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

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**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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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
(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\textsuperscript{13} and around 3% based on the Diabetes Atlas report from the International Diabetes Federation\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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)\textsuperscript{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\textsuperscript{5}. The age group of 10-14 years persistently had the highest incidence compared with other age groups over time\textsuperscript{5}. In addition, type 1 diabetes was reported to be more common in boys\textsuperscript{5} and this gender difference in incidence
was also observed in Sweden. 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. 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.

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. Therefore, type 1 diabetes will continue to be one of the most common chronic diseases in children in the following decades. 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. Although type 1 diabetes onset usually occurs in childhood, most prevalent cases (84%) in the United States are adults.

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. 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. 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. 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. 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.
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. The last stage is long-standing type 1 diabetes. The more β-cell autoantibody types present, the more rapid the progression from the preclinical stage to the overt type 1 diabetes stage.

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. 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. Another potential mechanism is the elevated endoplasmic reticulum stress of β-cells, leading to errors in mRNA splicing and protein translation and folding. These abnormal protein products will be considered as immunogenic antigens and exacerbate autoimmune responses in islets. 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.

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. 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. 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
associated with reduced risk of retinopathy and nephropathy and was also very important in avoiding hypoglycemia\textsuperscript{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\textsuperscript{46}. Moreover, the risk of type 1 diabetes varies slightly among individuals whose siblings (8\%), father (5\%) or (3\%) mother have type 1 diabetes\textsuperscript{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\textsuperscript{47-49}. Over 50\% of disease heritability can be attributed to these two haplotypes\textsuperscript{50}. These two haplotypes are also greatly prevalent among children with type 1 diabetes in Scandinavia (one or both, approximately 90\%)\textsuperscript{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\textsuperscript{53}. In contrast, there are also some haplotypes, which are associated with reduced risk of type 1 diabetes, including HLA-DR15-DQ6\textsuperscript{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. 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. 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, 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 and human studies. Not only have enteroviruses been detected in the islets of individuals who were recently diagnosed with type 1 diabetes, 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. 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. 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. 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. 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. In contrast, a meta-analysis of 23 studies has shown that routine vaccinations were not associated with childhood-onset type 1 diabetes.

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. 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. 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. 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 ≥ 4 months 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. 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. In the 1990s, many studies suggested that cow’s milk may trigger the development of type 1 diabetes, 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, a protective effect was not observed in the larger phase 3 trial. 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. 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. 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. Interestingly, practicing breastfeeding while introducing solid foods reduced risk in DAISY. 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.

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, 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. 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. 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, 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. 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. 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. As discussed in a scientific statement from the American Heart Association and American Diabetes Association, the age-adjusted risk of cardiovascular complications is approximately ten-fold higher in people with type 1 diabetes compared with the general population, and women are disproportionately affected compared with men. In addition, cardiovascular events occur earlier in people with type 1 diabetes than in the general population. 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, thus making it the leading cause of death in people with type 1 diabetes.

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. 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. 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. 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. 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)\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{119}. However, all-cause mortality in type 1 diabetes remains twofold to fourfold higher than in the general population\textsuperscript{120,121}, with a substantial loss of life expectancy (11 years for men and 13 years for women)\textsuperscript{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\textsuperscript{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\textsuperscript{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)\textsuperscript{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
\textsuperscript{124,125}. As such, they are more likely to experience disadvantages in employment and have a lower
income in adulthood \textsuperscript{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 \textsuperscript{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>
<thead>
<tr>
<th>Year</th>
<th>Cycle</th>
<th>Survey</th>
<th>Clinical Exams</th>
<th>Reproductive Questionnaires</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986-1988</td>
<td>C1</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>-1990</td>
<td>C2</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>-1992</td>
<td>C3</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>-1994</td>
<td>C4</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>-1996</td>
<td>C5</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>-1998</td>
<td>C6 (10-YEARS)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>-2000</td>
<td>C7</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2002</td>
<td>C8 - C9 Combined</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2004</td>
<td>-2006</td>
<td>C10 (18-YEARS)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>-2008</td>
<td>C11</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2010</td>
<td>C12</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>-2012</td>
<td>C13</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>-2014</td>
<td>C14 (25-YEARS)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>-2017</td>
<td>C15</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>-2019</td>
<td>C16 (30-YEARS)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Name</th>
<th>Start year</th>
<th>Country</th>
<th>Design</th>
<th>Research question</th>
<th>Population</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General population-based cohorts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIAMOND: The World Health Organization (WHO) Multinational Project for Childhood Diabetes (WHO DIAMOND Project)</td>
<td>1990</td>
<td>100 centers in 60 countries worldwide</td>
<td>Population-based study</td>
<td>To investigate and characterize global incidence, mortality and health care of T1D</td>
<td>Newborn babies from Southeast Sweden born between 1 October 1997 and 1 October 1999</td>
<td>17,055</td>
</tr>
<tr>
<td>ABIS: The All Babies In Southeast Sweden (ABIS) study</td>
<td>1997</td>
<td>Sweden</td>
<td>Population-based cohort study</td>
<td>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</td>
<td>Newborns of parents with type 1 diabetes</td>
<td>1,353</td>
</tr>
<tr>
<td><strong>Cohorts of children with genetic susceptibility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BABYDIAB</td>
<td>1989</td>
<td>Germany</td>
<td>Cohort</td>
<td>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</td>
<td>Newborns of parents with type 1 diabetes</td>
<td>1,353</td>
</tr>
<tr>
<td>DAISY: The Diabetes Autoimmunity Study in the Young (DAISY)</td>
<td>1993</td>
<td>US</td>
<td>Cohort</td>
<td>To examine the characteristics of individuals developing autoimmunity at age 8 years or later, compared with children with early-onset autoimmunity</td>
<td>Children (&lt;7 yrs) with first-degree relatives having type 1 diabetes or with HLA genotype screening at birth</td>
<td>2,547</td>
</tr>
<tr>
<td>DIPP: The Finnish Type 1 Diabetes Prediction and Prevention (DIPP)</td>
<td>1994</td>
<td>Finland</td>
<td>Cohort</td>
<td>To understand the pathogenesis of type 1 diabetes (T1D), predict the disease, and find preventive treatment</td>
<td>Newborn baby carrying a DR-DQ genotype associated with increased risk for T1D</td>
<td>16,193</td>
</tr>
<tr>
<td>TEDDY: The Environmental Determinants of Diabetes in the Young (TEDDY)</td>
<td>2003</td>
<td>Six clinical centers worldwide (Finland, Germany, Sweden and three in North America)</td>
<td>Cohort</td>
<td>To identify infectious agents, dietary factors, or other environmental factors, including psychosocial factors which trigger T1DM in genetically susceptible individuals or which protect against the disease</td>
<td>Newborns (&lt;4 months) with first-degree relatives having type 1 diabetes or with HLA genotype screening at birth</td>
<td>7,801</td>
</tr>
<tr>
<td><strong>Cohorts of individuals with type 1 diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDC: The Pittsburgh Epidemiology of Diabetes Complications (EDC) study</td>
<td>1986</td>
<td>US</td>
<td>Cohort</td>
<td>To investigate the natural history of childhood-onset type 1 diabetes and risk factors related to the development of diabetic macrovascular and microvascular complications</td>
<td>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</td>
<td>658</td>
</tr>
<tr>
<td>Study Name</td>
<td>Year</td>
<td>Country/Region</td>
<td>Study Type</td>
<td>Description</td>
<td>Key Details</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------------------------------</td>
<td>------</td>
<td>-----------------------------</td>
<td>-----------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>EURODIAB: The Eurodiab type 1 complications study 133</td>
<td>1989</td>
<td>16 European Countries</td>
<td>Cohort</td>
<td>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.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FinnDiane: The Finnish Diabetic Nephropathy (FinnDiane) Study 134</td>
<td>1997</td>
<td>Finland</td>
<td>Cohort</td>
<td>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 &lt;0.20 nmol/l.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEARCH: The SEARCH for Diabetes in Youth 135</td>
<td></td>
<td>Registra y 2000, cohort 2012</td>
<td>A registry and a cohort study</td>
<td>1. An active registry: to assess prevalence, annual incidence, and trends by age, race/ethnicity, sex, and diabetes type. 2. Longitudinal 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 &lt;20 years.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical trial and follow-up observational cohort: DCCT/EDIC</td>
<td></td>
<td></td>
<td></td>
<td>The DCCT was comprised of 1,441 research subjects with T1DM who were recruited between 1983 and 1989. 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.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCCT: Diabetes Control and Complications Trial <a href="https://edic.bsc.gwu.edu/web/edic/dcct-study">https://edic.bsc.gwu.edu/web/edic/dcct-study</a></td>
<td>1983</td>
<td>US</td>
<td>Clinical trial</td>
<td>To examine the effects of intensive compared with conventional diabetes treatment on the development and progression of early microvascular, neurologic and other complications.</td>
<td>1,441</td>
<td></td>
</tr>
<tr>
<td>EDIC: Epidemiology of Diabetes Interventions and Complications <a href="https://edic.bsc.gwu.edu/">https://edic.bsc.gwu.edu/</a></td>
<td>1994</td>
<td>US</td>
<td>EDIC is a multi-center, longitudinal, observational study</td>
<td>In 1994, 96% of the surviving cohort of DCCT agreed to participate in the EDIC study.</td>
<td>1,375</td>
<td></td>
</tr>
</tbody>
</table>
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. 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). These gonadotropins are the main driving force for ovary development, ovulation and secretion of sex steroids, including estrogens, progestogens, androgens and inhibin. 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 release and self-control their own secretion (autocrine feedback) (Figure 1.3).
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
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) \cite{141} and all components of the ovary, including the granulosa, thecal, stromal, and follicular \cite{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 \cite{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 \cite{144}. In hyperinsulinemia clamp studies in mice, increased circulating insulin was associated with a substantial rise in LH secretion \cite{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 \cite{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 \cite{148} or through enhancing steroidogenesis responses to gonadotropins \cite{149-152}. As such, many in vitro studies have shown that insulin stimulates the ovarian production of estrogens, progestogens, and androgens \cite{153,154}. In addition, insulin was positively correlated with ovarian volume in patients with polycystic ovary syndrome (PCOS) \cite{155}. In contrast, insulin has been shown to decrease the synthesis of sex hormone-binding globulin (SHBG) in both in vivo \cite{156} and in vitro studies \cite{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 \cite{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)\(^{139}\).

![Diagram](image)

**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. In women with type 1 diabetes, the pituitary function may decline with increasing duration of diabetes. In mice with streptozotocin-induced diabetes, hypogonadism was observed subsequently, along with the decreased level of LH, FSH, and estradiol. However, hypogonadism was reversed after continuous administration of insulin, which successfully counteracted most of the effects of diabetes on the gonadal axis. Moreover, the finding that the mice with brain IRs gene knockout developed hypogonadism, 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.

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. In the physiological state, endogenous insulin produced by the pancreas, goes through first-pass metabolism through the liver. After this process, 50% to 70% of endogenous insulin is eliminated before it goes to the peripheral circulation. 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 \(^\text{165}\). 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 \(^\text{153}\). Excessive insulin can also act as a co-gonadotropin (e.g., FSH) to drive ovarian growth, cyst formation, and recruitment and growth of follicles \(^\text{164}\), 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 \(^\text{166}\) whereas the prevalence of PCOS in the US general population is 4-12% \(^\text{167}\). The prevalence of polycystic ovarian morphology was approximately 50% in women with type 1 diabetes \(^\text{166}\). Meanwhile, the prevalence of hirsutism in adult women with type 1 diabetes (30.6%) was approximately four-fold higher \(^\text{168}\) in comparison with nondiabetic women (7.1%) from similar ethnic and genetic backgrounds \(^\text{169}\). 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 \(^\text{170}\) and hyperinsulinemia \(^\text{171}\). 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 \(^\text{172}\). 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 \(^\text{172}\). In addition, a recent human subjects study shown that the iatrogenic hyperinsulinemia is the predominant driver for insulin resistance in type 1 diabetes \(^\text{171}\). 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. The formation and accumulation of AGE progresses with aging and occurs at an accelerated rate in people with diabetes. 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. 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, 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. 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. 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. Meanwhile, RAGE is widely distributed in cells, including endothelial cells, monocytes, macrophages, pericytes, and podocytes. 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. 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). Menarche delay is a common finding in women with type 1 diabetes, especially when the type 1 diabetes onset precedes menarche onset, although not all studies agree (Table 1.3). 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.

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 and a 1.3 month greater delay among U.S. women. Evidence from animal studies also point to impaired reproductive function, including hypogonadism and impaired ovarian follicle maturation with insulin deficiency, defects which are ameliorated with insulin administration. Nevertheless, several studies indicated that glycemic control was not associated with age at menarche in type 1 diabetes. 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. These studies suggest that in addition to the delayed activation of gonadotropin secretion caused by insulin deficiency in puberty, 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, which inhibits the normal function of the HPO axis. In addition, elevated advanced glycation end products, sex hormone binding globulin (SHBG) levels and ovarian antibodies 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, which concurred with findings from the FinnDiane study. However, contrary to FinnDiane results of an association between delayed menarche and the presence of laser-treated retinopathy, we failed to detect consistent significant associations between delayed menarche and either PR or CDSP.

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. 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
to investigate the role of sex hormones in the development of ON in women with type 1 diabetes and delayed menarche.
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Age at Menarche (AAM, yrs)</th>
<th>T1D (n)</th>
<th>Controls (n)</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarson et al.</td>
<td>1985</td>
<td>Canada</td>
<td>13.2 ± 1.2</td>
<td>122</td>
<td>-</td>
<td>T1D onset before puberty; Participants displayed normal pubertal development; HbA1 levels was not a major determinant of pubertal development in T1D</td>
</tr>
<tr>
<td>Kjaer et al.</td>
<td>1992</td>
<td>Denmark</td>
<td>14.2 ± 1.5</td>
<td>245</td>
<td>13.4 ± 1.4*</td>
<td>T1D onset before the age of 10 yrs</td>
</tr>
<tr>
<td>Salerno et al.</td>
<td>1997</td>
<td>Italy</td>
<td>12.8 ± 1.4</td>
<td>30</td>
<td>12.5 ± 1.0</td>
<td>Children with T1D have normal onset of puberty</td>
</tr>
<tr>
<td>Strotmeyer et al.</td>
<td>2003</td>
<td>USA</td>
<td>13.5 ± 1.9</td>
<td>143</td>
<td>12.6 ± 1.4*</td>
<td>T1D onset before puberty</td>
</tr>
<tr>
<td>Codner et al.</td>
<td>2004</td>
<td>Chile</td>
<td>13.0 ± 0.2</td>
<td>100</td>
<td>12.5 ± 1.1*</td>
<td>BMI, but not HbA1c levels, was a determinant of pubertal development in T1D</td>
</tr>
<tr>
<td>Danielson et al.</td>
<td>2004</td>
<td>USA</td>
<td>12.8 ± 1.33</td>
<td>188</td>
<td>12.5 *</td>
<td>AAM was delayed 1.3 months with each 1% increase in HbA1c</td>
</tr>
<tr>
<td>Elamin et al.</td>
<td>2006</td>
<td>Sudan</td>
<td>15.1 ± 1.25</td>
<td>35</td>
<td>13.3 *</td>
<td>T1D onset at the age of 7-13 yrs; AAM was related to HbA1c and duration of T1D</td>
</tr>
<tr>
<td>Rohrer et al.</td>
<td>2007</td>
<td>Germany</td>
<td>13.22 ± 1.31</td>
<td>643</td>
<td>12.7 ± 1.09*</td>
<td>T1D onset at the age of 7-17 yrs; AAM was delayed 0.07 years with each 1% increase in HbA1c</td>
</tr>
<tr>
<td>Snell-Bergeon et al.</td>
<td>2008</td>
<td>USA</td>
<td>13.1 ± 1.8</td>
<td>293</td>
<td>12.8 ± 1.5*</td>
<td>T1D onset before the age of 30 yrs; AAM was delayed 0.07 years with each 1% increase in HbA1c</td>
</tr>
<tr>
<td>Lombardo et al.</td>
<td>2008</td>
<td>Italy</td>
<td>12.77 ± 1.13</td>
<td>73</td>
<td>12.0 ± 1.10*</td>
<td>T1D onset before menarche;</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Country</td>
<td>Mean AAM ± SD (N)</td>
<td>Menarche Duration</td>
<td>Observation</td>
<td></td>
</tr>
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</tr>
<tr>
<td>Picardi et al.</td>
<td>2009</td>
<td>Italy</td>
<td>12.6 ± 1.5 (162)</td>
<td>T1D onset before menarche; Menarche in T1D is still delayed despite good metabolic control.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deltsidou et al.</td>
<td>2009</td>
<td>Greece</td>
<td>12.2 ± 1.4 (100)</td>
<td>T1D onset before the age of 10 yrs;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schweiger et al.</td>
<td>2010</td>
<td>USA</td>
<td>12.81 ± 0.09 (185)</td>
<td>T1D onset before menarche;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zachurzok et al.</td>
<td>2013</td>
<td>Poland</td>
<td>13.1 ± 1.4 (47)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gomes et al.</td>
<td>2015</td>
<td>Brazil</td>
<td>12.7 ± 1.7 (1527)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harjutsalo et al.</td>
<td>2016</td>
<td>Finland</td>
<td>13.82 ± 1.65 (600)</td>
<td>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;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.89 ± 1.43 (704)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yi et al.</td>
<td>2019</td>
<td>USA</td>
<td>13.0 (315)</td>
<td>AAM was significantly associated with the prevalence and cumulative incidence of overt nephropathy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Age at Natural Menopause (ANM, yrs)</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorman et al.</td>
<td>2001</td>
<td>USA</td>
<td>T1D (n)</td>
<td>Controls (n)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41.6 (15)</td>
<td>49.9 (21 sisters); * 48.0 (15 unrelated controls) *</td>
</tr>
<tr>
<td>Sjoberg et al.</td>
<td>2011</td>
<td>Finland</td>
<td>52.5 (476)</td>
<td>51 (Finish population)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kim et al.</td>
<td>2014</td>
<td>USA</td>
<td>49.9 (Intensive therapy)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>49.0 (Conventional therapy)</td>
<td></td>
</tr>
<tr>
<td>Brand et al.</td>
<td>2015</td>
<td>Europe</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yarde et al.</td>
<td>2015</td>
<td>Dutch</td>
<td>49.89 ± 4.7 (140)</td>
<td>49.8 ± 4.1 (5426)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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, preeclampsia 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. 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. In addition, a Cochrane systematic review of three clinical trials 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. 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. 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.
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. 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. 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 macrovascular 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 and the granulosa, thecal, and stromal components in the ovarian system. 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, especially when the onset of type 1 diabetes precedes the onset of menarche.

The evidence regarding the effect of type 1 diabetes on age at natural menopause, however, is limited and conflicting. 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. First, early natural menopause is related to
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 $\geq 126$ 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 $\geq 126$ 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 $\geq 140/90$ 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\textsuperscript{276} while total cholesterol and triglycerides were analyzed by enzymatic methods (on a Hitachi 747 analyzer -Boehringer Mannheim Diagnostics, Indianapolis, Indiana)\textsuperscript{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\textsuperscript{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\textsuperscript{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 ($\geq 12$ months).
months without menses not due to surgery)\textsuperscript{276}. For women defined as postmenopausal, their age at menopause was calculated by subtracting date of birth from date of participants’ last menstrual period\textsuperscript{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.

\section*{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. 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. 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). 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. Furthermore, selection of controls from the same household may compromise the representativeness of the control sample, although it takes into account any genetic influences. 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. 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. 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.

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) and the ovaries. 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 but also on the downstream component - granulosa, thecal, and stromal in the ovaries. In mice with a neuron-specific destruction of the insulin receptor gene, INSR, female mice showed impaired ovarian follicle
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.
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.  

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). 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

<table>
<thead>
<tr>
<th>Characteristics at baseline</th>
<th>EDC (n=105)</th>
<th>SWAN (n=341)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, n=105, 341)</td>
<td>42.8 (7.3)</td>
<td>46.0 (2.5)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Age at diabetes onset (years, n=105, -)</td>
<td>9.3 (3.9)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age at menarche (years, n=105, 338)</td>
<td>13.2 (1.7)</td>
<td>12.6 (1.5)</td>
<td>0.0002</td>
</tr>
<tr>
<td>T1D onset before menarche (n=80, 338)</td>
<td>13.6 (1.7)</td>
<td>12.6 (1.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>T1D onset after menarche (n=25, 338)</td>
<td>12.2 (1.2)</td>
<td>12.6 (1.5)</td>
<td>0.173</td>
</tr>
<tr>
<td>Non-Hispanic white women only (n=101, 230)</td>
<td>13.2 (1.7)</td>
<td>12.6 (1.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>Data adding 18 EDC women (n=123, 338)*</td>
<td>13.2 (1.7)</td>
<td>12.6 (1.5)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diabetes duration (years, n=105)</td>
<td>33.5 (8.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Race (%), n=105, 341</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>96.2 (101)</td>
<td>68.3 (233)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Black</td>
<td>3.8 (4)</td>
<td>31.7 (108)</td>
<td></td>
</tr>
<tr>
<td>Marital status (%), n=104, 341</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single, never married</td>
<td>13.5 (14)</td>
<td>10.3 (35)</td>
<td>0.516</td>
</tr>
<tr>
<td>Currently married or cohabitating</td>
<td>69.2 (72)</td>
<td>66.3 (226)</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>12.5 (13)</td>
<td>16.1 (55)</td>
<td></td>
</tr>
<tr>
<td>Separated, Widowed, or others</td>
<td>4.8 (5)</td>
<td>7.3 (25)</td>
<td></td>
</tr>
<tr>
<td>Education (%), n=104, 341</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school or less</td>
<td>31.7 (33)</td>
<td>24.3 (83)</td>
<td>0.250</td>
</tr>
<tr>
<td>Some college or received bachelor's degree</td>
<td>51.0 (53)</td>
<td>53.1 (181)</td>
<td></td>
</tr>
<tr>
<td>Graduate education beyond bachelor's degree</td>
<td>17.3 (18)</td>
<td>22.6 (77)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m², n=105, 338)</td>
<td>25.2 (22.6, 28.1)</td>
<td>26.5 (23.7, 30.9)</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist to hip ratio (n=94, 334)</td>
<td>0.81 (0.76, 0.86)</td>
<td>0.79 (0.75, 0.84)</td>
<td>0.099</td>
</tr>
<tr>
<td>Smoking status (%), n=104, 339</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>67.3 (70)</td>
<td>48.1 (163)</td>
<td>0.002</td>
</tr>
<tr>
<td>Past smoker</td>
<td>16.4 (17)</td>
<td>28.9 (98)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>16.4 (17)</td>
<td>23.0 (78)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg, n=105, 341)</td>
<td>114.0 (102.0, 124.0)</td>
<td>111.0 (104.0, 123.0)</td>
<td>0.708</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg, n=105, 341)</td>
<td>65.0 (58.0, 71.0)</td>
<td>71.0 (66.0, 79.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Characteristics at baseline</td>
<td>EDC (n=105)</td>
<td>SWAN (n=341)</td>
<td>p-value</td>
</tr>
<tr>
<td>----------------------------------------------------------------</td>
<td>-------------</td>
<td>--------------</td>
<td>---------</td>
</tr>
<tr>
<td>Blood pressure medication use (%; n=105, 341)</td>
<td>16.2 (17)</td>
<td>8.8 (30)</td>
<td>0.031</td>
</tr>
<tr>
<td>Lipid medication use (%; n=105, 341)</td>
<td>19.05 (20)</td>
<td>0.29 (1)</td>
<td>&lt;0.0001 *</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl; n=105, 340)</td>
<td>181.0 (162.0, 197.0)</td>
<td>193.5 (173.5, 219.0)</td>
<td>0.0003</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl; n=105, 340)</td>
<td>61.0 (50.6, 73.0)</td>
<td>53.0 (46.0, 62.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl; n=84, 329)</td>
<td>100.9 (87.0, 127.4)</td>
<td>117.0 (101.0, 140.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides (mg/dl; n=86, 330)</td>
<td>74.0 (51.0, 100.0)</td>
<td>86.0 (65.0, 125.0)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

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

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

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

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

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.
Figure 2.1 Flow Chart of the Pittsburgh Site of the Study of Women’s Health Across the Nation (SWAN)
Figure 2.2 Determination of Menopausal Status in the EDC Study

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

- 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.
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\(^\text{126}\). 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.
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. Hypertension was defined as blood pressure ≥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) 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). 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)).

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
modification of the Lipid Research Clinics method \cite{126,275}. Total cholesterol and triglycerides were measured enzymatically \cite{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 \cite{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 \cite{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 \cite{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.
menopause (p=0.03). For every 30% increase in AER, age at natural menopause decreases by 0.18 years (back transformation: \(-0.67 \times \log(1.30) = -0.18\)). 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. 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 and promoting ovarian growth, cyst formation,
follicular recruitment and growth through its gonadotropin-like effect on the ovaries. In addition, insulin can up-regulate LH receptors and insulin-like growth factor (IGF)-1 receptors in the ovary. 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 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. 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.

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. 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. 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. 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 and a Finnish study 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)\textsuperscript{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\textsuperscript{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\textsuperscript{239}. On the contrary, no association between nephropathy and menopause was observed in the DCCT/EDIC study\textsuperscript{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)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline (years)</td>
<td>29.5 ± 6.2</td>
</tr>
<tr>
<td>Age at diabetes onset (years)</td>
<td>9.3 ± 3.9</td>
</tr>
<tr>
<td>Duration of type 1 diabetes at baseline (years)</td>
<td>20.2 ± 7.0</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>96.2 (101)</td>
</tr>
<tr>
<td>Black</td>
<td>3.8 (4)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
</tr>
<tr>
<td>Single, never married</td>
<td>37.1 (39)</td>
</tr>
<tr>
<td>Currently married or living as if married</td>
<td>48.6 (51)</td>
</tr>
<tr>
<td>Divorced</td>
<td>7.6 (8)</td>
</tr>
<tr>
<td>Separated, Widowed, or others</td>
<td>6.7 (7)</td>
</tr>
<tr>
<td>Education (n=103)</td>
<td></td>
</tr>
<tr>
<td>High school or less</td>
<td>35.0 (36)</td>
</tr>
<tr>
<td>Some college or received bachelor's degree</td>
<td>56.3 (58)</td>
</tr>
<tr>
<td>Graduate education beyond bachelor's degree</td>
<td>8.7 (9)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>67.6 (71)</td>
</tr>
<tr>
<td>Past smoker</td>
<td>13.3 (14)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>19.1 (20)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9 ± 2.9</td>
</tr>
<tr>
<td>Waist to hip ratio (n=104)</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>107.9 ± 11.7</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68.5 ± 9.6</td>
</tr>
<tr>
<td>Hypertension (n=105)</td>
<td>8.6 (9)</td>
</tr>
<tr>
<td>Blood pressure medication use (n=103)</td>
<td>6.8 (7)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>181.0 (162.0, 197.0)</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>61.0 (50.6, 73.0)</td>
</tr>
<tr>
<td>LDL-C (mg/dl) (n=100)</td>
<td>100.9 (87.0, 127.4)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl) (n=102)</td>
<td>74.0 (51.0, 100.0)</td>
</tr>
<tr>
<td>ACE/ARB use (n=104)</td>
<td>1.9 (2)</td>
</tr>
<tr>
<td>Lipid medication use (n=104)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>HbA1c (%) (n=104)</td>
<td>8.4 ± 1.2</td>
</tr>
<tr>
<td>HbA1c (mmol/mol, n=104)</td>
<td>68 ± 13.1</td>
</tr>
<tr>
<td>eGDR (mg<em>kg⁻¹</em>min⁻¹, n=103)</td>
<td>8.9±1.5</td>
</tr>
<tr>
<td>Insulin dose (units/day/kg, n=100)</td>
<td>0.71 ± 0.19</td>
</tr>
</tbody>
</table>
### Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AER (μg/min)</td>
<td>10.4 (6.2, 25.7)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>102.3 ± 27.9</td>
</tr>
<tr>
<td>WBC x 10³/mm²</td>
<td>6.4 ± 1.9</td>
</tr>
</tbody>
</table>

**At the last available follow-up**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent ever contraceptive use (n=104)</td>
<td>63.5 (66)</td>
</tr>
<tr>
<td>Percent ever pregnant</td>
<td>72.4 (76)</td>
</tr>
<tr>
<td>No. of pregnancies (n=76)</td>
<td>2.4 ± 1.3</td>
</tr>
<tr>
<td>No. of live births (n=76)</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>Age at last available follow-up (years)</td>
<td>58.7 ± 6.1</td>
</tr>
<tr>
<td>Age at natural menopause (years)</td>
<td>49.5 (4.1)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Time-weighted values</th>
<th>β coefficient (SE)</th>
<th>95% C.I.</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>0.001 (0.12)</td>
<td>-0.24, 0.25</td>
<td>0.99</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>-0.01 (0.04)</td>
<td>-0.09, 0.07</td>
<td>0.85</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>-0.04 (0.05)</td>
<td>-0.15, 0.06</td>
<td>0.43</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>-0.01 (0.01)</td>
<td>-0.04, 0.01</td>
<td>0.36</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>-0.01 (0.03)</td>
<td>-0.08, 0.06</td>
<td>0.76</td>
</tr>
<tr>
<td>Non-HDL-Cl (mg/dl)</td>
<td>-0.01 (0.01)</td>
<td>-0.04, 0.02</td>
<td>0.45</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>-0.01 (0.02)</td>
<td>-0.04, 0.02</td>
<td>0.58</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>-0.002 (0.01)</td>
<td>-0.02, 0.02</td>
<td>0.83</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>-0.61 (0.40)</td>
<td>-1.41, 0.18</td>
<td>0.13</td>
</tr>
<tr>
<td>eGDR (mg<em>kg⁻¹</em>min⁻¹)</td>
<td>-0.22 (0.28)</td>
<td>-0.78, 0.33</td>
<td>0.42</td>
</tr>
<tr>
<td>Insulin dose (units/kg/day)</td>
<td>-4.87 (2.3)</td>
<td>-9.46, -0.28</td>
<td>0.04*</td>
</tr>
<tr>
<td>ln(AER) (µg/min)</td>
<td>-0.62 (0.27)</td>
<td>-1.15, -0.10</td>
<td>0.02*</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>-0.02 (0.02)</td>
<td>-0.05, 0.02</td>
<td>0.35</td>
</tr>
<tr>
<td>WBC x 10³/mm²</td>
<td>0.06 (0.23)</td>
<td>-0.38, 0.51</td>
<td>0.78</td>
</tr>
</tbody>
</table>
Table 3.3 Multivariable Linear Regression Analysis for the Prediction of Age at Natural Menopause

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β coefficient</td>
<td>p-value</td>
<td>β coefficient</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>(s.e.)</td>
<td></td>
<td>(s.e.)</td>
<td></td>
</tr>
<tr>
<td>Ever smoked</td>
<td>0.50 (1.20)</td>
<td>0.68</td>
<td>0.41 (1.21)</td>
<td>0.74</td>
</tr>
<tr>
<td>Time-weighted BMI (kg/m²)</td>
<td>-0.12 (0.13)</td>
<td>0.39</td>
<td>-0.07 (0.13)</td>
<td>0.57</td>
</tr>
<tr>
<td>Time-weighted HDL-C (mg/dl)</td>
<td>-0.06 (0.04)</td>
<td>0.11</td>
<td>-0.05 (0.04)</td>
<td>0.25</td>
</tr>
<tr>
<td>Time-weighted non-HDL-C (mg/dl)</td>
<td>-0.01 (0.02)</td>
<td>0.52</td>
<td>-0.01 (0.02)</td>
<td>0.47</td>
</tr>
<tr>
<td>Time-weighted triglycerides (mg/dl)</td>
<td>0.02 (0.02)</td>
<td>0.21</td>
<td>0.01 (0.02)</td>
<td>0.53</td>
</tr>
<tr>
<td>Time-weighted HbA1c (%)</td>
<td>-0.76 (0.44)</td>
<td>0.09</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Time-weighted eGDR (mg<em>kg⁻¹</em>min⁻¹)</td>
<td>NA</td>
<td>NA</td>
<td>-0.43 (0.35)</td>
<td>0.21</td>
</tr>
<tr>
<td>Time-weighted insulin dose (units/day/kg)</td>
<td>-6.35 (2.49)</td>
<td>0.01*</td>
<td>-6.3 (2.51)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Time-weighted ln(AER) (μg/min)</td>
<td>-0.67 (0.31)</td>
<td>0.03*</td>
<td>-0.72 (0.31)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Ever contraceptive use</td>
<td>0.28 (0.85)</td>
<td>0.74</td>
<td>0.07 (0.86)</td>
<td>0.93</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>-0.41 (0.28)</td>
<td>0.14</td>
<td>-0.39 (0.28)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Characteristics at baseline</th>
<th>Women who were included in the analyses (n=105)</th>
<th>Women who died prior to reproductive assessment (n=75)</th>
<th>Women who have not reached menopause (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline (years)</td>
<td>29.5 ± 6.2</td>
<td>32.5 ± 7.7</td>
<td>18.3 ± 4.0</td>
</tr>
<tr>
<td>Age at diabetes onset (years)</td>
<td>9.3 ± 3.9</td>
<td>8.6 ± 3.9</td>
<td>6.6 ± 3.6</td>
</tr>
<tr>
<td>Duration of type 1 diabetes (years)</td>
<td>20.2 ± 7.0</td>
<td>23.9 ± 7.3</td>
<td>11.7 ± 3.5</td>
</tr>
<tr>
<td>Year of diabetes diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1950-1964</td>
<td>37.1% (39)</td>
<td>60% (45)</td>
<td>0</td>
</tr>
<tr>
<td>1965-1980</td>
<td>62.9% (66)</td>
<td>40% (30)</td>
<td>100% (37)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9 ± 2.9</td>
<td>23.8 ± 4.0</td>
<td>22.9 ± 2.8</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.77 ± 0.05</td>
<td>0.80 ± 0.07</td>
<td>0.78 ± 0.05</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>107.9 ± 11.7</td>
<td>117.8 ± 16.4</td>
<td>101.9 ± 8.1</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68.5 ± 9.6</td>
<td>73.4 ± 12.4</td>
<td>67.9 ± 8.2</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>181.0 (162.0, 197.0)</td>
<td>209.0 (176.0, 234.0)</td>
<td>170.0 (159.0, 207.0)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>61.0 (50.6, 73.0)</td>
<td>54.7 (45.5, 64.9)</td>
<td>55.7 (51.1, 63.9)</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>100.9 (87.0, 127.4)</td>
<td>122.6 (104.3, 146.9)</td>
<td>96.8 (87.0, 122.8)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>74.0 (51.0, 100.0)</td>
<td>111.0 (75.0, 173.0)</td>
<td>81.0 (61.0, 101.5)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.4 ± 1.2</td>
<td>9.06 ± 1.7</td>
<td>9.2 ± 1.6</td>
</tr>
<tr>
<td>Age at last available follow-up (years)</td>
<td>58.7 ± 6.1</td>
<td>59.9 ± 6.8</td>
<td>47.5 ± 3.7</td>
</tr>
</tbody>
</table>
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 examinations 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 (β±SE=-2.06±1.08) 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, atherosclerosis, and stroke. The ovaries are highly vascular and have high rates of blood flow; 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. 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. 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.

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, the age-adjusted risk of cardiovascular complications is approximately ten-fold higher in people with type 1 diabetes compared with the general population, and women are disproportionally affected, relative to their nondiabetic counterparts, than men. In addition, cardiovascular events occur earlier in
people with type 1 diabetes than in the general population. 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. Indeed, compared to non-diabetic women, women with a diagnosis of type 1 diabetes were shown to experience an earlier natural menopause, although not all studies agree.

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. 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. 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. 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 (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 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. Urinary albumin was measured by immunonephelometry.

4.2.3 Diabetes Complication Assessment

At baseline and biennial clinical examinations 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. PR was classified according to the modified Arlie House system. 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, 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 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 \( \geq 30 \) 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
(β±Standard Error (SE)=-2.4±1.01, p=0.02)). After multivariable adjustment, this association was slightly attenuated (β±SE=-2.06±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 (β±SE=2.19±0.86, p=0.01) compared with women without CDSP in the unadjusted model. This finding persisted (β±SE=2.95±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, as well as a
possible marker for kidney disease progression \textsuperscript{294}, although not all people with reduced glomerular filtration rate or diabetic kidney disease have or ever had MA \textsuperscript{295}. Moreover, MA is a risk marker for cardiovascular events in diabetes \textsuperscript{294,296} and also a strong predictor of all-cause mortality in patients with long-term type 1 diabetes \textsuperscript{297}. Thus, MA is considered as a marker for generalized vascular dysfunction in patients with diabetes \textsuperscript{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 \textsuperscript{294,296} and data from the Framingham Heart Study showed that cardiovascular risk factors accelerate menopause \textsuperscript{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\textsuperscript{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

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>n=105</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline (years)</td>
<td>29.5 ± 6.2</td>
</tr>
<tr>
<td>Age at diabetes onset (years)</td>
<td>9.3 ± 3.9</td>
</tr>
<tr>
<td>Duration of type 1 diabetes at baseline (years)</td>
<td>20.2 ± 7.0</td>
</tr>
<tr>
<td>Race (%, n)</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>96.2 (101)</td>
</tr>
<tr>
<td>Black</td>
<td>3.8 (4)</td>
</tr>
<tr>
<td>Marital status (%, n)</td>
<td></td>
</tr>
<tr>
<td>Single, never married</td>
<td>37.1 (39)</td>
</tr>
<tr>
<td>Currently married or living as if married</td>
<td>48.6 (51)</td>
</tr>
<tr>
<td>Divorced</td>
<td>7.6 (8)</td>
</tr>
<tr>
<td>Separated, Widowed, or others</td>
<td>6.7 (7)</td>
</tr>
<tr>
<td>Education (%, n=103)</td>
<td></td>
</tr>
<tr>
<td>High school or less</td>
<td>35.0 (36)</td>
</tr>
<tr>
<td>Some college or received bachelor's degree</td>
<td>56.3 (58)</td>
</tr>
<tr>
<td>Graduate education beyond bachelor's degree</td>
<td>8.7 (9)</td>
</tr>
<tr>
<td>Smoking status (%, n)</td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>67.6 (71)</td>
</tr>
<tr>
<td>Past smoker</td>
<td>13.3 (14)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>19.1 (20)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9 ± 2.9</td>
</tr>
<tr>
<td>Waist to hip ratio (n=104)</td>
<td>0.77 ± 0.05</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>107.9 ± 11.7</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68.5 ± 9.6</td>
</tr>
<tr>
<td>Hypertension (%, n)</td>
<td>8.6 (9)</td>
</tr>
<tr>
<td>Blood pressure medication use (%, n=103)</td>
<td>6.8 (7)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>181.0 (162.0, 197.0)</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>61.0 (50.6, 73.0)</td>
</tr>
<tr>
<td>LDL-C (mg/dl) (n=100)</td>
<td>100.9 (87.0, 127.4)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl) (n=102)</td>
<td>74.0 (51.0, 100.0)</td>
</tr>
<tr>
<td>Measure</td>
<td>Value</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>ACE/ARB use (%; n=104)</td>
<td>1.9 (2)</td>
</tr>
<tr>
<td>Lipid medication use (%; n=104)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>HbA1c (%) (n=104)</td>
<td>8.4 ± 1.2</td>
</tr>
<tr>
<td>eGDR (mg<em>kg⁻¹</em>min⁻¹; n=103)</td>
<td>8.9 ± 1.5</td>
</tr>
<tr>
<td>Insulin dose (units/day/kg; n=100)</td>
<td>0.71 ± 0.19</td>
</tr>
<tr>
<td>AER (µg/min)</td>
<td>10.4 (6.2, 25.7)</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>102.3 ± 27.9</td>
</tr>
<tr>
<td>WBC x 10⁹/mm²</td>
<td>6.4 ± 1.9</td>
</tr>
<tr>
<td>Microalbuminuria (%; n=99)</td>
<td>32.3 (32)</td>
</tr>
<tr>
<td>Overt nephropathy (%; n=97)</td>
<td>16.5 (16)</td>
</tr>
<tr>
<td>Proliferative retinopathy (%; n=103)</td>
<td>25.2 (26)</td>
</tr>
<tr>
<td>Confirmed distal symmetrical polyneuropathy (%; n=97)</td>
<td>23.7 (23)</td>
</tr>
<tr>
<td>Coronary artery disease (%; n=105)</td>
<td>3.8 (4)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Complications</th>
<th>Age at complication diagnosis Mean (SD)</th>
<th>Age at natural menopause Mean (SD)</th>
<th>Spearman Correlations r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalbuminuria (n=59)</td>
<td>33.3±8.1</td>
<td>49.1±4.1</td>
<td>0.28</td>
<td>0.03</td>
</tr>
<tr>
<td>Overt nephropathy (n=26)</td>
<td>31.4±6.7</td>
<td>49.1±4.9</td>
<td>0.32</td>
<td>0.12</td>
</tr>
<tr>
<td>Proliferative retinopathy (n=61)</td>
<td>34.2±6.7</td>
<td>49.6±4.0</td>
<td>0.10</td>
<td>0.44</td>
</tr>
<tr>
<td>Confirmed distal symmetrical polyneuropathy (n=50)</td>
<td>35.5±7.0</td>
<td>50.1±3.2</td>
<td>0.36</td>
<td>0.01</td>
</tr>
<tr>
<td>Coronary artery disease (n=25)</td>
<td>40.9±8.7</td>
<td>49.8±2.9</td>
<td>0.28</td>
<td>0.17</td>
</tr>
</tbody>
</table>
**Table 4.3 Linear Regression Models for the Association of Age at Complication Diagnosis with Age at Natural Menopause**

<table>
<thead>
<tr>
<th>Age at complication diagnosis</th>
<th>Age at natural menopause</th>
<th>Unadjusted</th>
<th>Adjusted[^]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>β coefficient (SE)</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>Overall P value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 yrs (n=25)</td>
<td>47.5±4.9</td>
<td>-2.40 (1.01)</td>
<td>0.02</td>
</tr>
<tr>
<td>≥30 yrs (n=34)</td>
<td>50.2±3.1</td>
<td>0.31 (0.92)</td>
<td>0.74</td>
</tr>
<tr>
<td>No (n=40)</td>
<td>49.9±4.0</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Overt nephropathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 yrs (n=12)</td>
<td>47.7±5.8</td>
<td>-1.78 (1.28)</td>
<td>0.17</td>
</tr>
<tr>
<td>≥30 yrs (n=14)</td>
<td>50.3±3.8</td>
<td>0.77 (1.20)</td>
<td>0.52</td>
</tr>
<tr>
<td>No (n=71)</td>
<td>49.5±3.8</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Proliferative retinopathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 yrs (n=17)</td>
<td>49.3±4.8</td>
<td>-0.03 (1.18)</td>
<td>0.98</td>
</tr>
<tr>
<td>≥30 yrs (n=44)</td>
<td>49.6±3.7</td>
<td>0.26 (0.88)</td>
<td>0.77</td>
</tr>
<tr>
<td>No (n=42)</td>
<td>49.4±4.1</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Confirmed distal symmetrical polyneuropathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 yrs (n=10)</td>
<td>48.0±3.4</td>
<td>-0.44 (1.39)</td>
<td>0.75</td>
</tr>
<tr>
<td>≥30 yrs (n=40)</td>
<td>50.6±3.0</td>
<td>2.19 (0.86)</td>
<td>0.01</td>
</tr>
<tr>
<td>No (n=47)</td>
<td>48.4±4.7</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 yrs (n=2)</td>
<td>46.0±4.2</td>
<td>-3.41 (2.90)</td>
<td>0.24</td>
</tr>
<tr>
<td>≥30 yrs (n=23)</td>
<td>50.1±2.7</td>
<td>0.68 (0.96)</td>
<td>0.48</td>
</tr>
<tr>
<td>No (n=80)</td>
<td>49.4±4.4</td>
<td>Ref</td>
<td>Ref</td>
</tr>
</tbody>
</table>

[^]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,
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
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 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
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; the two were especially highly concordant for classifying
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
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.
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