PROTOCOL

for the

DIABETES PREVENTION PROGRAM OUTCOMES STUDY-4 (DPPOS-4)

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1 EXECUTIVE SUMMARY

1.1 DPPOS-4

1.1.1 Background

The **DPP Outcomes Study-4 (DPPOS-4)** represents the continuing study of a cohort that began with prediabetes and has been studied continuously for more than 25 years. The **Diabetes Prevention Program** (DPP, 1996-2002) was a controlled clinical trial to examine whether diabetes development could be prevented or delayed in a population of high-risk adults. After demonstrating the effectiveness of the randomized intensive lifestyle intervention or metformin compared with placebo, a long-term follow-up, the **DPPOS** was initiated to take advantage of this valuable cohort. **DPPOS-1** (2002-2008) and **DPPOS-2** (2008-2015) evaluated the long-term effects of active DPP interventions on the further development of diabetes and composite diabetes-related microvascular complications. **DPPOS-3** (2015-2022) evaluated the long-term effects of the original DPP/DPPOS interventions on cancer and cardiovascular disease, with a particular focus on the effects of the metformin intervention. Cognitive impairment is increasingly recognized as a complication of diabetes. Cognition was evaluated during DPPOS-2 and DPPOS-3 with a limited cognitive battery. In addition to continuing the measurement of traditional complications of diabetes, **DPPOS-4** (2022-2027) will expand the evaluation of cognition to include cognitive diagnoses and brain health biomarkers. Detailed policies and procedures for DPPOS-4 are found in the Manuals of Operation.

The prevalence of Alzheimer's disease (AD) and AD related dementias (ADRD) is increasing without known prevention or cure. One in 11 persons over the age of 65 years in the United States has dementia presumed to be due to AD. Despite evidence that overall dementia incidence may be decreasing, the absolute number of cases is increasing, particularly among women and racial and ethnic minorities.

Persons with pre-diabetes (PreD) and type 2 diabetes (T2D) are important high-risk groups for AD/ADRD addressed in this phase of DPPOS. In addition, persons with PreD and T2D have been reported to be at higher risk of amnestic and nonamnestic cognitive decline, mild cognitive impairment (MCI), and dementia,[1,2] compared with populations without PreD and T2D, with the risk for incident dementia inversely related to the age of T2D onset.[3] Among US adults aged 18 years and older, PreD (96 million) and T2D (>35 million) are very common.[4] Over one-half of the US population 60 years and older, the age group most at risk for cognitive impairment, has PreD or T2D. The DPPOS cohort is a diverse cohort that started with PreD and has been carefully studied for approximately 25 years from PreD to the development of T2D in approximately two-thirds. The nature and determinants of risk of cognitive impairment in persons with PreD and T2D remain unknown and are the focus of **DPPOS-4**.

1.1.2 Objectives

In addition to continuing the study of diabetes and traditional diabetes-related complications, **DPPOS-4** (2022-2027) will expand its focus to study the determinants and nature of AD, ADRD, and cognitive impairment among persons with PreD and T2D in the DPPOS cohort, as described below. **DPPOS-4** will be carried out through four main projects that will:

1. Characterize the occurrence of AD, ADRD and other forms of cognitive impairment in the PreD and T2D cohort and examine their association with

biomarkers of neuropathology and brain insulin signaling and with sociodemographic and behavioral factors.
2. Examine the associations of cumulative glycemia, related metabolic factors, and microvascular and macrovascular complications, with cognitive syndromes and biomarkers of neuropathology.
3. Examine the association of cumulative exposure to metformin and other T2D medications with cognitive syndromes and biomarkers of neuropathology.
4. Evaluate the association of trajectories of physical activity, physical function and frailty with cognitive syndromes and biomarkers of neuropathology.

The primary outcome for DPPOS-4 will be adjudicated cognitive diagnoses based on the National Alzheimer's Coordinating Center Uniform Data Set (NACC-UDS[5]). Major secondary outcomes will include measures of cognitive decline and AD plasma biomarkers. Brain imaging (amyloid PET scanning and MRI) will be performed in a subset of the cohort to validate plasma biomarkers and provide additional measures of brain pathology.

1.1.3 Study population

All surviving DPPOS participants will be invited to join **DPPOS-4.** For participants who can no longer sign informed consent owing to infirmity, alternate informed consent processes have been put into place according to local practice and with institutional permission.

1.1.4 Study intervention

During **DPPOS-4**, no study interventions will be provided.

1.1.5 Outcomes

The primary outcome for **DPPOS-4** will be adjudicated cognitive diagnoses based on the NACC-UDS. Major secondary outcomes will include measures of cognitive decline, AD plasma biomarkers, and results of brain imaging.

Additional outcomes, some of which will be explored as risk factors for AD, ADRD, and other forms of cognitive impairment include (see outcomes table in Chapter 11 for complete listing):

- Diabetes and measures of glycemia
- Diabetic neuropathy
- Diabetic nephropathy
- Cardiovascular disease events
- Risk factors for cardiovascular disease
- Amputation in a lower extremity not resulting from major trauma
- Hospitalizations
- Physical activity, body weight and obesity
- Physical functioning, falls and frailty
- Quality of life indices
- Health care costs
- Urinary incontinence
- Mortality

1.1.6 Power

The main outcome for DPPOS-4 is the adjudicated cognitive diagnosis based on the NACC-UDS that classifies participants into the clinical dementia rating scale (CDR), normal (CDR=0), mild cognitive impairment (CDR=0.5), and dementia (CDR=1). The 4 projects will use this as their primary outcome with various exposures of interest. Assuming a conservative CDR distribution of 70% normal, 20% mild cognitive impairment, and 10% dementia and a 2-sided alpha of 0.05, the study of 1979 participants is able to detect a minimum of 1.31 odds ratio of MCI or dementia compared to normal associated with the various exposures of interest with 80% power using a proportional odds ordinal logistic regression.[6] Although DPPOS-4, an observational study, will use the main outcome above as the primary outcome for all 4 projects and for the calculation of power, other outcomes will be addressed, with appropriate statistical approaches, to take full advantage of the study cohort and numerous measurements. The study also has ample power to detect small effects for continuous outcomes and to detect a moderate effect for conditions with low prevalence. For outcomes in the form of continuous scores (e.g., measure of cognitive function) the sample size of 1979 provides 80% power at alpha level of 0.05 to detect effect sizes as small as Cohen's D=0.1 to 0.3 assuming exposure rates of 10-50% and similar variability in cognitive decline observed in the study and preliminary plasma biomarker data. That is, the minimum detectable difference between any two groups (e.g., ever metformin use vs. none) is 10% to 30% of the standard error of the residuals in the mixed model. In models with cumulative metformin exposure, we will have more power or smaller detectable effect size. Power calculations for continuous outcomes were conducted using r package simr[7] and software nQuery.[8]

1.1.7 Analyses

A detailed statistical analysis plan is developed in advance of beginning the analysis for all publications and presentations. Means and 95% confidence intervals and nominal p-values will be reported. Since the distributions of plasma and brain imaging biomarkers are often skewed, these variables will be transformed as needed and categories will be considered. We will use all available measures during the 25-year course of follow-up from DPP to DPPOS-4 to derive measures of trajectories of change over time and cumulative burden of exposure. General statistical methods for each project are described below.

Project 1 will assess cross-sectional differences in biomarkers of tau, neurodegeneration, neuroinflammation among the cognitive syndromes (MCI, dementia compared to normal) using ANCOVA adjusted for age, sex, and APOE-ε4. Secondary analyses will evaluate differences among cognitive syndromes in longitudinal measures of tau, neurodegeneration, neuroinflammation, analyzed as continuous measures in linear mixed effect models and as trajectory classes defined by latent class mixed effect models. Project 1 will also assess differences among cognitive syndromes in neuroimaging biomarkers of tau, neurodegeneration, neuroinflammation in the subset of participants with neuroimaging. We will explore consistency of the estimates from the subset compared to the full cohort using appropriate weighted models to evaluate potential selection bias. Horvitz-Thompson estimators in the inverse selection probability weighting method, propensity score methods will be used to correct biases due to unbalanced confounders in exposure and non-exposure groups.[9,10]

Project 2 will focus on the associations of type 2 diabetes-related factors with risk for cognitive impairment syndromes. We will use logistic regression for categorical outcomes and linear mixed models for continuous outcomes to test for the association between trajectories of change in HbA1c, log(1/fasting insulin), and proinsulin-to-insulin ratio, and advanced glycation end products (AGE) protein concentration as the main predictors, and in the full cohort, (a) amnestic and non-amnestic cognitive decline (categorical and continuous), MCI, and dementia diagnosis (categorical) outcomes and (b) trajectories of plasma biomarkers (continuous) as outcomes. In the sample of the cohort with imaging, we will examine summary measures from imaging biomarkers (continuous) as the outcomes. Among only those participants with a diagnosed cognitive impairment syndrome we will examine each of the cognitive impairment syndromes with associated pathology (AD continuum vs. non-AD pathologic change) as the outcome. All predictor variables will be included together in the same model to test for the independent effects of each.

Project 3 will examine the association of cumulative metformin exposure with amnestic and non-amnestic cognitive decline, MCI, and dementia, and explore these syndromes classified by AD pathologic change and vascular contributions to cognitive impairment and dementia (VCID). We will employ causal inference approaches to assess the association of metformin exposure (in-study and total) on the risk of cognitive impairment to adjust for potential confounding by indication in logistic models for cognitive syndromes and in linear mixed-effects models for measures over time such as amnestic and non-amnestic cognitive decline. We will also assess the association of cumulative metformin exposure with trajectories of amnestic and non-amnestic cognitive decline. All models will be adjusted for age, sex, race, education, and APOE-ε4.

Project 4 will examine the association between physical activity, physical function, and frailty, separately, with amnestic and non-amnestic cognitive decline using linear mixed models. We will fit semiparametric proportional rates model with Turnbull's algorithm[11] for interval-censored data to test the association between the summary measures of physical activity, physical function, and frailty, separately, and the risk of MCI and dementia. We will also fit stochastic Markov models[12] for the transition between normal to MCI, dementia or death between the two waves of data. These associations will be examined for amnestic and non-amnestic cognitive syndromes and AD continuum and non-AD pathology. We will compare the cognitive decline and risk of dementia and MCI across the joint classes of PA/PF/frailty using linear mixed, proportional rates or Markov models.

1.2 DPP and DPPOS-1, DPPOS-2 and DPPOS-3

See **Appendix 1** for detailed Executive Summaries of DPP and DPPOS 1, 2 and 3 and **Appendix 2** for a detailed description of DPPOS 1, 2 and 3 including study results.

The **Diabetes Prevention Program** (DPP, 1996-2002) (Figure 1, top panel) was a multicenter controlled clinical trial examining the efficacy of an intensive lifestyle intervention or metformin to prevent or delay the development of diabetes in a population selected to be at high risk due to the presence of impaired glucose tolerance (IGT). All 3,234 volunteers received standard lifestyle recommendations and were randomly assigned to one of three interventions: intensive lifestyle with the aim of losing and maintaining 7% weight loss and achieving ≥150

minutes per week of moderate intensity physical activity, metformin therapy with 850 mg twice per day, or placebo.

The intensive lifestyle cohort achieved a mean weight loss of 7% (14.5 lb.) and 224 minutes per week of physical activity by the end of the 16-session core curriculum (at approximately 6 months) and maintained a 5% weight loss (10.3 lb.) and 189 minutes of activity per week after a mean study duration of 2.8 years. Seventy-two percent of participants assigned to metformin and 80% of those assigned to placebo took at least 80% of assigned medications during the study. Based on a statistically significant and clinically compelling decrease in the development of diabetes in the lifestyle intervention and metformin-treated groups (58% and 31% reduction in hazards, respectively) compared with the placebo-treated group, the DPP Data Monitoring Board and NIDDK ended the masked treatment phase of the study in August 2001, one year earlier than originally planned.

The long-term follow-up study of the DPP, entitled the **Diabetes Prevention Program Outcomes Study** (DPPOS, 2002-2022, Figure 1, bottom 3 panels), was designed to take further advantage of the scientifically and clinically valuable cohort of DPP volunteers and the large volume of data collected during the study. Eighty-eight percent of the original surviving DPP cohort joined DPPOS. At the end of DPP, all participants were offered the intensive lifestyle intervention in a group setting and open-label metformin was continued in the original metformin treatment group. Placebo was discontinued. The primary objective of **DPPOS-1** (2002-2008) was to evaluate the long-term effects of active DPP interventions on the further development of diabetes. The primary objective of **DPPOS-2** (2008-2015) was to evaluate the long-term effects of the DPP interventions on the prevalence of composite diabetes-related microangiopathic and neuropathic outcomes. The primary objective of the **DPPOS-3** (2015-2022) was to evaluate the long-term effects of metformin compared with placebo on the incidence of cancer and major atherosclerotic cardiovascular events (MACE: fatal and nonfatal myocardial infarction or stroke, and other cardiovascular deaths), with the comparisons of lifestyle compared with placebo, and lifestyle compared with metformin, important secondary objectives.

Analyses through the first 6 years of DPPOS (**DPPOS-1**) revealed significant reductions from baseline in CVD risk factors in the lifestyle intervention group, with decreased utilization of glucose-lowering and lipid-lowering medications. Analyses following year 11 of DPPOS (**DPPOS-2**) revealed no significant differences in the prevalence of the aggregate microvascular outcome, comprised of fundus photography measured retinopathy, nephropathy based on estimated glomerular filtration rate (eGFR) or increased albuminuria (≥30 mg albumin/g creatinine), or neuropathy detected by abnormal monofilament sensation, among the treatment groups. However, in a pre-specified analysis by sex, women had a 21 and 22% reduced risk of this outcome in the lifestyle group compared with the placebo and metformin groups, respectively. Moreover, among the participants who had not developed diabetes during DPP/DPPOS, the prevalence of the aggregate microvascular outcome was 28% lower compared with those who had developed diabetes. **DPPOS-3** found no significant effects of metformin or lifestyle on the rates of total cancer cases or obesity-related cancers. Similarly, neither the original metformin nor lifestyle groups had a different cumulative incidence of aggregate CVD, its elements, or of total mortality from the original placebo group or from each other. Of note, conventional CVD risk factors (LDL cholesterol, triglyceride levels, blood pressure) generally improved over time with increasing use of blood pressure and cholesterol-lowering medications in all three groups. This

may have explained the relatively low rate of total CVD compared with the diabetes or pre-diabetes population in the US. The rates of nephropathy (either eGFR < 45 ml/min/1.73 m^2 or albuminuria > 30 mg/gm creatinine) and of distal symmetric neuropathy were also no different among the original randomized groups.

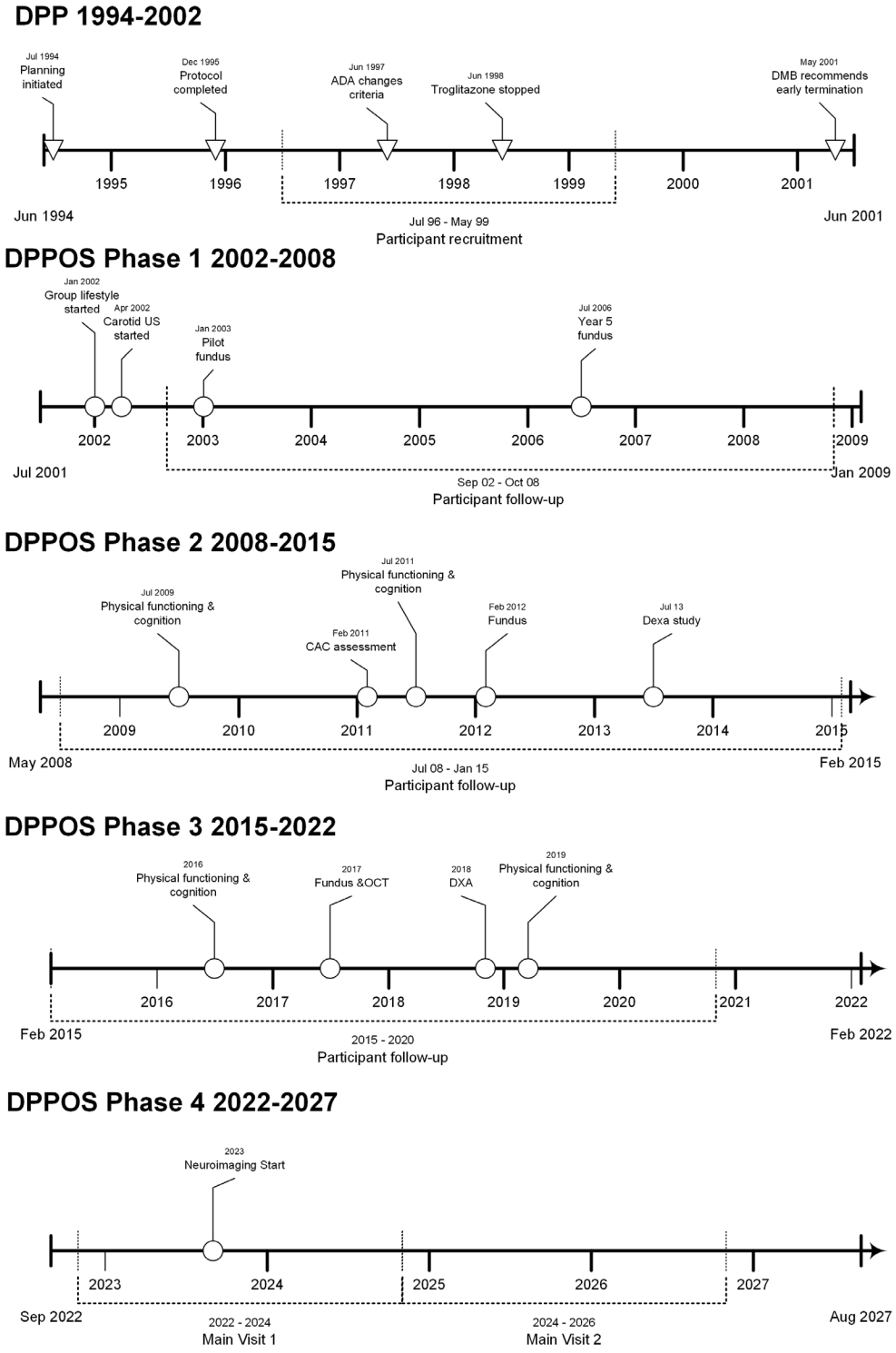

Figure 1. Timeline for DPP and DPPOS

Moreover, the development of these complications was generally associated with diabetes duration and higher HbA1c levels. Finally, the development of diabetes remained lower in the original lifestyle group than the metformin group until ~ year 17 of total follow-up, when they converged. Both the original metformin and lifestyle groups remained with lower cumulative incidence of diabetes than the placebo group over the entire follow-up, with ~65% of the total cohort developing diabetes over time.

As of December 2021, after 20 years of DPPOS and a mean of 23 (range 21-25) years of combined DPP/DPPOS, 84% of the surviving DPPOS cohort continue to attend annual follow-up visits.

2 DPPOS-4 BACKGROUND AND RATIONALE

The prevalence of Alzheimer's disease (AD) and AD-related dementias (ADRD) is increasing without known prevention or cure. One in 11 persons over 65 years has dementia presumed to be due to AD in the United States (US).[13] Despite evidence that overall dementia incidence may be decreasing,[14] the absolute number of cases is increasing, particularly among women and ethnic minorities.[13] Persons with pre-diabetes (PreD) and type 2 diabetes (T2D) are an important high-risk group for AD/ADRD addressed in this protocol. In addition, persons with PreD and T2D have been reported to be at higher risk of amnestic and non-amnestic cognitive decline, mild cognitive impairment (MCI), and dementia,[1,2] with the risk for incident dementia inversely related to the age of T2D onset.[3] Over one-half of the US population 60 years and older, the age group most at risk for cognitive impairment, has PreD or T2D.[4] Among US adults aged 18 years and older PreD (96 million) and T2D (>35 million) are also common.[4] The Diabetes Prevention Program (DPP) Outcomes Study (DPPOS) cohort is a diverse cohort that started with PreD and has been carefully studied for approximately 25 years from PreD to the development of T2D in approximately two-thirds. This study focuses on the high-risk group of persons with PreD and T2D; however, it does not address type 1 diabetes, which affects a smaller fraction of a younger population.[15] Despite the high prevalence of PreD and T2D and their increased risk for AD, ADRD and other cognitive impairment, the nature and determinants of risk of cognitive impairment in persons with PreD and T2D remain unknown,[16] and are the focus of DPPOS-4.

Hyperglycemia is the hallmark of PreD and T2D, but it is accompanied by other factors that may be important in AD and ADRD. T2D is defined as an elevation of glycemia sufficient to cause microvascular complications (e.g., peripheral neuropathy and nephropathy) and pharmacological and non-pharmacological management has been shown to ameliorate these complications.[17] PreD is defined by an elevation of glucose to a level that puts persons at a higher risk of progressing to T2D. PreD and T2D are also associated with an increased risk of the less diabetes-specific macrovascular complications, such as myocardial infarction and stroke.[18] The American Diabetes Association (ADA) currently defines T2D by a hemoglobin A1c (HbA1c) ≥ 6.5%, a fasting plasma glucose ≥ 126 mg/dl, or a 2-hour glucose after an oral glucose tolerance test (OGTT) ≥ 200 mg/dl. PreD is defined by a HbA1c between 5.7% and 6.4%, a fasting glucose between 100 and 125 mg/dl, or a two-hour OGTT glucose between 140 and 199 mg/dl.[19] Persons without PreD or T2D are defined as having normal glucose tolerance (NGT). Insulin, secreted by the pancreas, is the main hormone responsible for maintaining normal glucose levels.[20] For most, but not all, PreD and T2D are triggered by peripheral insulin resistance, i.e. resistance of tissues to the glucose-lowering actions of insulin, which is compounded by relative insulin deficiency.[21] These defects result in high glucose levels. The main determinant of insulin resistance in most people is adiposity (i.e. overweight and obesity).[21] In addition to hyperglycemia, PreD and T2D are accompanied by dyslipidemia, high blood pressure, endothelial dysfunction, inflammation, and advanced glycation end products (AGE), which have been identified as potential contributors to AD and ADRD.[16] The treatment of hyperglycemia in T2D and the prevention of conversion of PreD to T2D consists of pharmacological and non-pharmacological approaches.[17,22-25] The non-pharmacologic approaches are similar for both PreD and T2D, and consist mainly of physical activity and diet to promote weight loss.[25] The main pharmacological approach in T2D is the use of medications that lower glucose, some of which increase insulin levels, and some of which improve insulin resistance.[17] The central pharmacologic approach for prevention and treatment of

T2D is the drug metformin, although it is not approved by the FDA for prevention.[23] Both pharmacologic and non-pharmacologic approaches in PreD and T2D also have effects on dyslipidemia, blood pressure, endothelial function, inflammation and AGE. The complex relationships among factors related to hyperglycemia, its long-term complications and the prevention and management of T2D make it difficult to segregate the key determinants of cognitive impairment in PreD and T2D. DPPOS-4 will address this complexity by separately examining exposures including glycemia and related factors and outcomes, T2D medications, and physical activity, using longitudinal data from DPPOS. Vascular factors, dyslipidemia, inflammation, and AGE will be examined as important mediators. It is important to point out that we consider PreD and T2D as part of the continuum of hyperglycemia. Thus, we examine them together as a high-risk group, and make distinctions between them when appropriate.

Project 1 characterizes the occurrence of AD, ADRD, and other forms of cognitive impairment in the PreD and T2D cohort and addresses the neuropathologic, pathophysiologic, sociodemographic, and behavioral correlates of cognitive impairment. PreD and T2D are related to a higher risk of amnestic and non-amnestic forms of dementia and MCI in epidemiological studies[2], compared to persons without these conditions. It has long been assumed that amnestic syndromes are probably due to AD, characterized by amyloid plaques and tau neurofibrillary tangles, and that non-amnestic syndromes are due to non-AD pathologies,[13] including vascular contributions to cognitive impairment and dementia (VCID).[26,27] However, there is growing recognition that not all amnestic syndromes are due to AD, that non-amnestic syndromes can be due to AD, and that AD not always results in cognitive impairment.[13] Autopsy[28-32] and biomarker studies[33-35] are conflicting on whether PreD and T2D are related to AD neuropathology. This is important to clarify because most interventions in dementia prevention and treatment focus on amyloid,[36] which may not apply to persons with PreD and T2D. Although PreD and T2D are conditions of peripheral insulin resistance, it is not clear whether cognitive impairment in PreD and T2D is related to brain insulin signaling dysregulation, which can occur in neurodegenerative disorders.[37] T2D and cognitive impairment are more common in ethnic minorities[38] and in persons with lower education,[39,40] but there is a dearth of information on how these factors affect the risk of cognitive impairment in persons with PreD and T2D. Poor sleep quality, obstructive sleep apnea, and depressive symptoms are common in both T2D[41-43] and cognitive disorders,[44-46] but there is a dearth of studies examining them among persons with PreD and T2D.

Project 2 addresses the association of glycemia, related metabolic factors, and complications, with cognitive impairment and biomarkers of neuropathology**.** Hyperglycemia is the key characteristic that defines PreD and T2D, with risk for microvascular disease determining the diagnostic glycemia thresholds for T2D and risk for T2D defining glycemic thresholds for PreD.[17] Higher levels of glycemia over a continuum have been suggested to increase the risk for dementia.[47] However, hyperglycemia coexists and is correlated with microvascular disease as well as with other important cardiometabolic risk factors such as hyperinsulinemia, vascular dysfunction, and AGE.[16] Therefore, it is difficult to know if glycemia or other related factors account for the higher risk of cognitive impairment reported in PreD and T2D, and whether they are related to AD/ADRD pathology.

Project 3 addresses the association of T2D medications, primarily metformin, with cognitive impairment and biomarkers of neuropathology. Some laboratory[48,49] and human[50,51] studies suggest that metformin may increase the risk of AD, but this is countered by studies in

animals[52-54] and humans[55,56] indicating that metformin may be beneficial for AD. Given that metformin is the most frequently used medication for the treatment and prevention of T2D worldwide, it is key to answer whether metformin increases the risk of cognitive impairment and its effects on AD/ADRD. Other insulin-sensitizing medications such as dipeptidyl peptidase-4 inhibitors (DPP4i), glucagon-like peptide-1 receptor agonists (GLPR1a), and sodium-glucose cotransporter-2 inhibitors (SGLT2i), may also have beneficial effects on AD.[57,58] These medications are less commonly used than metformin and will be examined on an exploratory basis in this project.

Project 4 addresses the association of physical activity, physical function, and frailty with cognitive impairment and biomarkers of neuropathology. Physical activity is an integral part of non-pharmacological management and prevention of T2D[24] and may be beneficial for cognitive impairment.[30] However, persons with T2D have higher frailty levels[59] that may affect physical activity and may be part of cognitive impairment in PreD/T2D.[60] There is a dearth of studies examining the individual and aggregate relation of physical activity, physical function, and frailty, in relation to cognitive impairment and biomarkers of neuropathology in PreD/T2D.

3 DPPOS-4 OUTCOMES

DPPOS-4 will continue to measure many of the outcomes similar to those during DPP and DPPOS (see Chapter 11), with the addition of measurements specific to the cognitive aims of DPPOS-4.

3.1 Cognitive Outcomes

3.1.1 Cognitive tests performed during DPPOS-2 and DPPOS-3

- Verbal learning will be measured using the Spanish English Verbal Learning Test (SEVLT).[61]
- Executive function will be measured using the Digit Symbol Substitution Test (DSST).[62]
- Global cognition will be measured using the Mini-Mental Status Exam (3MS).[63]
- Telephone Interview for Cognitive Status (TICS)[64] and AD-8 (Dementia Screening Interview)[65] as needed.

3.1.2 New DPPOS-4 cognitive measures

DPPOS-4 will include the NACC-UDS cognitive battery, the NACC-UDS physician assessment, the study partner (informant) questionnaires, the NIH toolbox reading test as a measure of literacy, and the MAC-Q as a measure of subjective cognitive complaints.

- NACC-UDS neuropsychological battery.[5] We will implement the following tests from the NACC-UDS: The Craft-21, the trail-making tests (TMT) A and B, Benson visual retention test, digits forward and backward, category fluency for animals and vegetables, letter fluency, the Montreal Cognitive Assessment (MOCA), and the multilingual naming test (MINT). We will use norms developed for these tests to estimate percentiles of performance. The telephone version of these tests will be completed as needed.
- The NACC-UDS assessment includes a brief neurologic exam which will be recorded on video and graded by a central physician.
- Geriatric Depression Scale (GDS).[66]
- Study partner questionnaires will include the neuropsychiatric inventory (NPI), and the Functional Activities Questionnaire (FAQ),[67] which are part of the NACC-UDS, and the Quick Dementia Rating System (QDRS).[68] These questionnaires may be completed in person or by phone/video visit.
- The Reading Test from the NIH Toolbox for the Assessment of Behavioral and Neurological Function[69] will be used to assess literacy as a proxy of cognitive reserve.
- Memory Complaint Questionnaire (MAC-Q).[70]

Cognitive syndromes: A central committee of experts will adjudicate cognitive syndromes following criteria from the NIA/AA recommendations for MCI[71] and dementia[72] using the nomenclature found in the NACC-UDS and the information from the previously described cognitive and functional measures. The cognitive syndromes will include:

- Normal cognition.

- Dementia and dementia subtypes.
- Mild Cognitive impairment (MCI) and MCI subtypes.

3.1.3 AD and ADRD Biomarkers

AD and ADRD biomarkers will be measured on all participants during the first wave of DPPOS-4 as well as twice using stored samples from DPPOS. Biomarkers to be analyzed may include:

- amyloid (Aβ42/Aβ40 ratio)
- phosphorylated tau 181 (ptau-181)
- phosphorylated tau 217 (ptau-217)
- neurofilament light (NfL)
- neuroinflammation (glial fibrillary acidic protein [GFAP]).

3.1.4 Exosomes (extracellular vesicles)

Neuronal and peripheral exosomes will be extracted from plasma of all participants during the first wave of DPPOS-4. Assays in exosomes may include:

- pSer312-IRS1
- pTyr-IRS1
- pAKT/pGSK3B/pS6K
- p181Tau

3.1.5 Brain Imaging

Brain imaging (amyloid PET scanning and MRI) will be performed in a subset of participants to validate plasma biomarkers and provide additional measures of brain pathology. A separate informed consent will be obtained from participants selected to participate. Brain imaging will be conducted on a subsample of participants. There will be 2 types of study procedures for brain imaging outcomes: brain magnetic resonance imaging (MRI) and brain amyloid beta (Aβ) positron emission tomography (PET) with the radiological ligand 18^{F}-Florbetaben or 18^{F}-Florbetapir. The primary brain imaging outcomes will be amyloid burden defined continuously as amyloid Centiloid and defined categorically as amyloid positivity (yes/no); cortical thickness in regions of interest affected by AD; white matter hyperintensities (WMH), and infarcts.

3.2 Other Outcomes

Secondary outcomes represent diabetes-associated complications and their risk factors that have been studied longitudinally during DPP and DPPOS. Some of them will be explored as risk factors for AD, ADRD, and other forms of cognitive impairment. The timing of outcome assessments is described in Chapter 11.

3.2.1 Diabetes and Metabolic Measures

The effects of diabetes and glycemia on cognitive and other outcomes will be assessed categorically and continuously.

The categorical criteria for the diabetes outcome during DPPOS-4 follow 2022 ADA guidelines:

- FPG ≥ 126 mg/dL or HbA1c ≥ 6.5%, confirmed by a second of either test. The goal interval for retesting is 42-days; however, any subsequent retest is eligible for confirmation.
- HbA1c ≥ 6.5% and FPG ≥ 126 mg/dl on the same day

Metabolic measures that will be examined as continua include:

- Fasting Glucose
- Fasting Insulin and proinsulin
- HbA1c
- Other metabolic assays such as Advanced Glycation Endproducts

3.2.2 **Nephropathy**

- Albumin excretion: Urinary albumin corrected for creatinine concentrations from a spot collection.
- Serum creatinine
- History of end-stage kidney disease treated with dialysis or transplantation

Nephropathy was adapted from KDIGO[73] for categories of moderately increased albuminuria or moderately to severely decreased eGFR and defined as development of any of the following: (1) moderately increased albuminuria (≥30 mg/g creatinine) based on spot urine; (2) eGFR by CKD-epi[74] <45 ml/min; (3) kidney transplant; or (4) dialysis for end-stage renal disease.

3.2.3 **Neuropathy**

- Semmes Weinstein 10 gram monofilament examination
- Examination of vibration sensation
- Examination of pinprick sensation
- Michigan Neuropathy Screening Instrument Questionnaire (MNSI)

Symptomatic distal symmetric polyneuropathy (DSPN) is measured with the symptom questionnaire from the Michigan Neuropathy Screening Instrument used in previous years and signs of neuropathy are detected using quantitative measurements of vibration, light touch and sharp (pinprick) sensation, as recommended in the 2017 ADA Position statement.[75] DSPN is defined as the presence of DSPN symptoms (score ≥4 on the MNSI questionnaire)[76] or any signs of bilateral DSPN. The signs of bilateral DSPN require abnormal findings on both toes for pinprick testing (score ≥5)[77], for vibration testing (score≥5)[77], or for light touch sensing (<8 detected from 10 monofilament applications on each toe). Secondary analyses will include the presence separately of DSPN by symptoms or by signs, the presence of symptomatic DSPN confirmed by signs, and the individual elements of the neurologic sensory examination.

3.2.4 **Cardiovascular Disease**

Major cardiovascular disease (MACE) is defined as one or more of the following: fatal and non-fatal myocardial infarction and stroke or other cardiovascular death.

Extended MACE is defined as one or more of the following: a) cardiovascular disease (CVD) events (CVD death, fatal and non-fatal myocardial infarction and stroke); b) silent myocardial infarction on EKG;[78] c) coronary artery stenosis ≥ 50% documented by angiography; d) coronary revascularization; e) hospitalized congestive heart failure (CHF); f) hospitalized

unstable angina/acute coronary syndrome; or g) revascularization or amputation in lower extremity not caused by major trauma.

All CVD events are determined at the time of their report using a standardized medical history questionnaire and adjudicated using available medical records by an outcomes committee.

3.2.5 Mortality

Cause of death is adjudicated by the Outcomes Committee based on available medical records including death certificates. For participants who are lost to follow-up, a search of the National Death Index (NDI) or an external people-search service may be used to search for death and cause of death. The NDI is a central computerized index of death record information from State vital statistics offices nationwide. If permitted by the IRB, follow-back investigations will be undertaken to obtain medical records and next-of-kin interviews as required.

3.2.6 Medicare and other databases

The DPPOS may continue to search the Centers for Medicare and Medicaid Services (CMS) database and other large health and healthcare-related databases to determine diagnosis codes and dates for health care utilization to supplement medical records.

3.2.7 Cardiovascular disease risk factors:

- Blood pressure
- Heart rate
- Inflammatory, coagulation, and endothelial function biomarkers
- Lipid profile (total cholesterol, total triglyceride, HDL-cholesterol and LDL-cholesterol)

3.2.8 Body weight and obesity:

- Height, weight, waist circumference

3.2.9 Other assessments:

- Updated health history including COVID diagnoses
- Family health history
- Beck Depression Inventory
- MOS SF-36
- Social Determinants of Health (including transportation, financial and food security, living situation, social status ladder, social isolation, resilience, discrimination)
- Short Physical Performance Battery (SPPB)
- Physical activity using the Modifiable Activity Questionnaire (MAQ)
- Urinary incontinence
- Activities of Daily Living and Instrumental Activities of Daily Living
- Hearing and vision
- Sleep: STOP BANG OSA screener and PROMIS sleep disturbance questionnaires
- History of falls
- Serious adverse medical events

- Quality of Well Being (QWB) Scale

3.2.10 Resource Utilization, Costs, Health Utilities, and Effectiveness of treatments

DPPOS-4 will continue to collect information on resource utilization and costs, and medical care received outside of the study. The information used in the health economics analyses will be obtained with:

- Resource utilization instruments: Questionnaires to capture resource utilization from the perspectives of the participant and the DPPOS staff.
- Hospitalizations

3.2.11 Stored specimens

- Sample storage: Samples of plasma, serum, and urine will be stored for possible future analyses related to IGT and Type 2 Diabetes and their complications, Alzheimer's Disease plasma biomarkers, exosomes, and other associated disorders. Samples for DNA were collected during the DPP. New samples will be collected for future genomic studies (for example gene sequencing, epigenetics, and gene expression).

3.2.12 Other chemistries

- Vitamin B12 levels
- Myokines

4 DPPOS-4 STUDY DESIGN

4.1 Overall Design

In addition to continuing the study of diabetes and traditional diabetes-related complications, DPPOS- 4 is a prospective, observational study which will characterize the nature of AD, ADRD, and cognitive impairment, and their determinants, among persons with PreD and T2D in the DPPOS cohort.

4.2 Participation Criteria

All surviving participants will be recruited to enroll in DPPOS-4. Characteristics of the DPP/DPPOS cohort since the beginning of DPP are shown in Table 1.

Table 1. Characteristics of the DPP/DPPOS cohort through in the last 25 years and projected for DPPOS-4

	DPP	DPPOS-1	DPPOS-2	DPPOS-3	DPPOS-4@
Calendar Years	1996-2002	2002-2009	2009-2015	2015-2022	2022-2027
DPPOS Years	-	1-7	8-13	14-20	21-24 (Waves 1&2)
Total enrolled participants	3234	2766 (86%)#	2493 (93%)#	2261 (96%)#	~1979
Withdrew consent	42	27	31	10	--
Died during period**	28	97	144	277	-
Mean age in years +	53	61	67	72	77
Age ≥ 65 years (%) +	10%	26%	48%	74%	93%
Sex (% women)	2191 (68)	1878 (68)	1694 (68)	1572 (70)	1402 (71)
Ethnic and racial group (%)					
Hispanic	508 (16)	424 (15)	368 (14)	347 (15)	312 (15)
Non-Hispanic White	1768 (54)	1506 (54)	1350 (54)	1194 (52)	1030 (52)
Non-Hispanic Black	645 (20)	559 (20)	511 (20)	472 (20)	406 (20)
American-Indian	171 (6)	153 (5)	148 (5)	144 (6)	132 (6)
Asian American/Pacific Islander	142 (5)	124 (4)	116 (4)	104 (5)	99 (5)
Diabetes (%) +	851 (26)	1338 (48)	1501 (60)	1504 (67)	--
Diabetes duration (years)+^	1.6	5.8	10	14.3	~19

*#Of surviving members from prior study period. @Projected at end of visits in 2026. **Some participants died during study period without enrolling for that period. +At end of period or projected. ^Among participants with diagnosed T2D.*

4.3 Procedures for obtaining informed consent

All participants are provided information about the study prior to providing informed consent. The informed consent is approved by all required IRBs, which may include both the single IRB as well as local IRBs. Informed consent is obtained by approved local study staff. If there is concern about the participant's capacity to consent, we will assess the capacity of consent using a questionnaire such as the University of California, San Diego Brief Assessment of Capacity to Consent (UBACC[79]), adapted for DPPOS-4. The UBACC is a 10-item scale that assesses understanding and appreciation of the information related to a research protocol. The UBACC will be available in English and Spanish.

4.4 Procedures for designating a surrogate (legally authorized representative)

Each participant will be asked to name a surrogate (legally authorized representative, LAR) who may provide consent for their participation in the study should they lose capacity in the future. The procedures to designate a LAR depends on local regulations and may vary between study sites. If a participant chooses not to have a surrogate/LAR and loses the capacity to consent, the participant will be excluded from further participation.

4.5 Procedures for obtaining study partners

Each participant in DPPOS-4 will be asked to name a study partner who can provide additional information about the participant. The study partner should be a family member or close friend of the participant. The DPPOS staff will contact the study partner to collect additional information about the participant's health status and functioning. Study partners will not be asked to provide any information about their own health status. The study partner may also serve as the LAR (see above). Participants who are not able to name a study partner may still participate in DPPOS-4.

5 DPPOS-4 PARTICIPANT MANAGEMENT PROTOCOLS

DPPOS-4 is an observational study without any interventions. Participants will complete follow-up visits according to the schedule in Chapter 11 during two waves of visits as shown in Figure 2 below.

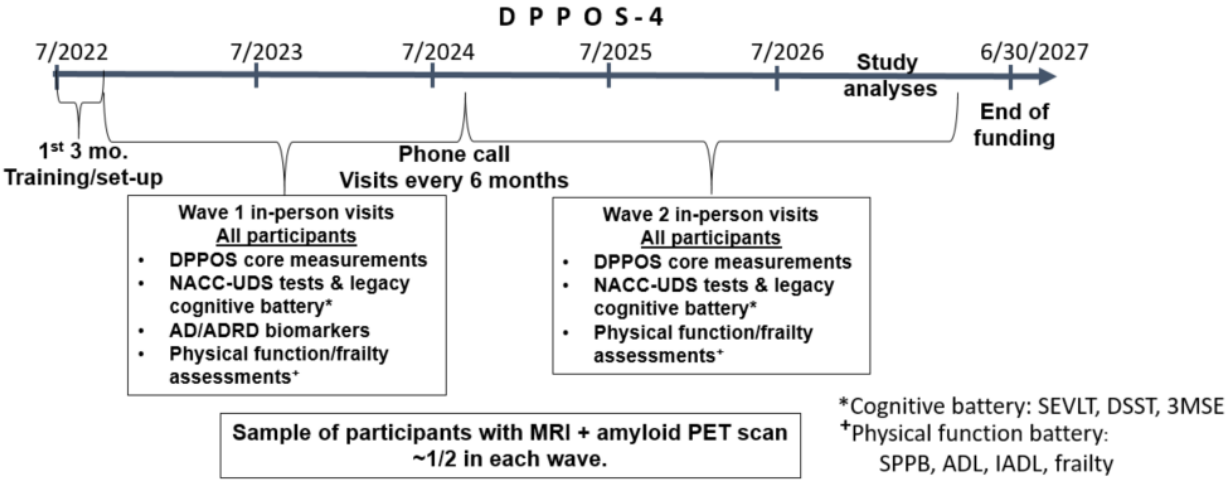

Figure 2. DPPOS-4 visit schedule

5.1 Main Study Visits

During DPPOS-4, the main study in-person visits to collect outcome assessments are scheduled during two 24-month waves and completed at the clinical centers. When possible, data collection and questionnaires that can be completed by phone or video will be done in advance of the in-person assessments. All assessments will be performed by study staff who are certified to perform the outcomes. For those individuals who cannot travel and for whom a home visit (see below) is not possible, a remote (phone or video) visit may be conducted to collect updated health history, administer questionnaires, and collect serious adverse events and other event information. Participants who move may be transferred to a DPPOS clinic that is more convenient to their new location.

5.2 Remote Check-in Visits

Scheduled remote (phone or video) check-in visits will be held approximately every six-months in between main study visits. These remote visits will be conducted to collect updated health history and serious adverse events, may include administration of some questionnaires, and will promote retention.

5.3 Interim Visits

An interim visit refers to all visits other than scheduled follow-up visits. Interim visits may be required to repeat procedures that were found to be deficient at a previous visit or requiring confirmation of a test result. Interim visits will be held as necessary.

5.4 Home and Non-Clinical Center Visits

A home or non-clinical center visit is defined as any visit outside the DPPOS clinical center performed by DPPOS-4 study staff. Home visits may be used when a participant is unable to attend a main study visit at the clinical center. A study staff person who is certified to perform the study outcomes will perform the home visit. The purpose of a home visit is to retain and/or reactivate participants and to collect important outcome data on participants who are having difficulty attending an in-person clinic visit.

Clinic staff will decide whether or not a specific home visit is feasible, taking into account time, cost, and risk. When a home visit is not considered feasible, portions of the main study visit, e.g., physical examination measurements, blood draws and urine collection, might be performed by a non-DPPOS-4 health technician who is trained and certified in DPPOS-4 procedures.

5.5 Limited Participation Visits

As the DPP/DPPOS participants age, conducting even limited in-clinic, home or non-clinical center visits are sometimes not possible. In such situations, participants will be asked to continue in DPPOS-4 by providing only limited data through periodic phone contact to collect an updated medical history and contact information. In addition, limited participation participants will be consented to allow medical record review. Participants will also have the option to agree to a blood draw at their place of residence one time during DPPOS-4. The purpose of limited participation visits is to collect important outcome data through medical records and via the study partner on participants who are unable to continue with regular study visits.

5.6 Retention Monitoring and Recovery of Inactive Participants

Retention of participants throughout the study period is key to both the power and generalizability of DPPOS-4 findings. Retention of DPPOS-4 participants is encouraged through the provision of social support from study staff during clinic visits, group meetings, and other incentives. All participants receive a small reimbursement at specified visits, in recognition of the time and effort spent in DPPOS-4.

Participants' attendance at scheduled clinic visits is monitored, with the goal of maximizing retention and completion of study activities. Missing scheduled data collection visits trigger a graded hierarchy of recovery efforts designed to maintain participants' involvement in DPPOS-4.

5.7 Suspension of Study Participation

The occurrence or presence of the following will constitute suspension of further study participation: voluntary withdrawal by the participant, loss of capacity to consent without an available LAR, or a condition that, in the opinion of the investigator, makes it unsafe or not feasible for the participant to continue data collection and participation at any level. Efforts to return participants to an active status will be made, as appropriate.

6 DPPOS-4 ADVERSE EVENT REPORTING

The DPPOS-4 study design is observational and involves data collection only – i.e., no treatments, interventions, or management are included. Most DPPOS-4 data collection procedures represent known clinical tests and procedures that may be used for adults with or at risk for T2D, AD or ADRD, along with PET and MRI scans on a subset of participants. All assessments and procedures are performed according to standard instructions by appropriately trained individuals.

6.1 Definitions of Events

The definition of Serious Adverse Events (SAE) listed below represent a continuation of the reporting undertaken during DPP and DPPOS with some modifications. In DPPOS-4, we are particularly interested in SAEs that have been established or are putatively associated with cognitive or physical dysfunction that we are studying. In DPPOS-4, SAEs have been defined to include any adverse experience that results in any of the following outcomes:

- The event results in an inpatient hospitalization (any overnight stay associated with an admission).
- The event results in the prolongation of a hospital stay.
- The event results in permanent or severe disability.
- The event results in death.
- The event is life-threatening.
- Treatment is required to prevent a serious event

Detailed definitions are provided in the Manuals of Operation.

6.2 Eliciting and Recording Serious Adverse Events

Reporting of SAEs will be accomplished by collecting information on these adverse experiences during all remote check-in or in-person main follow-up visits. In order to avoid bias in eliciting serious adverse events, these adverse events will be assessed using a standardized checklist. In addition, participants are instructed to contact the clinical center with any serious adverse event meeting the above criteria. SAEs will be reported to the DPPOS-4 Coordinating Center (CoC) as they occur through routine data entry (within 2-3 days).

An SAE in DPPOS-4 occurring as a consequence of performing any study-related data collection procedure are reported to the IRB and will be reported by the clinic to any local IRB(s) as required. Adverse reactions that are determined by the investigator to be unrelated to research-specific procedures need not be reported to any overseeing IRB, unless the specific IRB requires otherwise.

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7 DPPOS-4 DATA PROCESSING

7.1 Data Forms

DPPOS-4 data forms are completed to document protocol performance and to collect participant data relevant to the research questions. Chapter 11 is the schedule of outcomes collection. The list of DPPOS data forms appears in the study Manual of Operations and includes administrative as well as data collection instruments.

The CoC creates the DPPOS data form templates. At each clinic, the clinic staff, directed by the program coordinator, reviews completed data forms prior to data entry. Completed forms are edited as they are entered into the data management system, and then again via the central data management system at the CoC.

7.2 Data Entry and Management System

7.2.1 Clinical Centers

A secure, web-based, password-protected data entry and management software corresponding to the data forms completed at a clinical center is developed and maintained by the staff at the CoC. Reports are developed for use at the clinical centers to assist clinical center staff in participant management, data collection, and study management. DPPOS computers are kept in a safe location that can be locked when not attended.

7.2.2 CoC Centralized Data Management System

Data entered via the web-based electronic data collection system is securely transmitted and stored on the CoC's server. Data are converted to SAS data sets after being uploaded to the CoC's server. All new data are edited for unavailable, out of range, or inconsistent values. Weekly audit programs produce more detailed edits across forms for an individual participant. Summaries are prepared for reports to the Steering Committee and Protocol Oversight Program. The CoC maintains confidentiality of participant data and emerging results per a confidentiality policy, which every staff member is required to sign.

Data including participant's personal information (such as name, Social Security Number, Medicare number, and zip code) that is shared with the DPPOS Coordinating Center will be used to access data sources such as Medicare and Medicaid. Such data are stored securely and separately from the research record, and only limited, authorized research personnel have access to this information.

The CoC adheres to the Biostatistics Center's data back-up and security policies to ensure the safety and confidentiality of the data. Back-up procedures include twice-weekly system back-up, daily incremental back-up, and off-site disaster recovery back-up. Security procedures include logon and link password protection, and for internet access, separate Web servers which use SSL and encryption algorithms. Virus and malware protection software is used on all computers and implemented on an hourly basis. All portable computers employ full disk encryption. University computing facilities provide support in the event of a disaster. Access to the server and databases is secured by use of login user accounts and passwords. Remote access is granted only to authorized users and is accomplished using a secure virtual private network (VPN). Appropriate filtering/firewall setup is used to prevent unauthorized access.

7.2.3 Central Biochemistry Laboratory Data Management System

The Central Biochemistry Laboratory (CBL) at the University of Minnesota Advanced Research and Diagnostics Laboratory (ARDL) uses a relational database to manage analyses performed within the laboratory using a custom-developed Laboratory Information Management System (LIMS). Automated analyzers are connected to the database via communication interfaces developed and maintained by the CBL staff. Reports to the CoC are transmitted via secure FTP to the CoC with the original reports stored in the relational database as well as on a file server. All storage media containing clinical data in use at the laboratory utilize hardware fault redundancy, and the data are backed up daily to a secure and remote data storage facility.

7.2.4 Neuroimaging Reading Center Data Management System

Image data transfer from each site to the University of Pennsylvania analysis lab will be via the American College of Radiology TRIAD system, a HIPAA-compliant, secure data transfer and storage resource. Site users will be trained on image upload procedures. University of Pennsylvania staff will download new scans from TRIAD and check for study completeness and quality. Image analysis will be performed on a state-of-the-art computing cluster housed in the University of Pennsylvania data center. Results are transmitted to the CoC via secure FTP.

7.3 Performance Monitoring

7.3.1 Training Workshop and Site Visits

The CoC and Central Units, with appropriate investigator subcommittee members, have established procedures to train and certify clinical investigators in the protocol, manuals of operation, and data processing procedures. In-person and virtual workshops are held for training personnel from the clinical centers to address DPPOS-4 procedures, including the use of the DPPOS-4 data forms and data processing systems. CBL personnel instruct the program coordinators and study staff on the proper collection, packaging, and mailing of specimens for analysis by the CBL. Central units also instruct, train, and certify the program coordinators and technicians (as needed) to promote standardized, uniform assessments across the clinical sites. The CoC maintains close contact with the program coordinators and provides additional training or review as needed.

Based on clinic performance monitoring, appropriate representatives from the CoC, the NIA, and clinic investigators will visit the clinical centers virtually or in person, as required. These site visits will review procedures with the program coordinators/technicians, assess proficiency in executing the DPPOS-4 protocol, review deficiencies detected in monitoring the performance of the clinical centers, review the utilization of personnel relative to the amounts budgeted, and receive feedback on the adequacy of the centralized support operations.

7.3.2 Periodic Performance Reports

During the DPPOS, the CoC will monitor the performance of the clinical centers and produce periodic reports summarizing protocol performance for the Protocol Oversight Program (POP) committee.

7.3.3 Retention

The CoC and the POP will monitor the performance of the clinical centers in retaining participants. The CoC prepares reports on participant compliance with the DPPOS protocol and participants on inactive follow-up.

7.3.4 DPPOS Data Form Completion

The CoC prepares reports for data completion and quality. Missing data, particularly on outcome variables, will effectively reduce the power of analyses. Systematic patterns of missing data could bias the study results. Therefore, many of the procedural details outlined in the Manuals of Operation are designed to minimize the amount of missing data.

7.3.5 Other Reports

Other reports are developed, as needed, based on requests from the Steering Committee and associated subcommittees.

8 DPPOS-4 STUDY ADMINISTRATION

8.1 Organizational Units

The following five Cores and Centers have been established to manage DPPOS-4 operations.

8.1.1 Administrative Core

The Administrative Core provides budgetary and administrative support to the other four cores and four projects in DPPOS-4. The Administrative Core will leverage the current governance structure of DPPOS and will interact with leadership at NIA and other national AD and ADRD research and research education programs. The Administrative Core will support the governance structure of DPPOS-4, including:

- Executive Committee manages the day-to-day operations of the study
- Steering Committee provides the scientific direction to the study
- External Advisory Board reviews the progress of the study and provides non-binding recommendations
- Ancillary Studies Committee reviews and approves ancillary proposals
- Publications and Presentations Committee reviews and approves all abstracts, posters, presentations, and publications

8.1.1.1 External Advisory Board

External Advisory Board (EAB) members will serve as external reviewers and advisors to the DPPOS-4 Steering Committee. The EAB consists of experts in relevant biomedical fields, biostatistics, and medical ethics.

8.1.2 Clinical Operations and Procedures Core (Clinical Core)

The Clinical Core will supervise training, certification, quality assurance (QA) and quality control (QC) of DPPOS-4 procedures and measures with the exception of cognitive measures. The Clinical Core will provide oversight and management of all DPPOS clinical centers, coordinate the training and certification of clinical center staff in the performance of standardized procedures and collection of DPPOS-4 measures, and support the central laboratory.

8.1.2.1 Clinical Centers

The clinical centers implement the DPPOS-4 protocol; assume responsibility for the completion of the assessments and measurements for each participant enrolled in the study; record participant data; review and enter information from data forms using the data entry and management system; and respond to edit queries from the CoC. Each clinical center has a Principal Investigator, a Program Coordinator, and additional staff as needed to carry out the protocol.

8.1.2.2 Central Biochemistry Laboratory

The Central Biochemistry Laboratory at the University of Minnesota Advanced Research and Diagnostics Laboratory (ARDL). ARDL is fully accredited as a high-complexity laboratory by the Clinical Laboratory Improvement Amendments (CLIA), the College of American Pathologists (CAP), and the New York State Department of Health. ARDL has served as a Central Laboratory for multicenter studies for over 30 years and began as the DPPOS CBL in 2020. All

routine laboratory measurements will take place at ARDL. ARDL will provide de-identified samples to other selected laboratories for measurements of plasma biomarkers and exosomes, and to the delegated NIH biorepository, as instructed by the CoC.

8.1.2.3 Working Committees

In addition to the administrative committees listed above, the Clinical Core will support working committees including:

- Outcomes Classification
- Program Coordinator
- Protocol Oversight Program
- Quality Control

Additional working committees will be formed as necessary.

8.1.3 Cognitive Assessment and Adjudication Core (Cognition Core)

The Cognition Core will conduct all training, certification, QA, and QC activities related to cognitive testing, including developing, maintaining, updating, and implementing training, certification, and QA procedures related to cognitive and functional testing.

8.1.4 Brain Imaging and Plasma Biomarkers Core (Biomarkers Core)

The Biomarkers Core will oversee brain imaging activities and plasma biomarker-related activities. A neuroimaging coordinating center will conduct all training, certification, QA, and QC activities related to structural and functional MRI, brain amyloid PET imaging. A plasma biomarkers laboratory will supervise and measure plasma biomarkers of amyloid, tau, neurodegeneration, and neuroinflammation.

8.1.5 Biostatistics and Data Management Core (Data Core)

The Data Core will collect and manage all study data, providing data infrastructure, a data warehouse, and statistical expertise for DPPOS-4. The Data Core will provide statistical expertise for the four projects to ensure rigor in study design, develop testable hypotheses with appropriate analyses using uniform statistical approaches and disseminate results, provide and maintain data infrastructure for the overall study, and disseminate data for analyses to facilitate the preparation of ancillary studies, promote training, and for submission to an NIH repository.

8.2 Funding Mechanism/Study Resources

DPPOS-4 is supported by the National Institutes of Health through the National Institute of Aging, other co-funding NIH Institutes and Centers, and other DHHS co-funding Agencies using the U19 mechanism.

The NIA program official and project scientist provide program involvement in the scientific efforts of the DPP Research Group through the development of protocols and assistance in the conduct and oversight of the DPPOS-4.

8.3 Policies

8.3.1 Publications

The Publications and Presentations Subcommittee (PPS) coordinates, monitors, reviews, and assumes responsibility for arranging the preparation of all study-wide communications (press releases, interviews, presentations, and publications) relating to the scientific aspects of the study. There will be no publication or presentation of study plans or results which have not been reviewed and approved by a majority of the PPS, and for some types of communications, a majority of the Steering Committee.

With respect to publications and presentations from DPPOS, the goals of the PPS are to:

1. Ensure accurate, uniform, timely, and high-quality reporting of the DPPOS activities and results;
2. Preserve the scientific integrity of the study;
3. Safeguard the rights and confidentiality of participants;
4. Assure that the timing of publications and presentations serves the right of the public to know the results of the program without jeopardizing its conduct.

Members of the writing group will include volunteers from the DPPOS investigators at large. The PPS approves a writing group for each publication or presentation proposed by the DPPOS investigators.

There will be several categories of publications and presentations, with different rules for authorship, ranging from publications of the main results of the study (with authorship by the entire research group) to other types of publications with named authors. The authorship rules balance the need to recognize the contributions of all investigators and staff with the need to recognize individuals for specific contributions to certain types of publications and presentations.

8.3.2 **Ancillary Studies**

The Ancillary Studies Subcommittee evaluates all proposals for studies that involve DPPOS participants or stored samples and that are not part of the protocol. These studies may be done only on a subset of participants in the DPPOS. However, studies that include all participants and studies that analyze study data in ways extracurricular to the protocol are also submitted to the Ancillary Studies Subcommittee. Supplemental funding required to complete an ancillary study is obtained independent of core study funding. Ancillary studies are approved by the Steering Committee following recommendation by the Ancillary Studies Committee.

Major factors in consideration of ancillary studies include:

- Clinical importance and scientific validity
- Compatibility of goals with those of DPPOS
- Amount of burden on study subjects and staff

9 DPPOS-4 STATISTICAL CONSIDERATIONS

9.1 Power

The main outcome for DPPOS-4 is the adjudicated cognitive diagnosis based on the NACC-UDS that classifies participants into the clinical dementia rating scale (CDR), normal (CDR=0), mild cognitive impairment (CDR=0.5), and dementia (CDR=1). The 4 projects will use this as their primary outcome with various exposures of interest. Assuming a conservative CDR distribution of 70% normal, 20% mild cognitive impairment, and 10% dementia with a 2-sided alpha of 0.05, the study of 1979 participants is able to detect a minimum of 1.31 odds ratio of MCI or dementia compared to normal cognitive function associated with the various exposures of interest with 80% power using a proportional odds ordinal logistic regression.[6] The study also has ample power to detect small effects for continuous outcomes and to detect a moderate effect for conditions with low prevalence. For outcomes in the form of continuous scores (e.g., measure of cognitive function) the sample size of 1979 provides 80% power at alpha level of 0.05 to detect effect sizes as small as Cohen's D=0.1 to 0.3 assuming exposure rates of 10-50% and similar variability in cognitive decline observed in the study and preliminary plasma biomarker data. That is, the minimum detectable difference between any two groups (e.g., ever metformin use vs. none) is 10% to 30% of the standard error of the residuals in the mixed model. In models with cumulative metformin exposure, we will have more power or smaller detectable effect size. Power calculations for continuous outcomes were conducted using r package simr[7] and software nQuery.[8]

9.2 Analyses

Cross-cutting statistical issues and strategies: Means and 95% confidence intervals and nominal P values will be reported. Model assumptions will be tested. For logistic models, these include testing for multicollinearity and establishing linearity between independent variables the log odds. Linear model assumptions, including tests for linear relationships between continuous outcomes and predictor variables and lack of homoscedasticity, will be tested. Residual analysis will be used to determine model fit, and transformations may be necessary. Since the distributions of plasma and brain imaging biomarkers are often skewed, the variables will be transformed as needed, and categories will be considered. We will use all available measures during the 25-year follow-up from DPP to DPPOS-4 to derive measures of trajectories of change over time and the cumulative burden of exposure.

Statistical considerations are provided for the main aims of each of the four projects. A statistical analysis plan is developed in advance of beginning the analysis for all publications and presentation. General statistical methods for each project are described below.

9.2.1 Project 1

Project 1 will assess cross-sectional differences in biomarkers of tau, neurodegeneration, neuroinflammation among the cognitive syndromes (MCI, dementia compared to normal) using ANCOVA adjusted for age, sex, and APOE-ε4. Secondary analyses will evaluate differences among cognitive syndromes in longitudinal measures of tau, neurodegeneration, neuroinflammation, analyzed as continuous measures in linear mixed effect models and as trajectory classes defined by latent class mixed effect models. Project 1 will also assess differences among cognitive syndromes in neuroimaging biomarkers of tau, neurodegeneration,

neuroinflammation in those with neuroimaging. We will explore the consistency of the estimates from the subset compared to the full cohort using appropriate weighted models to evaluate potential selection bias. Horvitz-Thompson estimators in the inverse selection probability weighting method, propensity score methods will be used to correct biases due to unbalanced confounders in exposure and non-exposure groups.[9,10]

9.2.2 Project 2

Project 2 will focus on the associations of type 2 diabetes-related factors with risk for cognitive impairment syndromes. We will use logistic regression for categorical outcomes and linear mixed models for continuous outcomes to test for the association between trajectories of change in HbA1c, log(1/fasting insulin), and proinsulin-to-insulin ratio, and AGE protein concentration as the main predictors, and in the full cohort, (a) amnestic and non-amnestic cognitive decline (categorical and continuous), MCI, and dementia diagnosis (categorical) outcomes and (b) trajectories of plasma biomarkers (continuous) as outcomes. In the sample of the cohort with imaging, we will examine summary measures from imaging biomarkers (continuous) as the outcomes. Among only those participants with a diagnosed cognitive impairment syndrome we will examine each of the cognitive impairment syndromes with associated pathology (AD continuum vs. non-AD pathologic change) as the outcome. All predictor variables will be included together in the same model to test for the independent effects of each.

9.2.3 Project 3

Project 3 will examine the association of cumulative metformin exposure with amnestic and non-amnestic cognitive decline, MCI, and dementia, and explore these syndromes classified by AD pathologic change and VCID. We will employ causal inference approaches to assess the association of metformin exposure (in-study and total) on the risk of cognitive impairment to adjust for potential confounding by indication in logistic models for cognitive syndromes and linear mixed-effects models for measures over time such as amnestic and non-amnestic cognitive decline. We will also assess the association of cumulative metformin exposure with trajectories of amnestic and non-amnestic cognitive decline. All models will be adjusted for age, sex, race, education and APOE-ε4.

9.2.4 Project 4

Project 4 will examine the association between physical activity, physical function, and frailty, separately, with amnestic and non-amnestic cognitive decline using linear mixed models. We will fit semiparametric proportional rates model with Turnbull's algorithm[11] for interval censored data to test the association between the summary measures of physical activity, physical function, and frailty, separately, and the risk of MCI and dementia. We will also fit stochastic Markov models[12] for the transition between normal to MCI, dementia, or death between the two waves of data. These associations will be examined for amnestic and non-amnestic cognitive syndromes and AD continuum and non-AD pathology. We will compare the cognitive decline and risk of dementia and MCI across the joint classes of PA/PF/frailty using linear mixed, proportional rates or Markov models.

9.3 Timing of Analyses

It is anticipated that analyses will occur after the end of Wave 1 data collection and again after Wave 2 data has been collected.

10 DPPOS-4 STUDY TIMETABLE

Diabetes Prevention Program-4

DPPOS- 4	September 2022	NIA 5-year funding awarded
	November 2022 – October 2024	Wave 1 participant follow-up
	November 2024 – October 2026	Wave 2 participant follow-up

11 DPPOS-4 Outcomes Schedule, Waves 1 and 2

Participant DPPOS-4 visit wave	Wave 1		Wave 2	
Visit calendar years	11/1/22-10/31/24*		11/1/24-10/31/26*	
Type of visit	Remote Check-in	Main§ (In-person or remote)	Remote Check-in	Main§ (In-person or remote)
Cognitive Function				
Legacy DPPOS Assessments		X		X
NACC UDS Assessments		X		X
AD/ADRD biomarkers				
Brain imaging+		X		X
AD/ADRD plasma biomarkers**		X		
Exosomes		X		
Medical history				
Updated medical history including adverse events, COVID, and falls	X	X	X	X
Prescription medications and OTC medications and supplements	X	X	X	X
Alcohol and smoking		X		X
Family health history	X			
Diabetes and metabolic measures				
Fasting glucose		X		X
HbA1c		X		X
Fasting insulin		X		X
Fasting pro-insulin**		X		X

Participant DPPOS-4 visit wave	Wave 1		Wave 2	
Visit calendar years	11/1/22-10/31/24*		11/1/24-10/31/26*	
Type of visit	Remote Check-in	Main§ (In-person or remote)	Remote Check-in	Main§ (In-person or remote)
Others such as advanced glycation end products		X		X
Lipids				
Lipid profile		X		X
Kidney Function				
Urine Albumin & Creatinine		X		X
Serum Creatinine		X		X
Other laboratory measures				
Urine Albumin & Creatinine		X		X
Serum Creatinine		X		X
Inflammatory, coagulation, and endothelial function biomarkers		X		X
Vitamin B12		X		X
Myokines		X		X
Physical				
Weight		X		X
Height		X		X
Waist Circumference		X		X
Arm Blood Pressure		X		X
Heart rate		X		X
Short Physical Performance Battery (SPPB)		X		X

Participant DPPOS-4 visit wave	Wave 1		Wave 2	
Visit calendar years	11/1/22-10/31/24*		11/1/24-10/31/26*	
Type of visit	Remote Check-in	Main§ (In-person or remote)	Remote Check-in	Main§ (In-person or remote)
Neuropathy assessments (monofilament, vibration, pinprick)		X		X
Quality of Life				
Beck Depression Inventory		X		X
SF-36		X		X
Quality-of-well being	X		X	
Urinary Incontinence		X		X
Social determinants of health (including transportation, financial and food security, living situation, social status ladder, social isolation, resilience, discrimination)	X		X	
Activities of Daily Living and Instrumental Activities of Daily Living		X		X
Other Questionnaires				
Hearing and vision		X		X
MNSI		X		X
STOP BANG OSA screener and PROMIS sleep disturbance questionnaires		X		X
Modifiable Activity Questionnaire (MAQ)		X		X
Resource Utilization	X		X	

* Visit windows on either side of the calendar dates will allow for flexibility in scheduling visit waves
** May also be measured on stored samples from prior DPPOS Phases
+ Brain imaging PET and MRI scans will be completed on a sample of participants one time across the two waves

[§]Special note: Due to the covid-19 pandemic, main in-person clinic visits may be replaced by phone or video visits as needed for participant safety or as required locally or nationally.

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