental increase in age at menopause onset would have been expected from women without CDSP, to those with CDSP occurring after age 30 to women in whom CDSP occurred before 30 years of age. We did not detect a dose-response relationship which may suggest that the association of CDSP and age at natural menopause in the present study is a chance finding.

Strengths of this study include the well-characterized cohort of childhood-onset type 1 diabetes, including comprehensive ascertainment of vascular diabetes complications, and the long (mean of 29.2 years) follow-up. To the best of our knowledge, this is the first study assessing the impact of timing of complication onset on menopause by using age at complications diagnosis. Among the limitations, selection or survival bias may have been introduced by excluding from analyses women who died or dropped out prior to menopause onset. In addition, the

generalizability of these findings may be limited due to lack of racial diversity in participants and a relatively small sample size.

In conclusion, women with type 1 diabetes in whom MA presented before age 30 experienced an earlier natural menopause compared to those with normoalbuminuria. A similar pattern was observed for ON and CAD, although results did not reach statistical significance. While it is necessary to validate these results in future studies with a larger sample size, our findings suggest that premature ovarian aging may be a further manifestation of vascular damage in women with type 1 diabetes. Our study findings also further emphasize the importance of preventing vascular impairment or delay the onset of vascular complications in type 1 diabetes by glycemic management and improvements in lifestyle.

Table 4.1 Baseline Characteristics of EDC Female Participants Who Experienced Natural Menopause during the Study Follow-up

Baseline Characteristics	n=105
Age at baseline (years)	29.5 ± 6.2
Age at diabetes onset (years)	9.3 ± 3.9
Duration of type 1 diabetes at baseline (years)	20.2 ± 7.0
Race (%, n)	
Non-Hispanic White	96.2 (101)
Black	3.8 (4)
Marital status (%, n)	
Single, never married	37.1 (39)
Currently married or living as if married	48.6 (51)
Divorced	7.6 (8)
Separated, Widowed, or others	6.7 (7)
Education (%, n=103)	
High school or less	35.0 (36)
Some college or received bachelor's degree	56.3 (58)
Graduate education beyond bachelor's degree	8.7 (9)
Smoking status (%, n)	
Never smoker	67.6 (71)
Past smoker	13.3 (14)
Current smoker	19.1 (20)
BMI (kg/m^2)	22.9 ± 2.9
Waist to hip ratio (n=104)	0.77 ± 0.05
Systolic blood pressure (mmHg)	107.9 ± 11.7
Diastolic blood pressure (mmHg)	68.5 ± 9.6
Hypertension (%, n)	8.6 (9)
Blood pressure medication use (%, n=103)	6.8 (7)
Total cholesterol (mg/dl)	181.0 (162.0, 197.0)
HDL-C (mg/dl)	61.0 (50.6, 73.0)
LDL-C (mg/dl) (n=100)	100.9 (87.0, 127.4)
Triglycerides (mg/dl) (n=102)	74.0 (51.0, 100.0)

ACE/ARB use (%, n=104)	1.9 (2)
Lipid medication use (%, n=104)	0 (0)
HbA1c (%, n=104)	8.4 ± 1.2
eGDR (mg*kg-1*min-1, n=103)	8.9±1.5
Insulin dose (units/day/kg, n=100)	0.71 ± 0.19
AER (μg/min)	10.4 (6.2, 25.7)
eGFR (mL/min/1.73 m^2)	102.3 ± 27.9
WBC x 10^3/mm^2	6.4 ± 1.9
Microalbuminuria (%, n=99)	32.3 (32)
Overt nephropathy (%, n=97)	16.5 (16)
Proliferative retinopathy (%, n=103)	25.2 (26)
Confirmed distal symmetrical polyneuropathy (%, n=97)	23.7 (23)
Coronary artery disease (%, n=105)	3.8 (4)

Data are means (SD), median (IQR) or percent (n)

AER, albumin excretion rate; eGDR, estimated glucose disposal rate; eGFR, estimated glomerular filtration rate; WBC, white blood cell count

Table 4.2 Age at Complication Diagnosis and Its Association with Age at Natural Menopause among Women

Who Had a Complication Diagnosed before Menopause

Complications	Age at complication diagnosis	Age at natural menopause	Spearman Correlations	
	Mean (SD)	Mean (SD)	r	p-value
Microalbuminuria (n=59)	33.3±8.1	49.1±4.1	0.28	0.03
Overt nephropathy (n=26)	31.4±6.7	49.1±4.9	0.32	0.12
Proliferative retinopathy (n=61)	34.2±6.7	49.6±4.0	0.10	0.44
Confirmed distal symmetrical polyneuropathy (n=50)	35.5±7.0	50.1±3.2	0.36	0.01
Coronary artery disease (n=25)	40.9±8.7	49.8±2.9	0.28	0.17

Table 4.3 Linear Regression Models for the Association of Age at Complication Diagnosis with Age at

Natural Menopause

Age at complication diagnosis	Age at natural menopause		Unadjusted		Adjusted^	
	Mean (SD)	Overall P value	β coefficient (SE)	P value	β coefficient (SE)	P value
Microalbuminuria						
<30 yrs (n=25)	47.5±4.9	0.02	-2.40 (1.01)	0.02	-2.06 (1.08)	0.06
≥30 yrs (n=34)	50.2±3.1		0.31 (0.92)	0.74	0.60 (0.99)	0.55
No (n=40)	49.9±4.0		Ref		Ref	
Overt nephropathy						
<30 yrs (n=12)	47.7±5.8	0.27	-1.78 (1.28)	0.17	-1.42 (1.38)	0.31
≥30 yrs (n=14)	50.3±3.8		0.77 (1.20)	0.52	1.20 (1.28)	0.35
No (n=71)	49.5±3.8		Ref		Ref	
Proliferative retinopathy						
<30 yrs (n=17)	49.3±4.8	0.95	-0.03 (1.18)	0.98	0.24 (1.22)	0.85
≥30 yrs (n=44)	49.6±3.7		0.26 (0.88)	0.77	0.63 (0.94)	0.50
No (n=42)	49.4±4.1		Ref		Ref	
Confirmed distal symmetrical polyneuropathy						
<30 yrs (n=10)	48.0±3.4	0.02	-0.44 (1.39)	0.75	0.26 (1.50)	0.87
≥30 yrs (n=40)	50.6±3.0		2.19 (0.86)	0.01	2.95 (0.91)	0.002
No (n=47)	48.4±4.7		Ref		Ref	
Coronary artery disease						
<30 yrs (n=2)	46.0±4.2	0.37	-3.41 (2.90)	0.24	-3.46 (3.00)	0.25
≥30 yrs (n=23)	50.1±2.7		0.68 (0.96)	0.48	0.93 (0.99)	0.35
No (n=80)	49.4±4.4		Ref		Ref	

^Model allowed for baseline BMI, smoking status, HDL, and HbA1c

5.0 OVERALL CONCLUSIONS AND DISCUSSION

5.1 SUMMARY OF FINDINGS AND PUBLIC HEALTH SIGNIFICANCE

This dissertation focused on the comprehensive assessment of the reproductive lifespan and risk prediction of premature ovarian aging in women with type 1 diabetes. Using data from a large prospective cohort study on individuals with childhood-onset type 1 diabetes and a study on women's health, I: 1) demonstrated that women with type 1 diabetes onset before menarche have shorter reproductive lifespan compared to non-diabetic women, 2) identified two modifiable factors related to ovarian aging (exogenous insulin dose and AER), and 3) identified a predictor of early age at menopause (presence of MA before 30 years of age) in type 1 diabetes. This comprehensive work has great public health significance and clinical implications as, upon replication, it could constitute the foundation for future recommendations in clinical reproductive counseling and prevention of early menopause-induced health issues (e.g., increased risks of cardiovascular events, osteoporosis, fracture, and mortality) in women with type 1 diabetes.

5.1.1 Shorter Reproductive Period in Women with Type 1 Diabetes

The evidence regarding the effect of type 1 diabetes on age at natural menopause is limited by studies of small sample size [236], failing to distinguish the effect of type 1 and type 2 diabetes [237] or to control for confounding factors [239]. Moreover, existing findings regarding premature natural menopause in type 1 diabetes are conflicting [236-239] potentially due to failure to stratify analyses by the timing of type 1 diabetes onset being before or after menarche. Finally, although

it is known that reproductive characteristics are associated with late-age survival (later age at natural menopause and longer reproductive lifespan are significantly associated with increased longevity) [300], no study directly assessed the length of the reproductive period by including both the age at menarche and the age at menopause in women with type 1 diabetes. Hence, our first paper aimed to fill these research gaps by assessing the length of the reproductive years of women with type 1 diabetes from the Pittsburgh Epidemiology of Diabetes Complication (EDC) study and of women without diabetes from the Pittsburgh site of the Study of Women's Health Across the Nation (SWAN). The Pittsburgh EDC Study recruited childhood-onset (<17 years) type 1 diabetes patients diagnosed, or seen within one year of diagnosis, at Children's Hospital of Pittsburgh between 1950 and 1980. SWAN is a multi-site longitudinal epidemiologic study designed to examine the health of midlife women during their transitioning to menopause period. SWAN female participants from the Pittsburgh site were selected as they comprised an excellent comparison group to the Pittsburgh EDC cohort for assessing the length of the reproductive period. Since EDC participants were younger at study entry compared with participants of the SWAN cohort, covariate data for women with type 1 diabetes were taken from the follow-up visit preceding menopause in which chronological age was closest to the mean baseline age (i.e., 46.0 years) in SWAN.

In paper 1, after stratifying data by the timing of type 1 diabetes onset being before or after menarche, we found that the length of the reproductive period of women with type 1 diabetes onset prior to menarche was 2.9 years shorter compared to women without diabetes after adjustments. The shorter reproductive period in women with type 1 diabetes related to both a delayed menarche (by 1 year) and premature natural menopause (by 1.8 years) compared with women without diabetes. However, no association between type 1 diabetes and age at menarche, age at natural

menopause, or length of reproductive years was found when type 1 diabetes developed after menarche, although the small sample size (n=25) may have undermined the statistical power to detect a difference in this group. Another possible explanation of why our findings of a shorter reproductive period may have been restricted to women whose type 1 diabetes onset occurred prior to menarche may be that type 1 diabetes occurring before menarche leads to a greater disruption of the female reproductive system compared with type 1 diabetes occurring after menarche.

Findings of Paper 1 support the hypothesis of a disruption of the normal function of the female reproductive system in the presence of type 1 diabetes and extends the available evidence. Meanwhile, they underscore the need for determining modifiable factors that contribute to early age at menopause, leading to the identification of novel intervention strategies to improve reproductive health in women with type 1 diabetes.

5.1.2 Two Novel Predictors of Menopause in Type 1 Diabetes – Exogenous Insulin Dose and Albumin Excretion Rate

Given our findings of a greater likelihood of experiencing menopause earlier among women with type 1 diabetes (Paper 1), and the enormous impact on health that early menopause has, we next set to identify modifiable factors which contribute to a younger age at menopause in type 1 diabetes, as such data are scarce. In the general population, it is well known that smoking accelerates natural menopause onset [240,241], whereas high parity is associated with later natural menopause onset [222]. However, little is known about the effects of traditional risk factors, such as smoking and BMI, or diabetes-specific factors, such as glycemic control, insulin dose, and diabetes complications, on the age at natural menopause in women with type 1 diabetes. Therefore, the objective of our second paper was to identify metabolic factors (HbA1c, lipids, blood pressure,

insulin dose, etc.) that are independently associated with age at natural menopause in the EDC cohort of women with childhood-onset type 1 diabetes. Time-weighted means for all metabolic factors of interest were used to represent their average levels over time, up to the exam cycle prior to the onset of menopause.

In paper 2, we observed that higher average levels of insulin dose and AER over time were significantly associated with an earlier age at natural menopause in type 1 diabetes after multivariable adjustments, including for HbA1c. Each 0.1 unit increase in insulin dose per day per kilogram was associated with 0.64 years younger age at natural menopause (p=0.01), while for every 30% increase in AER, age at natural menopause decreased by 0.18 years (p=0.03). Interestingly, despite insulin dose being a predictor after accounting for HbA1c levels, insulin sensitivity (eGDR) did not predict age at natural menopause in a separate model. These findings suggest that the adverse effect of insulin dose does not reflect insulin resistance but rather may indicate that the higher insulin concentrations have a direct, deleterious effect on ovarian aging in women with type 1 diabetes.

Findings of Paper 2 raise significant questions relating to a potentially deleterious effect of high exogenous insulin doses, in addition to that of kidney disease, and may (upon replication) constitute a useful source in clinical reproductive counseling for women with type 1 diabetes. Moreover, these findings have significant clinical implications as they support the need for identifying an optimal insulin dose administration which could achieve glucose control goals and at the same time, minimize its potential deleterious effect on the reproductive system for female patients with type 1 diabetes. Additionally, should further studies confirm that diabetic kidney complications play a role in ovarian function, women with these two risks of early menopause,

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type 1 diabetes and kidney complications, could be targeted for timely interventions to prevent the health consequences (e.g., CVD) of premature ovarian aging.

5.1.3 Diabetes Vascular Complications and Menopause in Type 1 Diabetes

A quantitative review of observational studies suggested a protective effect of hormone replacement therapy (HRT) on coronary heart disease (CHD) risk [301]. Nevertheless, findings from two large-scale clinical trials were not supportive of any beneficial effects of estrogen therapy [302,303]. These conflicting findings on the effects of HRT brought about the hypothesis that existing premenopausal vascular damage plays a role in premature ovarian aging, and that earlier menopause itself may not be the culprit leading to increased cardiovascular risk.

The ovaries are highly vascular organs and have high rates of blood flow [289]; thus, it is logical to postulate that vascular injury may lead to earlier age at menopause. Individuals with type 1 diabetes are at high risk of vascular damage which is manifested by macrovascular and microvascular complications. Previously, conflicting findings of the association of vascular complications and age at natural menopause among women with type 1 diabetes were reported. A Finnish study found that proliferative retinopathy (n=17) and end-stage renal disease (n=9) were independently associated with earlier menopause[239]. On the other hand, data from the Diabetes Control and Complications Trial (DCCT) and its observational follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study suggested that microvascular complications did not affect the risk of menopause, although age at menopause was not directly assessed in this study[243]. However, no study has assessed the impact of the timing or age at complication development on the age at menopause. Yet, it is possible that the early development

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of complications and/or the longer duration of exposure to diabetes complications may affect the age at which menopause manifests.

In Paper 3, we attempted to fill this research gap by assessing the ability of the age of diabetes complication onset to predict the age at natural menopause in female participants of the EDC cohort of childhood-onset type 1 diabetes. We observed that although age at natural menopause did not differ between women with normoalbuminuria and those diagnosed with MA after age 30, menopause occurred 2.06 years earlier in women with MA diagnosed before 30 years of age compared with women without MA after adjustment for baseline BMI, smoking status, HDL, and HbA1c ($\beta\pm SE=-2.06\pm1.08$, $p=0.06$). A similar pattern was observed for overt nephropathy (ON) ($p=0.27$), and coronary artery disease (CAD) ($p=0.37$), although results did not reach statistical significance. In contrast, age at menopause did not differ by presence or timing of PR, whereas menopause appeared later in women with CDSP diagnosed after age 30 compared with women without CDSP. Our finding raises attention to the potential vascular dysfunction in the ovary when vascular complications develop early in type 1 diabetes. MA is considered as a marker for generalized vascular dysfunction in patients with diabetes [296] and early development of microalbuminuria represents vascular damage which may also adversely affects age at natural menopause, i.e., vascular damage leads to both early presentation of complications and early menopause. The vascular damage occurring in the ovaries is likely not caused by diabetes complications but is rather another phenotype of systemic vascular damage.

Findings of Paper 3 have great public health significance given the high prevalence of complications in type 1 diabetes. While it is necessary to validate these results in future studies with larger sample sizes, our findings suggest that premature ovarian aging could be one manifestation of this vascular damage. These findings further emphasize the importance of

preventing vascular impairment or delay the onset of vascular complications in type 1 diabetes by glycemic management and lifestyle improvement.

5.2 STRENGTHS

This dissertation has a number of strengths that add value to the literature. First, we used data from the Pittsburgh EDC study, a well-characterized type 1 diabetes cohort. The EDC study recruited childhood-onset type 1 diabetes patients with good representativeness of the type 1 diabetes population in Allegheny County, PA, US. Subsequently, the EDC study prospectively followed participants for up to 30 years with a detailed biennial surveys and comprehensive clinical examinations during which data on biological markers and diabetes complications were assessed, providing a large amount of valuable data for analysis. These longitudinal data with repeated measurements allowed us to have a more accurate estimation of the characteristics of the EDC participants over time, compared with a single measurement at baseline. Thus, data used in Paper 2 reflect the cumulative effect of metabolic factors over time on age at menopause. Second, the data selected from the Pittsburgh site of the SWAN study comprised an excellent non-diabetic comparison group to the Pittsburgh EDC study for assessing age at menopause (Paper 1). The participants from the EDC study and SWAN Pittsburgh site live in the same geographic region and have similar demographic characteristics at baseline. Third, for the ascertainment of our main outcome – age at natural menopause, we combined self-reported reproductive history information and sex hormone results based on the Women's Ischemia Syndrome Evaluation (WISE) algorithms, which improved the accuracy of menopausal status classification, compared to use of

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only self-reported information. Last but not least, to the best of our knowledge, Paper 3 is the first study assessing the impact of timing of complications onset on menopause. Previous research simply assessed the role of complications status as a dichotomous variable (yes/no) on age at menopause.

5.3 LIMITATIONS

The major limitation of this dissertation was the unavailability of detailed data on reproductive health (especially hormone levels) prior to the 12th follow-up visit of the EDC study. Thus, selection or survival bias may have been introduced given that women who did not participate in assessments post 2009 due to death, dropout, or other reasons, were excluded from analyses. However, early loss due to death or ill health would also be more likely to be associated with an earlier age at menopause; thus, these exclusions may have underestimated the main finding of Paper 1. In addition, despite the 30-year follow-up of the EDC study, some female participants (n=37) are young enough to have not yet reached menopause. We, therefore, were unable to include them in the current analyses. However, in the sensitivity analysis, we observed that results did not change significantly when we assigned the mean age at natural menopause in SWAN to these 37 EDC women who had not yet reached menopause (0.4-year delay in menarche, 1.8 years earlier in natural menopause, and 2.9 fewer reproductive years in women with type 1 diabetes).

Another limitation was the different method of ascertainment of menopause status in the EDC study and SWAN for analyses in Paper 1. However, a previous investigation suggested that 73%-77% of SWAN participants received a concordant menopausal status classification by the WISE and SWAN algorithms [280]; the two were especially highly concordant for classifying

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postmenopausal status (30.5% of women were classified as postmenopausal by the SWAN algorithm and 32.7% of women were classified as postmenopausal by the WISE algorithm) [280].

Additionally, the relatively small sample size of the EDC women having gone through natural menopause by the 30-year assessment (n=105) may have undermined the statistical power (analyses in Paper 3) and thus these results require validation in large cohorts of type 1 diabetes. Lastly, since participants in the EDC study were mainly Non-Hispanic white, the lack of racial diversity in this cohort may limit the generalizability of dissertation findings.

5.4 DIRECTIONS FOR FUTURE RESEARCH

This dissertation highlights several key questions that remain unanswered regarding menopause in women with type 1 diabetes.

First, whether the timing of type 1 diabetes onset relative to menarche modifies the association of age at menopause and type 1 diabetes status is unclear. Is it true that there is no significant difference in age at natural menopause between women with type 1 diabetes onset after menarche and women without diabetes? The EDC cohort does not have optimal numbers of participants with the onset of diabetes following menarche (n=25); thus, this finding should be confirmed in a larger study. Should this result be replicated, it would identify the subgroup of women with type 1 diabetes who have a high likelihood of experiencing early age at natural menopause so that efforts to unearth the biologic rationale and target potential prevention practices would be better focused.

Second, the potentially deleterious effect of high exogenous insulin doses, in addition to that of kidney disease, on premature ovarian aging requires validation in additional (larger) cohorts

of women with type 1 diabetes, including in cohorts with greater racial diversity. If the deleterious effect truly exists, the biologic mechanisms underlying the observed associations between exogenous insulin dose and AER on the reproductive health of women with type 1 diabetes should be investigated in both human and animal model studies. Moreover, a study aiming to determine the optimal insulin dose to achieve glucose control goals while at the same time minimizing its potential deleterious effects on the reproductive system would have significant clinical implications. These investigations are quite clinically relevant as it may provide important evidence or resources for clinicians to consider when starting or adjusting insulin dose among women with type 1 diabetes.

Third, validation of current findings with different analytic techniques which require larger sample sizes could be another area of future study. Compared to the use of time-weighted means of risk factors in relation to age at menopause (as presented in Paper 2 of this dissertation), longitudinal data analysis approaches such as linear mixed model or trajectory analysis could be used to capture the changes or trajectories of insulin dose, AER, or HbA1c over time and assess whether specific patterns or trajectories relate to age at natural menopause in type 1 diabetes. This is important in identifying potential additional risk factors of premature ovarian aging and subsequently making practical intervention plans to prevent premature ovarian aging in type 1 diabetes.

Additionally, studies should be conducted to further understand diabetic vascular damage and explore whether premature ovarian aging is one manifestation of diabetes vascular complications. Should further studies confirm the biologic rationale that premature ovarian aging is also one phenotype of diabetes vascular complications, it would be helpful in increasing awareness of diabetes complications and further emphasizing the importance of preventing

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vascular damage in type 1 diabetes by exercising regularly, monitoring blood glucose closely, and taking medications (pill and/or insulin) as prescribed, etc.

5.5 CONCLUSIONS

This dissertation provided new information as it pertains to the length of the reproductive period of women with type 1 diabetes, suggesting that it is shorter, exhibiting delayed menarche and earlier natural menopause compared with non-diabetic women. It is important to note, however, that these findings appeared restricted to women who were diagnosed with type 1 diabetes before reaching menarche. This dissertation also showed two novel diabetes-specific predictors of earlier age at natural menopause in type 1 diabetes – a higher exogenous insulin dose and albumin excretion rate (AER) over time. In addition, we demonstrated that women with microalbuminuria (MA) that occurred before 30 years of age were more likely to experience natural menopause earlier than women without MA, whereas age at natural menopause did not differ between those with normoalbuminuria and those diagnosed with MA after age 30 in type 1 diabetes. Taken together, our findings suggested that ovarian aging may be another manifestation of vascular complications in type 1 diabetes and the exogenous insulin administration may play a role for this pathogenesis through its gonadotropic function which accelerates the depletion of the ovarian reserve. This dissertation extends current knowledge regarding the reproductive health of women with type 1 diabetes and fills multiple gaps in the existing literature, as well as highlights areas where further research is needed.

Bibliography

1. Atkinson MA, Eisenbarth GS. Type 1 diabetes: New perspectives on disease pathogenesis and treatment. *Lancet.* 2001;358(9277):221-229.

2. Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature.* 2010;464(7293):1293-1300.

3. Harjutsalo V, Sjöberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *The Lancet.* 2008;371(9626):1777-1782.

4. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *The Lancet.* 2014;383(9911):69-82.

5. Mayer-Davis EJ, Lawrence JM, Dabelea D, et al. Incidence trends of type 1 and type 2 diabetes among youths, 2002-2012. *New England Journal of Medicine.* 2017;376(15):1419-1429.

6. Diaz-Valencia PA, Bougnères P, Valleron AJ. Global epidemiology of type 1 diabetes in young adults and adults: A systematic review. *BMC Public Health.* 2015;15(1).

7. 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. *The Lancet Diabetes and Endocrinology.* 2018;6(2):122-129.

8. Hope SV, Wienand-Barnett S, Shepherd M, et al. Practical Classification Guidelines for Diabetes in patients treated with insulin: A cross-sectional study of the accuracy of diabetes diagnosis. *British Journal of General Practice.* 2016;66(646):e315-e322.

9. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. *Lancet.* 2018;391(10138):2449-2462.

10. Dabelea D, Rewers A, Stafford JM, et al. Trends in the prevalence of ketoacidosis at diabetes diagnosis: The search for diabetes in youth study. *Pediatrics.* 2014;133(4):e938-e945.

11. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. *Endocrinology and Metabolism Clinics of North America.* 2010;39(3):481-497.

12. Harjutsalo V, Sund R, Knip M, Groop PH. Incidence of type 1 diabetes in Finland. *JAMA.* 2013;310(4):427-428.

13. Group DP. Incidence and trends of childhood Type 1 diabetes worldwide 1990-1999. *Diabet Med.* 2006;23(8):857-866.

14. Bruno G, Maule M, Biggeri A, et al. More than 20 years of registration of type 1 diabetes in Sardinian children: temporal variations of incidence with age, period of diagnosis, and year of birth. *Diabetes.* 2013;62(10):3542-3546.

15. International Diabetes Federation. IDF Diabetes Atlas, 9th edn. Brussels, Belgium: 2019. Available at: http://www.diabetesatlas.org. Accessed on 12/05/2019. Accessed.

16. Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. *The Lancet.* 2009;373(9680):2027-2033.

17. Östman J, Lönnberg G, Arnqvist HJ, et al. Gender differences and temporal variation in the incidence of type 1 diabetes: Results of 8012 cases in the nationwide Diabetes Incidence Study in Sweden 1983-2002. *Journal of Internal Medicine.* 2008;263(4):386-394.

18. Imperatore G, Boyle JP, Thompson TJ, et al. Projections of type 1 and type 2 diabetes burden in the U.S. population aged <20 years through 2050: dynamic modeling of incidence, mortality, and population growth. *Diabetes Care.* 2012;35(12):2515-2520.

19. Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, Tuomilehto J. Incidence of childhood type 1 diabetes worldwide. *Diabetes Care.* 2000;23(10):1516-1526.

20. Gale EAM. Type 1 diabetes in the young: The harvest of sorrow goes on. *Diabetologia.* 2005;48(8):1435-1438.

21. Atkinson MA. The pathogenesis and natural history of type 1 diabetes. *Cold Spring Harb Perspect Med.* 2012;2(11).

22. Menke A, Orchard TJ, Imperatore G, Bullard KM, Mayer-Davis E, Cowie CC. The prevalence of type 1 diabetes in the United States. *Epidemiology.* 2013;24(5):773-774.

23. Chiang JL, Kirkman MS, Laffel LMB, Peters AL. Type 1 diabetes through the life span: A position statement of the American Diabetes Association. *Diabetes Care.* 2014;37(7):2034-2054.

24. Campbell-Thompson ML, Atkinson MA, Butler AE, et al. The diagnosis of insulitis in human type 1 diabetes. *Diabetologia.* 2013;56(11):2541-2543.

25. McLaughlin KA, Richardson CC, Ravishankar A, et al. Identification of tetraspanin-7 as a target of autoantibodies in type 1 diabetes. *Diabetes.* 2016;65(6):1690-1698.

26. Eisenbarth GS. Update: Update in type 1 diabetes. *Journal of Clinical Endocrinology and Metabolism.* 2007;92(7):2403-2407.

27. Ziegler AG, Rewers M, Simell O, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA - Journal of the American Medical Association.* 2013;309(23):2473-2479.

28. Insel RA, Dunne JL, Atkinson MA, et al. Staging presymptomatic type 1 diabetes: A scientific statement of jdrf, the endocrine society, and the American diabetes association. *Diabetes Care.* 2015;38(10):1964-1974.

29. Greenbaum CJ, Speake C, Krischer J, et al. Strength in Numbers: Opportunities for Enhancing the Development of Effective Treatments for Type 1 Diabetes-The TrialNet Experience. *Diabetes.* 2018;67(7):1216-1225.

30. Pociot F, Lernmark A. Genetic risk factors for type 1 diabetes. *Lancet.* 2016;387(10035):2331-2339.

31. Atkinson MA, Bluestone JA, Eisenbarth GS, et al. How does type 1 diabetes develop? The notion of homicide or β-cell suicide revisited. *Diabetes.* 2011;60(5):1370-1379.

32. Richardson SJ, Rodriguez-Calvo T, Gerling IC, et al. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. *Diabetologia.* 2016;59(11):2448-2458.

33. Marhfour I, Lopez XM, Lefkaditis D, et al. Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes. *Diabetologia.* 2012;55(9):2417-2420.

34. Tersey SA, Nishiki Y, Templin AT, et al. Islet β-cell endoplasmic reticulum stress precedes the onset of type 1 diabetes in the nonobese diabetic mouse model. *Diabetes.* 2012;61(4):818-827.

35. Eizirik DL, Sammeth M, Bouckenooghe T, et al. The human pancreatic islet transcriptome: Expression of candidate genes for type 1 diabetes and the impact of pro-inflammatory cytokines. *PLoS Genetics.* 2012;8(3).

36. Bogdani M, Johnson PY, Potter-Perigo S, et al. Hyaluronan and hyaluronan-binding proteins accumulate in both human type 1 diabetic islets and lymphoid tissues and associate with inflammatory cells in insulitis. *Diabetes.* 2014;63(8):2727-2743.

37. Akirav EM, Baquero MT, Opare-Addo LW, et al. Glucose and inflammation control islet vascular density and β-cell function in NOD mice control of islet vasculature and vascular endothelial growth factor by glucose. *Diabetes.* 2011;60(3):876-883.

38. Keenan HA, Sun JK, Levine J, et al. Residual insulin production and pancreatic β-cell turnover after 50 years of diabetes: Joslin medalist study. *Diabetes.* 2010;59(11):2846-2853.

39. Lam CJ, Jacobson DR, Rankin MM, Cox AR, Kushner JA. β Cells persist in T1D pancreata without evidence of ongoing β-Cell turnover or neogenesis. *Journal of Clinical Endocrinology and Metabolism.* 2017;102(8):2647-2659.

40. Davis AK, DuBose SN, Haller MJ, et al. Prevalence of detectable c-peptide according to age at diagnosis and duration of type 1 diabetes. *Diabetes Care.* 2015;38(3):476-481.

41. Oram RA, Jones AG, Besser REJ, et al. The majority of patients with long-duration type 1 diabetes are insulin microsecretors and have functioning beta cells. *Diabetologia.* 2014;57(1):187-191.

42. Oram RA, McDonald TJ, Shields BM, et al. Most people with long-duration type 1 diabetes in a large population-based study are insulin microsecretors. *Diabetes Care.* 2015;38(2):323-328.

43. Rother KI, Spain LM, Wesley RA, et al. Effects of exenatide alone and in combination with daclizumab on β-cell function in long-standing type 1 diabetes. *Diabetes Care.* 2009;32(12):2251-2257.

44. Lachin JM, McGee P, Palmer JP. Impact of c-peptide preservation on metabolic and clinical outcomes in the diabetes control and complications trial. *Diabetes.* 2014;63(2):739-748.

45. Steffes MW, Sibley S, Jackson M, Thomas W. β-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care.* 2003;26(3):832-836.

46. Redondo MJ, Jeffrey J, Fain PR, Eisenbarth GS, Orban T. Concordance for islet autoimmunity among monozygotic twins. *New England Journal of Medicine.* 2008;359(26):2849-2850.

47. Krischer JP, Lynch KF, Schatz DA, et al. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. *Diabetologia.* 2015;58(5):980-987.

48. Ilonen J, Hammais A, Laine AP, et al. Patterns of beta-cell autoantibody appearance and genetic associations during the first years of life. *Diabetes.* 2013;62(10):3636-3640.

49. Ziegler R, Alper CA, Awdeh ZL, et al. Specific association of HLA-DR4 with increased prevalence and level of insulin autoantibodies in first-degree relatives of patients with type I diabetes. *Diabetes.* 1991;40(6):709-714.

50. Noble JA. Immunogenetics of type 1 diabetes: A comprehensive review. *Journal of Autoimmunity.* 2015;64:101-112.

51. Sanjeevi CB, Landin-Olsson M, Kockum I, Dahlquist G, Lernmark A. Effects of the second HLA-DQ haplotype on the association with childhood insulin-dependent diabetes mellitus. *Tissue Antigens.* 1995;45(2):148-152.

52. Graham J, Hagopian WA, Kockum I, et al. Genetic effects on age-dependent onset and islet cell autoantibody markers in type 1 diabetes. *Diabetes.* 2002;51(5):1346-1355.

53. Bottini N, Vang T, Cucca F, Mustelin T. Role of PTPN22 in type 1 diabetes and other autoimmune diseases. *Semin Immunol.* 2006;18(4):207-213.

54. Kondrashova A, Reunanen A, Romanov A, et al. A six-fold gradient in the incidence of type 1 diabetes at the eastern border of Finland. *Annals of Medicine.* 2005;37(1):67-72.

55. Oilinki T, Otonkoski T, Ilonen J, Knip M, Miettinen P. Prevalence and characteristics of diabetes among Somali children and adolescents living in Helsinki, Finland. *Pediatric Diabetes.* 2012;13(2):176-180.

56. Söderström U, Åman J, Hjern A. Being born in Sweden increases the risk for type 1 diabetes - A study of migration of children to Sweden as a natural experiment. *Acta Paediatrica, International Journal of Paediatrics.* 2012;101(1):73-77.

57. Gundersen E. Is diabetes of infectious origin? *Journal of Infectious Diseases.* 1927;41(3):197-202.

58. Gamble DR, Fitzgerald MG, Bolton R, Taylor KW, Kinsley ML. Viral Antibodies in Diabetes Mellitus. *British Medical Journal.* 1969;3(5671):627-630.

59. Yoon JW, Austin M, Onodera T, Notkins AL. Virus-Induced Diabetes Mellitus: Isolation of a Virus from the Pancreas of a Child with Diabetic Ketoacidosis. *New England Journal of Medicine.* 1979;300(21):1173-1179.

60. Coppieters KT, Wiberg A, Tracy SM, von Herrath MG. Immunology in the clinic review series: Focus on type 1 diabetes and viruses: The role of viruses in type 1 diabetes: A difficult dilemma. *Clinical and Experimental Immunology.* 2012;168(1):5-11.

61. Stene LC, Rewers M. Immunology in the clinic review series; focus on type 1 diabetes and viruses: The enterovirus link to type 1 diabetes: Critical review of human studies. *Clinical and Experimental Immunology.* 2012;168(1):12-23.

62. Tracy S, Drescher KM, Chapman NM. Enteroviruses and type 1 diabetes. *Diabetes/Metabolism Research and Reviews.* 2011;27(8):820-823.

63. Richardson SJ, Willcox A, Bone AJ, Foulis AK, Morgan NG. The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes. *Diabetologia.* 2009;52(6):1143-1151.

64. Krogvold L, Edwin B, Buanes T, et al. Detection of a low-grade enteroviral infection in the islets of langerhans of living patients newly diagnosed with type 1 diabetes. *Diabetes.* 2015;64(5):1682-1687.

65. Rešić Lindehammer S, Honkanen H, Nix WA, et al. Seroconversion to islet autoantibodies after enterovirus infection in early pregnancy. *Viral Immunology.* 2012;25(4):254-261.

66. Dahlquist GG, Ivarsson S, Lindberg B, Forsgren M. Maternal enteroviral infection during pregnancy as a risk factor for childhood IDDM: A population-based case-control study. *Diabetes.* 1995;44(4):408-413.

67. Viskari H, Ilonen J, Knip M, et al. Maternal enterovirus infection as a risk factor for type 1 diabetes in the exposed offspring. *Diabetes Care.* 2012;35(6):1328-1332.

68. Honeyman MC, Stone NL, Harrison LC. T-cell epitopes in type 1 diabetes autoantigen tyrosine phosphatase IA-2: potential for mimicry with rotavirus and other environmental agents. *Mol Med.* 1998;4(4):231-239.

69. Rogers MAM, Basu T, Kim C. Lower Incidence Rate of Type 1 Diabetes after Receipt of the Rotavirus Vaccine in the United States, 2001-2017. *Scientific Reports.* 2019;9(1):7727.

70. Brown CT, Davis-Richardson AG, Giongo A, et al. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS ONE.* 2011;6(10).

71. Kostic AD, Gevers D, Siljander H, et al. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host and Microbe.* 2015;17(2):260-273.

72. Morgan E, Halliday SR, Campbell GR, Cardwell CR, Patterson CC. Vaccinations and childhood type 1 diabetes mellitus: a meta-analysis of observational studies. *Diabetologia.* 2016;59(2):237-243.

73. Borch-Johnsen K, Joner G, Mandrup-Poulsen T, et al. Relation between breast-feeding and incidence rates of insulin-dependent diabetes mellitus. A hypothesis. *Lancet.* 1984;2(8411):1083-1086.

74. Ziegler AG, Schmid S, Huber D, Hummel M, Bonifacio E. Early Infant Feeding and Risk of Developing Type 1 Diabetes-Associated Autoantibodies. *Journal of the American Medical Association.* 2003;290(13):1721-1728.

75. Holmberg H, Whalberg J, Vaarala O, Ludvigsson J. Short duration of breast-feeding as a risk-factor for β-cell autoantibodies in 5-year-old children from the general population. *British Journal of Nutrition.* 2007;97(1):111-116.

76. Harrison LC, Honeyman MC. Cow's milk and type 1 diabetes: the real debate is about mucosal immune function. *Diabetes.* 1999;48(8):1501-1507.

77. Vaarala O, Klemetti P, Juhela S, Simell O, Hyoty H, Ilonen J. Effect of coincident enterovirus infection and cows' milk exposure on immunisation to insulin in early infancy. *Diabetologia.* 2002;45(4):531-534.

78. Knip M, Veijola R, Virtanen SM, Hyöty H, Vaarala O, Åkerblom HK. Environmental triggers and determinants of type 1 diabetes. *Diabetes.* 2005;54(SUPPL. 2):S125-S136.

79. Dahl-Jorgensen K, Joner G, Hanssen KF. Relationship between cows' milk consumption and incidence of IDDM in childhood. *Diabetes Care.* 1991;14(11):1081-1083.

80. Verge CF, Howard NJ, Irwig L, Simpson JM, Mackerras D, Silink M. Environmental factors in childhood IDDM: A population-based, case-control study. *Diabetes Care.* 1994;17(12):1381-1390.

81. Virtanen SM, Hypponen E, Laara E, et al. Cow's milk consumption, disease-associated autoantibodies and type 1 diabetes mellitus: a follow-up study in siblings of diabetic children. Childhood Diabetes in Finland Study Group. *Diabet Med.* 1998;15(9):730-738.

82. Knip M, Virtanen SM, Seppä K, et al. Dietary intervention in infancy and later signs of beta-cell autoimmunity. *New England Journal of Medicine.* 2010;363(20):1900-1908.

83. Writing Group for the TSG, Knip M, Akerblom HK, et al. Effect of Hydrolyzed Infant Formula vs Conventional Formula on Risk of Type 1 Diabetes: The TRIGR Randomized Clinical Trial. *JAMA.* 2018;319(1):38-48.

84. Norris JM, Barriga K, Klingensmith G, et al. Timing of Initial Cereal Exposure in Infancy and Risk of Islet Autoimmunity. *Journal of the American Medical Association.* 2003;290(13):1713-1720.

85. Frederiksen B, Kroehl M, Lamb MM, et al. Infant exposures and development of type 1 diabetes mellitus: The Diabetes Autoimmunity Study in the Young (DAISY). *JAMA Pediatrics.* 2013;167(9):808-815.

86. Virtanen SM, Takkinen HM, Nevalainen J, et al. Early introduction of root vegetables in infancy associated with advanced ß-cell autoimmunity in young children with human leukocyte antigen-conferred susceptibility to Type 1 diabetes. *Diabetic Medicine.* 2011;28(8):965-971.

87. Sørensen IM, Joner G, Jenum PA, Eskild A, Torjesen PA, Stene LC. Maternal serum levels of 25-hydroxy-vitamin D during pregnancy and risk of type 1 diabetes in the offspring. *Diabetes.* 2012;61(1):175-178.

88. Miettinen ME, Reinert L, Kinnunen L, et al. Serum 25-hydroxyvitamin D level during early pregnancy and type 1 diabetes risk in the offspring. *Diabetologia.* 2012;55(5):1291-1294.

89. Marjamäki L, Niinistö S, Kenward MG, et al. Maternal intake of vitamin D during pregnancy and risk of advanced beta cell autoimmunity and type 1 diabetes in offspring. *Diabetologia.* 2010;53(8):1599-1607.

90. Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. *Archives of Disease in Childhood.* 2008;93(6):512-517.

91. Simpson M, Brady H, Yin X, et al. No association of vitamin D intake or 25-hydroxyvitamin D levels in childhood with risk of islet autoimmunity and type 1 diabetes: The Diabetes Autoimmunity Study in the Young (DAISY). *Diabetologia.* 2011;54(11):2779-2788.

92. Soedamah-Muthu SS, Fuller JH, Mulnier HE, Raleigh VS, Lawrenson RA, Colhoun HM. High risk of cardiovascular disease in patients with type 1 diabetes in the U.K.: a cohort study using the general practice research database. *Diabetes Care.* 2006;29(4):798-804.

93. Pambianco G, Costacou T, Ellis D, Becker DJ, Klein R, Orchard TJ. The 30-year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study experience. *Diabetes.* 2006;55(5):1463-1469.

94. de Ferranti SD, de Boer IH, Fonseca V, et al. Type 1 diabetes mellitus and cardiovascular disease: a scientific statement from the American Heart Association and American Diabetes Association. *Diabetes Care.* 2014;37(10):2843-2863.

95. Deckert T, Poulsen JE, Larsen M. Prognosis of diabetics with diabetes onset before the age of thirtyone - I. Survival, causes of death, and complications. *Diabetologia.* 1978;14(6):363-370.

96. Krolewski AS, Kosinski EJ, Warram JH, et al. Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus. *The American Journal of Cardiology.* 1987;59(8):750-755.

97. Libby P, Nathan DM, Abraham K, et al. Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on cardiovascular complications of type 1 diabetes mellitus. *Circulation.* 2005;111(25):3489-3493.

98. Huxley RR, Peters SAE, Mishra GD, Woodward M. Risk of all-cause mortality and vascular events in women versus men with type 1 diabetes: A systematic review and meta-analysis. *The Lancet Diabetes and Endocrinology.* 2015;3(3):198-206.

99. Miller RG, Mahajan HD, Costacou T, Sekikawa A, Anderson SJ, Orchard TJ. A contemporary estimate of total mortality and cardiovascular disease risk in young adults with type 1 diabetes: The pittsburgh epidemiology of diabetes complications study. *Diabetes Care.* 2016;39(12):2296-2303.

100. Secrest AM, Becker DJ, Kelsey SF, LaPorte RE, Orchard TJ. Cause-specific mortality trends in a large population-based cohort with long-standing childhood-onset type 1 diabetes. *Diabetes.* 2010;59(12):3216-3222.

101. Costacou T, Orchard TJ. Cumulative Kidney Complication Risk by 50 Years of Type 1 Diabetes: The Effects of Sex, Age, and Calendar Year at Onset. *Diabetes Care.* 2018;41(3):426-433.

102. Krolewski AS. Progressive renal decline: The new paradigm of diabetic nephropathy in type 1 diabetes. *Diabetes Care.* 2015;38(6):954-962.

103. Parving HH, Jacobsen P, Rossing K, Smidt UM, Hommel E, Rossing P. Benefits of long-term antihypertensive treatment on prognosis in diabetic nephropathy. *Kidney International.* 1996;49(6):1778-1782.

104. Svensson M, Sundkvist G, Arnqvist HJ, et al. Signs of nephropathy may occur early in young adults with diabetes despite modern diabetes management: Results from the nationwide

population-based Diabetes Incidence Study in Sweden (DISS). *Diabetes Care.* 2003;26(10):2903-2909.

105. Brenner BM, Cooper ME, De Zeeuw D, et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *New England Journal of Medicine.* 2001;345(12):861-869.

106. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The Effect of Angiotensin-Converting-Enzyme Inhibition on Diabetic Nephropathy. *New England Journal of Medicine.* 1993;329(20):1456-1462.

107. Lewis EJ, Hunsicker LG, Clarke WR, et al. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *New England Journal of Medicine.* 2001;345(12):851-860.

108. Orchard TJ, Secrest AM, Miller RG, Costacou T. In the absence of renal disease, 20 year mortality risk in type 1 diabetes is comparable to that of the general population: A report from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetologia.* 2010;53(11):2312-2319.

109. Groop PH, Thomas MC, Moran JL, et al. The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. *Diabetes.* 2009;58(7):1651-1658.

110. Nathan DM, Genuth S, Lachin J, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *The New England journal of medicine.* 1993;329(14):977-986.

111. Aiello LP, Group DER. Diabetic retinopathy and other ocular findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes Care.* 2014;37(1):17-23.

112. de Boer IH, Group DER. Kidney disease and related findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes Care.* 2014;37(1):24-30.

113. Martin CL, Albers JW, Pop-Busui R, Group DER. Neuropathy and related findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes Care.* 2014;37(1):31-38.

114. Lachin JM, Orchard TJ, Nathan DM. Update on cardiovascular outcomes at 30 years of the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes Care.* 2014;37(1):39-43.

115. Nathan DM, Zinman B, Cleary PA, et al. Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: The diabetes control and complications trial/epidemiology of diabetes interventions and complications and Pittsburgh epidemiology of diabetes complications experience (1983-2005). *Archives of Internal Medicine.* 2009;169(14):1307-1316.

116. Secrest AM, Becker DJ, Kelsey SF, LaPorte RE, Orchard TJ. All-cause mortality trends in a large population-based cohort with long-standing childhood-onset type 1 diabetes: The Allegheny County type 1 diabetes registry. *Diabetes care.* 2010;33(12):2573-2579.

117. Morgan E, Cardwell CR, Black CJ, McCance DR, Patterson CC. Excess mortality in Type 1 diabetes diagnosed in childhood and adolescence: a systematic review of population-based cohorts. *Acta Diabetologica.* 2015;52(4):801-807.

118. Rawshani A, Rawshani A, Franzen S, et al. Mortality and Cardiovascular Disease in Type 1 and Type 2 Diabetes. *The New England journal of medicine.* 2017;376(15):1407-1418.

119. Miller RG, Secrest AM, Sharma RK, Songer TJ, Orchard TJ. Improvements in the life expectancy of type 1 diabetes: The pittsburgh epidemiology of diabetes complications study cohort. *Diabetes.* 2012;61(11):2987-2992.

120. Livingstone SJ, Levin D, Looker HC, et al. Estimated life expectancy in a scottish cohort with type 1 diabetes, 2008-2010. *JAMA - Journal of the American Medical Association.* 2015;313(1):37-44.

121. Eeg-Olofsson K, Cederholm J, Nilsson PM, et al. Glycemic control and cardiovascular disease in 7,454 patients with type 1 diabetes: An observational study from the Swedish National Diabetes Register (NDR). *Diabetes Care.* 2010;33(7):1640-1646.

122. American Diabetes A. Economic Costs of Diabetes in the U.S. in 2017. *Diabetes Care.* 2018;41(5):917-928.

123. Dall TM, Mann SE, Zhang Y, et al. Distinguishing the economic costs associated with type 1 and type 2 diabetes. *Population Health Management.* 2009;12(2):103-110.

124. Milton B, Holland P, Whitehead M. The social and economic consequences of childhood-onset Type 1 diabetes mellitus across the lifecourse: a systematic review. *Diabet Med.* 2006;23(8):821-829.

125. Tao B, Pietropaolo M, Atkinson M, Schatz D, Taylor D. Estimating the cost of type 1 diabetes in the U.S.: A propensity score matching method. *PLoS ONE.* 2010;5(7).

126. Orchard TJ, Dorman JS, Maser RE, et al. Prevalence of complications in IDDM by sex and duration. Pittsburgh Epidemiology of Diabetes Complications Study II. *Diabetes.* 1990;39(9):1116-1124.

127. WHO Multinational Project for Childhood Diabetes. WHO Diamond Project Group. *Diabetes Care.* 1990;13(10):1062-1068.

128. Russell JT, Roesch LFW, Ordberg M, et al. Genetic risk for autoimmunity is associated with distinct changes in the human gut microbiome. *Nature communications.* 2019;10(1):3621.

129. Ziegler AG, Hummel M, Schenker M, Bonifacio E. Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB Study. *Diabetes.* 1999;48(3):460-468.

130. Rewers M, Norris JM, Eisenbarth GS, et al. Beta-cell autoantibodies in infants and toddlers without IDDM relatives: diabetes autoimmunity study in the young (DAISY). *J Autoimmun.* 1996;9(3):405-410.

131. ClinicalTrials.gov. Available at https://clinicaltrials.gov/ct2/show/NCT03269084. Accessed on Jan 20th. Accessed.

132. Group TS. The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. *Pediatr Diabetes.* 2007;8(5):286-298.

133. Soedamah-Muthu SS, Chaturvedi N, Witte DR, et al. Relationship between risk factors and mortality in type 1 diabetic patients in Europe: the EURODIAB Prospective Complications Study (PCS). *Diabetes Care.* 2008;31(7):1360-1366.

134. Thorn LM, Forsblom C, Fagerudd J, et al. Metabolic syndrome in type 1 diabetes: association with diabetic nephropathy and glycemic control (the FinnDiane study). *Diabetes Care.* 2005;28(8):2019-2024.

135. Hamman RF, Bell RA, Dabelea D, et al. The SEARCH for Diabetes in Youth study: rationale, findings, and future directions. *Diabetes Care.* 2014;37(12):3336-3344.

136. Oakley AE, Clifton DK, Steiner RA. Kisspeptin signaling in the brain. *Endocrine Reviews.* 2009;30(6):713-743.

137. Roa J, Navarro VM, Tena-Sempere M. Kisspeptins in reproductive biology: Consensus knowledge and recent developments. *Biology of Reproduction.* 2011;85(4):650-660.

138. Constantin S. Physiology of the gonadotrophin-releasing hormone (GnRH) neurone: Studies from embryonic GnRH neurones. *Journal of Neuroendocrinology.* 2011;23(6):542-553.

139. Codner E, Merino PM, Tena-Sempere M. Female reproduction and type 1 diabetes: From mechanisms to clinical findings. *Human Reproduction Update.* 2012;18(5):568-585.

140. Nandi A, Poretsky L. Diabetes and the Female Reproductive System. *Endocrinology and Metabolism Clinics of North America.* 2013;42(4):915-946.

141. Bruning JC, Gautam D, Burks DJ, et al. Role of brain insulin receptor in control of body weight and reproduction. *Science.* 2000;289(5487):2122-2125.

142. Poretsky L, Grigorescu F, Seibel M, Moses AC, Flier JS. Distribution and characterization of insulin and insulin-like growth factor i receptors in normal human ovary. *Journal of Clinical Endocrinology and Metabolism.* 1985;61(4):728-734.

143. Poretsky L, Smith D, Seibel M, Pazianos A, Moses AC, Flier JS. Specific insulin binding sites in human ovary. *Journal of Clinical Endocrinology and Metabolism.* 1984;59(4):809-811.

144. Okamoto H, Nakae J, Kitamura T, Park BC, Dragatsis I, Accili D. Transgenic rescue of insulin receptor-deficient mice. *Journal of Clinical Investigation.* 2004;114(2):214-223.

145. Burcelin R, Thorens B, Glauser M, Gaillard RC, Pralong FP. Gonadotropin-releasing hormone secretion from hypothalamic neurons: Stimulation by insulin and potentiation by leptin. *Endocrinology.* 2003;144(10):4484-4491.

146. Salvi R, Castillo E, Voirol MJ, et al. Gonadotropin-releasing hormone-expressing neurons immortalized conditionally are activated by insulin: Implication of the mitogen-activated protein kinase pathway. *Endocrinology.* 2006;147(2):816-826.

147. Pralong FP. Insulin and NPY pathways and the control of GnRH function and puberty onset. *Molecular and Cellular Endocrinology.* 2010;324(1-2):82-86.

148. Poretsky L, Kalin MF. The gonadotropic function of insulin. *Endocrine Reviews.* 1987;8(2):132-141.

149. Garzo VG, Dorrington JH. Aromatase activity in human granulosa cells during follicular development and the modulation by follicle-stimulating hormone and insulin. *American Journal of Obstetrics and Gynecology.* 1984;148(5):657-662.

150. Willis D, Mason H, Gilling-Smith C, Franks S. Modulation by insulin of follicle-stimulating hormone and luteinizing hormone actions in human granulosa cells of normal and polycystic ovaries. *Journal of Clinical Endocrinology and Metabolism.* 1996;81(1):302-309.

151. Cara JF, Rosenfield RL. Insulin-like growth factor i and insulin potentiate luteinizing hormone-induced androgen synthesis by rat ovarian thecal-interstitial cells. *Endocrinology.* 1988;123(2):733-739.

152. Davoren JB, Hsueh AJW. Insulin enhances FSH-stimulated steroidogenesis by cultured rat granulosa cells. *Molecular and Cellular Endocrinology.* 1984;35(2-3):97-105.

153. Barbieri RL, Makris A, Ryan KJ. Effects of insulin on steroidogenesis in cultured porcine ovarian theca. *Fertility and Sterility.* 1983;40(2):237-241.

154. Joslin EP, Root HF, White P. The growth, development and prognosis of diabetic children. *Journal of the American Medical Association.* 1925;85(6):420-422.

155. Markussis V, Goni MH, Tolis G. The role of insulin in ovarian size in patients with the polycystic ovary syndrome. *Gynecological Endocrinology.* 1994;8(3):197-202.

156. Pasquali R, Casimirri F, De Iasio R, et al. Insulin regulates testosterone and sex hormone-binding globulin concentrations in adult normal weight and obese men. *The Journal of clinical endocrinology and metabolism.* 1995;80(2):654-658.

157. Strain G, Zumoff B, Rosner W, Pi-Sunyer X. The relationship between serum levels of insulin and sex hormone-binding globulin in men: the effect of weight loss. *The Journal of clinical endocrinology and metabolism.* 1994;79(4):1173-1176.

158. Ding EL, Song Y, Manson JE, et al. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *The New England journal of medicine.* 2009;361(12):1152-1163.

159. Griffin ML, South SA, Yankov VI, et al. Insulin-dependent diabetes mellitus and menstrual dysfunction. *Annals of Medicine.* 1994;26(5):331-340.

160. Bestetti GE, Junker U, Locatelli V, Rossi GL. Continuous subtherapeutic insulin counteracts hypothalamopituitary-gonadal alterations in diabetic rats. *Diabetes.* 1987;36(11):1315-1319.

161. Steger RW, Amador A, Lam E, Rathert J, Weis J, Smith MS. Streptozotocin-induced deficits in sex behavior and neuroendocrine function in male rats. *Endocrinology.* 1989;124(4):1737-1743.

162. Chang AS, Dale AN, Moley KH. Maternal diabetes adversely affects preovulatory oocyte maturation, development, and granulosa cell apoptosis. *Endocrinology.* 2005;146(5):2445-2453.

163. Chabrolle C, Jean Pierre E, Tosca L, Ramé C, Dupont J. Effects of high levels of glucose on the steroidogenesis and the expression of adiponectin receptors in rat ovarian cells. *Reproductive Biology and Endocrinology.* 2008;6.

164. Poretsky L, Clemons J, Bogovich K. Hyperinsulinemia and human chorionic gonadotropin synergistically promote the growth of ovarian follicular cysts in rats. *Metabolism.* 1992;41(8):903-910.

165. Codner E, Soto N, Merino PM. Contraception and pregnancy in adolescents with type 1 diabetes: A review. *Pediatric Diabetes.* 2012;13(1):108-123.

166. Codner E, Escobar-Morreale HF. Clinical review: Hyperandrogenism and polycystic ovary syndrome in women with type 1 diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism.* 2007;92(4):1209-1216.

167. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *The Journal of clinical endocrinology and metabolism.* 2004;89(6):2745-2749.

168. Escobar-Morreale HF, Roldán B, Barrio R, et al. High prevalence of the polycystic ovary syndrome and hirsutism in women with type 1 diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism.* 2000;85(11):4182-4187.

169. Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S, Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *The Journal of clinical endocrinology and metabolism.* 2000;85(7):2434-2438.

170. Dunaif A. Insulin resistance and the polycystic ovary syndrome: Mechanism and implications for pathogenesis. *Endocrine Reviews.* 1997;18(6):774-800.

171. Gregory JM, Smith TJ, Slaughter JC, et al. Iatrogenic Hyperinsulinemia, Not Hyperglycemia, Drives Insulin Resistance in Type 1 Diabetes as Revealed by Comparison With GCK-MODY (MODY2). *Diabetes.* 2019;68(8):1565-1576.

172. Rossetti L, Giaccari A, DeFronzo RA. Glucose toxicity. *Diabetes care.* 1990;13(6):610-630.

173. John WG, Lamb EJ. The Maillard or browning reaction in diabetes. *Eye.* 1993;7 (Pt 2):230-237.

174. Dyer DG, Dunn JA, Thorpe SR, et al. Accumulation of Maillard reaction products in skin collagen in diabetes and aging. *Journal of Clinical Investigation.* 1993;91(6):2463-2469.

175. Paul RG, Bailey AJ. The effect of advanced glycation end-product formation upon cell-matrix interactions. *International Journal of Biochemistry and Cell Biology.* 1999;31(6):653-660.

176. Chiarelli F, De Martino M, Mezzetti A, et al. Advanced glycation end products in children and adolescents with diabetes: Relation to glycemic control and early microvascular complications. *Journal of Pediatrics.* 1999;134(4):486-491.

177. Berg TJ, Dahl-Jørgensen K, Torjesen PA, Hanssen KF. Increased serum levels of advanced glycation end products (AGEs) in children and adolescents with IDDM. *Diabetes care.* 1997;20(6):1006-1008.

178. Sims TJ, Rasmussen LM, Oxlund H, Bailey AJ. The role of glycation cross-links in diabetic vascular stiffening. *Diabetologia.* 1996;39(8):946-951.

179. Miyata S, Monnier V. Immunohistochemical detection of advanced glycosylation end products in diabetic tissues using monoclonal antibody to pyrraline. *Journal of Clinical Investigation.* 1992;89(4):1102-1112.

180. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. *Diabetologia.* 2001;44(2):129-146.

181. Furth AJ. Glycated proteins in diabetes. *British Journal of Biomedical Science.* 1997;54(3):192-200.

182. McCance DR, Dyer DG, Dunn JA, et al. Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. *Journal of Clinical Investigation.* 1993;91(6):2470-2478.

183. Thornalley PJ. Cell activation by glycated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs. *Cellular and molecular biology (Noisy-le-Grand, France).* 1998;44(7):1013-1023.

184. Bodiga VL, Eda SR, Bodiga S. Advanced glycation end products: Role in pathology of diabetic cardiomyopathy. *Heart Failure Reviews.* 2014;19(1):49-63.

185. Ramasamy R, Schmidt AM. Receptor for advanced glycation end products (RAGE) and implications for the pathophysiology of heart failure. *Current Heart Failure Reports.* 2012;9(2):107-116.

186. Vlassara H, Palace MR. Diabetes and advanced glycation endproducts. *Journal of Internal Medicine.* 2002;251(2):87-101.

187. Schmidt AM, Yan SD, Wautier JL, Stern D. Activation of receptor for advanced glycation end products: A mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. *Circulation Research.* 1999;84(5):489-497.

188. Diamanti-Kandarakis E, Piperi C, Patsouris E, et al. Immunohistochemical localization of advanced glycation end-products (AGEs) and their receptor (RAGE) in polycystic and normal ovaries. *Histochem Cell Biol.* 2007;127(6):581-589.

189. Diamanti-Kandarakis E, Katsikis I, Piperi C, et al. Increased serum advanced glycation end-products is a distinct finding in lean women with polycystic ovary syndrome (PCOS). *Clinical Endocrinology.* 2008;69(4):634-641.

190. Pertynska-Marczewska M, Diamanti-Kandarakis E. Aging ovary and the role for advanced glycation end products. *Menopause.* 2017;24(3):345-351.

191. Ceriello A. Hyperglycaemia: the bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. *Diabetes, nutrition & metabolism.* 1999;12(1):42-46.

192. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. *Reproductive biology and endocrinology : RB&E.* 2005;3:28.

193. Yi Y, Denic-Roberts H, Rubinstein D, Orchard TJ, Costacou T. Effect of age at menarche on microvascular complications among women with Type 1 diabetes. *Diabet Med.* 2019.

194. McDowell MA, Brody DJ, Hughes JP. Has age at menarche changed? Results from the National Health and Nutrition Examination Survey (NHANES) 1999-2004. *J Adolesc Health.* 2007;40(3):227-231.

195. Rohrer T, Stierkorb E, Grabert M, et al. Delayed menarche in young German women with type 1 diabetes mellitus: Recent results from the DPV diabetes documentation and quality management system. *European Journal of Pediatrics.* 2008;167(7):793-799.

196. Deltsidou A. Age at Menarche and Menstrual Irregularities of Adolescents with Type 1 Diabetes. *Journal of Pediatric and Adolescent Gynecology.* 2010;23(3):162-167.

197. Strotmeyer ES, Steenkiste AR, Foley Jr TP, Berga SL, Dorman JS. Menstrual cycle differences between women with type 1 diabetes and women without diabetes. *Diabetes Care.* 2003;26(4):1016-1021.

198. Danielson KK, Palta M, Allen C, D'Alessio DJ. The association of increased total glycosylated hemoglobin levels with delayed age at menarche in young women with type 1 diabetes. *Journal of Clinical Endocrinology and Metabolism.* 2005;90(12):6466-6471.

199. Schweiger BM, Snell-Bergeon JK, Roman R, McFann K, Klingensmith GJ. Menarche delay and menstrual irregularities persist in adolescents with type 1 diabetes. *Reproductive Biology and Endocrinology.* 2011;9.

200. Harjutsalo V, Maric-Bilkan C, Forsblom C, Groop PH, FinnDiane Study G. Age at menarche and the risk of diabetic microvascular complications in patients with type 1 diabetes. *Diabetologia.* 2016;59(3):472-480.

201. Salerno M, Argenziano A, Di Maio S, et al. Pubertal growth, sexual maturation, and final height in children with IDDM: Effects of age at onset and metabolic control. *Diabetes Care.* 1997;20(5):721-724.

202. Clarson C, Daneman D, Ehrlich RM. The relationship of metabolic control to growth and pubertal development in children with insulin-dependent diabetes. *Diabetes Research.* 1985;2(5):237-241.

203. Rohrer T, Stierkorb E, Grabert M, et al. Delayed menarche in young German women with type 1 diabetes mellitus: recent results from the DPV diabetes documentation and quality management system. *Eur J Pediatr.* 2008;167(7):793-799.

204. Rohrer T, Stierkorb E, Heger S, et al. Delayed pubertal onset and development in German children and adolescents with type 1 diabetes: Cross-sectional analysis of recent data from the DPV diabetes documentation and quality management system. *European Journal of Endocrinology.* 2007;157(5):647-653.

205. Bruning JC, Gautam D, Burks DJ, et al. Role of brain insulin receptor in control of body weight and reproduction. *Science.* 2000;289(5487):2122-2125.

206. Picardi A, Cipponeri E, Bizzarri C, Fallucca S, Guglielmi C, Pozzilli P. Menarche in type 1 diabetes is still delayed despite good metabolic control. *Fertil Steril.* 2008;90(5):1875-1877.

207. Lombardo F, Salzano G, Crisafulli G, et al. Menarcheal timing in intensively treated girls with type 1 diabetes mellitus. *Nutrition, Metabolism and Cardiovascular Diseases.* 2009;19(1):35-38.

208. Remer T, Maser-Gluth C, Boye KR, Hartmann MF, Heinze E, Wudy SA. Exaggerated adrenarche and altered cortisol metabolism in Type 1 diabetic children. *Steroids.* 2006;71(7):591-598.

209. Codner E, Mook-Kanamori D, Bazaes RA, et al. Ovarian function during puberty in girls with type 1 diabetes mellitus: Response to leuprolide. *Journal of Clinical Endocrinology and Metabolism.* 2005;90(7):3939-3945.

210. Šnajderová M, Martínek J, Hořejší J, Nováková D, Lebl J, Koloušková S. Premenarchal and postmenarchal girls with insulin-dependent diabetes mellitus: Ovarian and other organ-specific autoantibodies, menstrual cycle. *Journal of Pediatric and Adolescent Gynecology.* 1999;12(4):209-214.

211. Agarwal M, Selvan V, Freedman BI, Liu Y, Wagenknecht LE. The relationship between albuminuria and hormone therapy in postmenopausal women. *Am J Kidney Dis.* 2005;45(6):1019-1025.

212. Szekacs B, Vajo Z, Varbiro S, et al. Postmenopausal hormone replacement improves proteinuria and impaired creatinine clearance in type 2 diabetes mellitus and hypertension. *BJOG.* 2000;107(8):1017-1021.

213. Hadjadj S, Gourdy P, Zaoui P, et al. Effect of raloxifene -- a selective oestrogen receptor modulator -- on kidney function in post-menopausal women with Type 2 diabetes: results from a randomized, placebo-controlled pilot trial. *Diabet Med.* 2007;24(8):906-910.

214. KjÆr K, Hagen C, Sandø SH, Eshøj O. Epidemiology of menarche and menstrual disturbances in an unselected group of women with insulin-dependent diabetes mellitus compared to controls. *Journal of Clinical Endocrinology and Metabolism.* 1992;75(2):524-529.

215. Codner E, Barrera A, Mook-Kanamori D, et al. Ponderal gain, waist-to-hip ratio, and pubertal development in girls with type-1 diabetes mellitus. *Pediatric Diabetes.* 2004;5(4):182-189.

216. Elamin A, Hussein O, Tuvemo T. Growth, puberty, and final height in children with Type 1 diabetes. *Journal of Diabetes and its Complications.* 2006;20(4):252-256.

217. Snell-Bergeon JK, Dabelea D, Ogden LG, et al. Reproductive history and hormonal birth control use are associated with coronary calcium progression in women with type 1 diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism.* 2008;93(6):2142-2148.

218. Lombardo F, Salzano G, Crisafulli G, et al. Menarcheal timing in intensively treated girls with type 1 diabetes mellitus. *Nutrition, metabolism, and cardiovascular diseases : NMCD.* 2009;19(1):35-38.

219. Picardi A, Cipponeri E, Bizzarri C, Fallucca S, Guglielmi C, Pozzilli P. Menarche in type 1 diabetes is still delayed despite good metabolic control. *Fertility and Sterility.* 2008;90(5):1875-1877.

220. Zachurzok A, Deja G, Gawlik A, Drosdzol-Cop A, Malecka-Tendera E. Hyperandrogenism in adolescent girls with type 1 diabetes mellitus treated with intensive and continuous subcutaneous insulin therapy. *Endokrynologia Polska.* 2013;64(2):121-128.

221. Gomes MB, Negrato CA, Calliari LEP. Early age at menarche: A risk factor for overweight or obesity in patients with type 1 diabetes living in urban areas? *Diabetes Research and Clinical Practice.* 2015;107(1):23-30.

222. Gold EB. The timing of the age at which natural menopause occurs. *Obstetrics and gynecology clinics of North America.* 2011;38(3):425-440.

223. Luoto R, Kaprio J, Uutela A. Age at natural menopause and sociodemographic status in Finland. *American Journal of Epidemiology.* 1994;139(1):64-76.

224. te Velde ER, Pearson PL. The variability of female reproductive ageing. *Hum Reprod Update.* 2002;8(2):141-154.

225. Jacobsen BK, Heuch I, Kvale G. Age at natural menopause and all-cause mortality: a 37-year follow-up of 19,731 Norwegian women. *Am J Epidemiol.* 2003;157(10):923-929.

226. Jacobsen BK, Knutsen SF, Fraser GE. Age at natural menopause and total mortality and mortality from ischemic heart disease: the Adventist Health Study. *J Clin Epidemiol.* 1999;52(4):303-307.

227. Hu FB, Grodstein F, Hennekens CH, et al. Age at natural menopause and risk of cardiovascular disease. *Arch Intern Med.* 1999;159(10):1061-1066.

228. Joakimsen O, Bonaa KH, Stensland-Bugge E, Jacobsen BK. Population-based study of age at menopause and ultrasound assessed carotid atherosclerosis: The Tromso Study. *J Clin Epidemiol.* 2000;53(5):525-530.

229. Lisabeth LD, Beiser AS, Brown DL, Murabito JM, Kelly-Hayes M, Wolf PA. Age at natural menopause and risk of ischemic stroke: the Framingham heart study. *Stroke.* 2009;40(4):1044-1049.

230. Ossewaarde ME, Bots ML, Verbeek AL, et al. Age at menopause, cause-specific mortality and total life expectancy. *Epidemiology.* 2005;16(4):556-562.

231. Kritz-Silverstein D, Barrett-Connor E. Early menopause, number of reproductive years, and bone mineral density in postmenopausal women. *American journal of public health.* 1993;83(7):983-988.

232. van Der Voort DJ, van Der Weijer PH, Barentsen R. Early menopause: increased fracture risk at older age. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA.* 2003;14(6):525-530.

233. Franceschi S, La Vecchia C, Booth M, et al. Pooled analysis of 3 European case-control studies of ovarian cancer: II. Age at menarche and at menopause. *International journal of cancer.* 1991;49(1):57-60.

234. Schildkraut JM, Cooper GS, Halabi S, Calingaert B, Hartge P, Whittemore AS. Age at natural menopause and the risk of epithelial ovarian cancer. *Obstet Gynecol.* 2001;98(1):85-90.

235. Monninkhof EM, van der Schouw YT, Peeters PH. Early age at menopause and breast cancer: are leaner women more protected? A prospective analysis of the Dutch DOM cohort. *Breast cancer research and treatment.* 1999;55(3):285-291.

236. Dorman JS, Steenkiste AR, Foley TP, et al. Menopause in type 1 diabetic women: is it premature? *Diabetes.* 2001;50(8):1857-1862.

237. Brand JS, Onland-Moret NC, Eijkemans MJC, et al. Diabetes and onset of natural menopause: Results from the European Prospective Investigation into Cancer and Nutrition. *Human Reproduction.* 2015;30(6):1491-1498.

238. Yarde F, Van Der Schouw YT, De Valk HW, et al. Age at menopause in women with type 1 diabetes mellitus: The OVADIA study. *Human Reproduction.* 2015;30(2):441-446.

239. Sjöberg L, Pitkäniemi J, Harjutsalo V, et al. Menopause in women with type 1 diabetes. *Menopause.* 2011;18(2):158-163.

240. Van Asselt KM, Kok HS, Van Der Schouw YT, et al. Current smoking at menopause rather than duration determines the onset of natural menopause. *Epidemiology.* 2004;15(5):634-639.

241. Parente RC, Faerstein E, Celeste RK, Werneck GL. The relationship between smoking and age at the menopause: A systematic review. *Maturitas.* 2008;61(4):287-298.

242. Do KA, Treloar SA, Pandeya N, et al. Predictive factors of age at menopause in a large Australian twin study. *Human Biology.* 1998;70(6):1073-1091.

243. Kim C, Cleary PA, Cowie CC, et al. Effect of glycemic treatment and microvascular complications on menopause in women with type 1 diabetes in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort. *Diabetes Care.* 2014;37(3):701-708.

244. Sowers MR, Eyvazzadeh AD, McConnell D, et al. Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. *The Journal of clinical endocrinology and metabolism.* 2008;93(9):3478-3483.

245. Soto N, Iñiguez G, López P, et al. Anti-Müllerian hormone and inhibin B levels as markers of premature ovarian aging and transition to menopause in type 1 diabetes mellitus. *Human Reproduction.* 2009;24(11):2838-2844.

246. Yarde F, Spiering W, Franx A, et al. Association between vascular health and ovarian ageing in type 1 diabetes mellitus. *Human Reproduction.* 2016;31(6):1354-1362.

247. Kim C, Karvonen-Gutierrez C, Kong S, et al. Antimullerian hormone among women with and without type 1 diabetes: the Epidemiology of Diabetes Interventions and Complications Study and the Michigan Bone Health and Metabolism Study. *Fertil Steril.* 2016;106(6):1446-1452.

248. Kim C, Dunn RL, Braffett B, et al. Ovarian reserve in women with Type 1 diabetes in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study. *Diabet Med.* 2016;33(5):691-692.

249. American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* 2009;32 Suppl 1:S62-67.

250. Albrecht SS, Kuklina EV, Bansil P, et al. Diabetes trends among delivery hospitalizations in the U.S., 1994-2004. *Diabetes Care.* 2010;33(4):768-773.

251. Mackin ST, Nelson SM, Kerssens JJ, et al. Diabetes and pregnancy: national trends over a 15 year period. *Diabetologia.* 2018;61(5):1081-1088.

252. Feig DS, Hwee J, Shah BR, Booth GL, Bierman AS, Lipscombe LL. Trends in incidence of diabetes in pregnancy and serious perinatal outcomes: a large, population-based study in Ontario, Canada, 1996-2010. *Diabetes Care.* 2014;37(6):1590-1596.

253. Owens LA, Sedar J, Carmody L, Dunne F. Comparing type 1 and type 2 diabetes in pregnancy- similar conditions or is a separate approach required? *BMC Pregnancy Childbirth.* 2015;15:69.

254. Jensen DM, Damm P, Moelsted-Pedersen L, et al. Outcomes in type 1 diabetic pregnancies: a nationwide, population-based study. *Diabetes Care.* 2004;27(12):2819-2823.

255. Persson M, Norman M, Hanson U. Obstetric and perinatal outcomes in type 1 diabetic pregnancies: A large, population-based study. *Diabetes Care.* 2009;32(11):2005-2009.

256. Platt MJ, Stanisstreet M, Casson IF, et al. St Vincent's Declaration 10 years on: outcomes of diabetic pregnancies. *Diabet Med.* 2002;19(3):216-220.

257. Penney GC, Mair G, Pearson DW, Scottish Diabetes in Pregnancy G. Outcomes of pregnancies in women with type 1 diabetes in Scotland: a national population-based study. *BJOG.* 2003;110(3):315-318.

258. Casson IF, Clarke CA, Howard CV, et al. Outcomes of pregnancy in insulin dependent diabetic women: results of a five year population cohort study. *BMJ.* 1997;315(7103):275-278.

259. Evers IM, de Valk HW, Visser GH. Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in the Netherlands. *BMJ.* 2004;328(7445):915.

260. Macintosh MC, Fleming KM, Bailey JA, et al. Perinatal mortality and congenital anomalies in babies of women with type 1 or type 2 diabetes in England, Wales, and Northern Ireland: population based study. *BMJ.* 2006;333(7560):177.

261. Abell SK, Boyle JA, de Courten B, et al. Contemporary type 1 diabetes pregnancy outcomes: impact of obesity and glycaemic control. *The Medical journal of Australia.* 2016;205(4):162-167.

262. Maresh MJ, Holmes VA, Patterson CC, et al. Glycemic targets in the second and third trimester of pregnancy for women with type 1 diabetes. *Diabetes Care.* 2015;38(1):34-42.

263. Damm P, Mersebach H, Rastam J, et al. Poor pregnancy outcome in women with type 1 diabetes is predicted by elevated HbA1c and spikes of high glucose values in the third trimester. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet.* 2014;27(2):149-154.

264. Klemetti MM, Laivuori H, Tikkanen M, Nuutila M, Hiilesmaa V, Teramo K. White's classification and pregnancy outcome in women with type 1 diabetes: a population-based cohort study. *Diabetologia.* 2016;59(1):92-100.

265. Nielsen GL, Moller M, Sorensen HT. HbA1c in early diabetic pregnancy and pregnancy outcomes: a Danish population-based cohort study of 573 pregnancies in women with type 1 diabetes. *Diabetes Care.* 2006;29(12):2612-2616.

266. Murphy HR, Bell R, Cartwright C, et al. Improved pregnancy outcomes in women with type 1 and type 2 diabetes but substantial clinic-to-clinic variations: a prospective nationwide study. *Diabetologia.* 2017;60(9):1668-1677.

267. Pregnancy outcomes in the Diabetes Control and Complications Trial. *Am J Obstet Gynecol.* 1996;174(4):1343-1353.

268. Mills JL, Knopp RH, Simpson JL, et al. Lack of relation of increased malformation rates in infants of diabetic mothers to glycemic control during organogenesis. *N Engl J Med.* 1988;318(11):671-676.

269. Sacks DA, Feig DS, Liu IL, Wolde-Tsadik G. Managing type I diabetes in pregnancy: how near normal is necessary? *J Perinatol.* 2006;26(8):458-462.

270. Farrag OA. Prospective study of 3 metabolic regimens in pregnant diabetics. *Aust N Z J Obstet Gynaecol.* 1987;27(1):6-9.

271. Demarini S, Mimouni F, Tsang RC, Khoury J, Hertzberg V. Impact of metabolic control of diabetes during pregnancy on neonatal hypocalcemia: a randomized study. *Obstet Gynecol.* 1994;83(6):918-922.

272. Middleton P, Crowther CA, Simmonds L. Different intensities of glycaemic control for pregnant women with pre-existing diabetes. *Cochrane Database Syst Rev.* 2016(5):CD008540.

273. Sowers MF, Crawford SL, Sternfeld B. SWAN: A multi-center, multi-ethnic, community-based cohort study of women and the menopausal transition. *Menopause: Biology and Pathobiology.* 2000:175-188.

274. The hypertension detection and follow-up program: Hypertension detection and follow-up program cooperative group. *Preventive medicine.* 1976;5(2):207-215.

275. Orchard TJ, Dorman JS, Maser RE, et al. Factors associated with avoidance of severe complications after 25 yr of IDDM. Pittsburgh Epidemiology of Diabetes Complications Study I. *Diabetes Care.* 1990;13(7):741-747.

276. Thurston RC, El Khoudary SR, Sutton-Tyrrell K, et al. Vasomotor symptoms and lipid profiles in women transitioning through menopause. *Obstet Gynecol.* 2012;119(4):753-761.

277. Johnson BD, Merz CN, Braunstein GD, et al. Determination of menopausal status in women: the NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE) Study. *Journal of women's health.* 2004;13(8):872-887.

278. Samargandy S, Matthews KA, Brooks MM, et al. Arterial Stiffness Accelerates Within 1 Year of the Final Menstrual Period: The SWAN Heart Study. *Arterioscler Thromb Vasc Biol.* 2020;40(4):1001-1008.

279. Poretsky L, Kalin MF. The gonadotropic function of insulin. *Endocr Rev.* 1987;8(2):132-141.

280. Johnston JM, Colvin A, Johnson BD, et al. Comparison of SWAN and WISE menopausal status classification algorithms. *J Womens Health (Larchmt).* 2006;15(10):1184-1194.

281. Borhani NO KE, Langford HG, Payne GH, Remington RD, Stamler J. The hypertension detection and follow-up program. *Prev Med.* 1976;5(2):207-215.

282. Prince CT, Becker DJ, Costacou T, Miller RG, Orchard TJ. Changes in glycaemic control and risk of coronary artery disease in type 1 diabetes mellitus: findings from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC). *Diabetologia.* 2007;50(11):2280-2288.

283. Williams KV, Erbey JR, Becker D, Arslanian S, Orchard TJ. Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes.* 2000;49(4):626-632.

284. Ellis D, Coonrod BA, Dorman JS, et al. Choice of Urine Sample Predictive of Microalbuminuria in Patients With Insulin-Dependent Diabetes Mellitus. *American Journal of Kidney Diseases.* 1989;13(4):321-328.

285. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Annals of internal medicine.* 2009;150(9):604-612.

286. Isik S, Ozcan HN, Ozuguz U, et al. Evaluation of ovarian reserve based on hormonal parameters, ovarian volume, and antral follicle count in women with type 2 diabetes mellitus. *The Journal of clinical endocrinology and metabolism.* 2012;97(1):261-269.

287. Cheung KL, Stefanick ML, Allison MA, et al. Menopausal symptoms in women with chronic kidney disease. *Menopause.* 2015;22(9):1006-1011.

288. Stenvinkel P, Larsson TE. Chronic kidney disease: a clinical model of premature aging. *Am J Kidney Dis.* 2013;62(2):339-351.

289. Reynolds LP, Grazul-Bilska AT, Redmer DA. Angiogenesis in the female reproductive organs: pathological implications. *International journal of experimental pathology.* 2002;83(4):151-163.

290. Kok HS, van Asselt KM, van der Schouw YT, et al. Heart disease risk determines menopausal age rather than the reverse. *J Am Coll Cardiol.* 2006;47(10):1976-1983.

291. Zhu D, Chung HF, Pandeya N, et al. Premenopausal cardiovascular disease and age at natural menopause: a pooled analysis of over 170,000 women. *Eur J Epidemiol.* 2019;34(3):235-246.

292. Feldman EL, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA. A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. *Diabetes Care.* 1994;17(11):1281-1289.

293. Tuttle KR, Bakris GL, Bilous RW, et al. Diabetic kidney disease: a report from an ADA Consensus Conference. *Diabetes Care.* 2014;37(10):2864-2883.

294. Bakris GL, Molitch M. Microalbuminuria as a risk predictor in diabetes: the continuing saga. *Diabetes Care.* 2014;37(3):867-875.

295. Costacou T, Ellis D, Fried L, Orchard TJ. Sequence of progression of albuminuria and decreased GFR in persons with type 1 diabetes: a cohort study. *Am J Kidney Dis.* 2007;50(5):721-732.

296. Karalliedde J, Viberti G. Microalbuminuria and cardiovascular risk. *American journal of hypertension.* 2004;17(10):986-993.

297. Arun CS, Stoddart J, Mackin P, MacLeod JM, New JP, Marshall SM. Significance of microalbuminuria in long-duration type 1 diabetes. *Diabetes Care.* 2003;26(7):2144-2149.

298. Kagitani F, Uchida S, Hotta H. Effects of electrical stimulation of the superior ovarian nerve and the ovarian plexus nerve on the ovarian estradiol secretion rate in rats. *J Physiol Sci.* 2008;58(2):133-138.

299. Uchida S, Kagitani F. Effects of electrical stimulation of autonomic nerves to the ovary on the ovarian testosterone secretion rate in rats. *Auton Neurosci.* 2014;180:48-52.

300. Shadyab AH, Macera CA, Shaffer RA, et al. Ages at menarche and menopause and reproductive lifespan as predictors of exceptional longevity in women: the Women's Health Initiative. *Menopause.* 2017;24(1):35-44.

301. Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. *Preventive medicine.* 1991;20(1):47-63.

302. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA.* 1998;280(7):605-613.

303. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA.* 2002;288(3):321-333.