

Validation of C-Peptide Responses to Oral Glucose Tolerance Tests in Healthy Dogs: A Foundation for Assessing Residual β -cell Mass in Dogs with Diabetes Mellitus

Brief Introduction:

Canine diabetes mellitus (CDM) is common and has been managed only with exogenous insulin therapy since the 1930s, yet 10–40 % of newly diagnosed dogs are euthanized, underscoring the difficulties and limits of current therapy. Early in the disease many dogs retain β -cell function. Developing an assay to detect residual insulin secretory capacity would open a window for therapeutic intervention. The only practical surrogate for endogenous insulin is C-peptide, which is released in equimolar amounts with insulin, minimally extracted by the liver, and unaffected by exogenous insulin.

In humans, 68 % of patients newly diagnosed with type 1 diabetes (T1D) still secrete insulin and C-peptide. Those with residual β -cell function experience fewer hypoglycemic episodes, reduced glucose variability, and lower long-term complications. However, β -cell activity declines rapidly after onset indicating the ongoing autoimmune destruction of the insulin-producing cells. Interventions that preserve this residual function are therefore critical in T1D.

Prior canine studies have produced inconsistent results because of variable assay platforms and stimulation protocols, preventing the establishment of reference ranges or clear clinical uses, posing a barrier to progress in CDM.

Problem Description and Significance: Canine diabetes mellitus (CDM) is common and its management relies almost exclusively on insulin, yet 10–40 % of newly diagnosed dogs are euthanized, underscoring the inadequacy of current care. At diagnosis many dogs retain residual β -cell function—a therapeutic window that cannot be exploited because no reliable, standardized biomarker exists to identify or monitor insulin secretory capacity. This work addresses a **critical gap**: the absence of validated biomarkers to guide disease-modifying interventions in CDM. Preliminary assessment of stimulated C-peptide as a biomarker to identify residual β -cell activity in healthy dogs will enable: 1) a larger study to develop reference ranges in healthy dogs, 2) future assessment of stimulated C-peptide testing in diabetic dogs, 3) development of targeted immunotherapy trials in CDM, 4) expand treatment options and improve quality of life in CDM, and 5) provide a translational platform relevant to human type 1 diabetes research.

Hypothesis: An optimized canine C-peptide assay combined with a standardized mixed-meal tolerance test (MMTT) will produce measurable C-peptide responses that indicate residual β -cell function in dogs with diabetes mellitus (DM).

Objective: Generate pre-clinical feasibility data on canine insulin secretion in healthy dogs through C-peptide testing, laying the groundwork for detecting residual β -cell function in diabetic dogs.

Study Design and Methods: Ten healthy dogs (5–13 y, >10 kg) from UF CVM faculty, staff and students will be recruited. Exclusions: glucocorticoid use in past 2 mo, acute/chronic disease in past 6 mo, or any history of diabetes or pancreatitis. The study, IACUC-approved (IACUC202500000412), will follow two visits. **Visit 1:** A complete physical exam, medical history, age, weight, BCS and sex will be recorded and blood and urine collected (CBC, chemistry panel, urinalysis, fructosamine). Dogs meeting all criteria are included. **Visit 2** (\leq 6 weeks later): Following i.v. placement in the cephalic or saphenous vein, the 10 dogs will undergo a mixed-meal tolerance test (MMTT) after a 12-h fast.¹ A glucose rich liquid (Ensure Plus Vanilla) will be fed at time 0 and finished within 10 min; syringe feeding is used if needed. Blood samples (1 mL) will be collected into EDTA tubes at –10, 0, 2, 5, 10, 15, 30, 60, 90, and 120 min relative to the meal, placed on ice, centrifuged to separate plasma, and then stored at -80°C . Plasma will be assayed in duplicate for C-peptide (ELISA, Sigma Aldrich EZCCP-47k), C-peptide (RIA, Millipore Sigma CCP-24HK), and insulin (ELISA, Mercodia 10-1203-01).²

Role of the Veterinary Student: The veterinary student will recruit and screen healthy dogs, collect demographics, blood, and urine, and participate in both performing the MMTT. The student will run the C-peptide ELISA/RIA assays under supervision. They will handle sample labeling, storage, and database entry while ensuring IACUC compliance and good laboratory practice. The student will also assist with statistical analysis, manuscript preparation, and future study planning, gaining hands-on training in clinical research methodology, laboratory techniques, and data management essential for translational veterinary science.

Citations for methods cited:

1. Pascutti KM, O’Kell AL, Hill RC, Castro RA, Salute ME, Gilor C. The effect of capromorelin on glycemic control in healthy dogs. *Domest Anim Endocrinol*. 2022 Oct 1;81.
2. Öberg J, Fall T, Lilliehöök I. Validation of a species-optimized enzyme-linked immunosorbent assay for determination of serum concentrations of insulin in dogs. *Vet Clin Pathol*. 2011 Mar;40(1):66–73.