A Look at Old and New Insulin Formations
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When choosing an insulin formulation, factors like duration of action, potency, species-source, convenience and price should be considered. Another important factor that had not received much consideration is day-to-day variability in insulin action. While onset of action, duration of action and overall time-action profile are important when attempting to mimic physiological needs, minimizing day-to-day variability is critical for minimizing the risk of hypoglycemia while still maintaining acceptable glycemic control throughout the day. With most formulations, a peak insulin action occurs after injection and that peak should coincide with post-prandial glucose absorption. Post-peak levels should meet the basal insulin requirements (fasting requirements) but should not exceed them. However, achieving complete congruity between exogenous insulin action and endogenous insulin requirements during these 2 different phases is challenging at best, even if assuming complete consistency between meals, post-meal activity levels, stress, etc. Thus, to avoid hypoglycemia with a practical twice-daily insulin therapy and relatively infrequent monitoring, there has to be a tradeoff. In veterinary medicine this tradeoff is typically loose glycemic control with blood glucose concentrations ranging between 100 – 300 mg/dL and some residual glycosuria and incomplete reduction of clinical signs. In people, because of the critical importance of tight glycemic control, the tradeoff is often a combination of more intensive blood glucose monitoring with a more intensive treatment protocol that combines ultra-short acting formulations with long-acting ones.

Insulin formulations: General principles
Suspensions versus solutions
Insulin molecules tend to form dimers and hexamers (especially in the presence of zinc) but only the monomers are biologically active. Protamine and zinc were traditionally used to prolong the duration of action of insulin by promoting formation of insulin crystals (Zinc in Lente formulations, Protamine in NPH formulations, and both Protamine and zinc in PZI formulations). These traditional intermediate-acting formulations (Lente, NPH, PZI) are injected as suspension: They need to be re-suspended by gentle rolling (or thorough mixing in the case of Vetsulin) prior to drawing up a dose from the vial. This contributes to imprecision in dosing of these formulations. The process of de-precipitation in the SQ depot is relatively erratic and unpredictable for Lente, NPH and PZI which also contributes to their relatively variable time-action profile when compared to novel insulin formulations. In contrast to traditional formulations, synthetic insulin analogs (Glargine, detemir, degludec) are supplied as solutions (and not suspensions) and have a more predictable time-action profile, a result of their more precise dosing and more predictable absorption from the SQ depot.

Insulin glargine
Recombinant human insulin analog (Asparagine at A21 is replaced by glycine and 2 arginines are added at B31 and B32). This synthetic molecule does not tent to hexamerize at pH of 4.0 but strongly crystalizes at pH = 7.2. Considered in people long-acting and “peakless”.

Insulin detemir
Recombinant human insulin analog (B30 replaced by myristic acid – a 14-carbon fatty acid). Considered in people long-acting but not “peakless” and still as effective as insulin glargine as basal insulin (fewer side effects because more predictable). The fatty acid bound to insulin Levemir prevents formation of regular hexamers and allows hydrophobic interactions between detemir molecules and with albumin. These interactions allow more predictable absorption from the SQ depot and buffering of detemir concentrations by albumin which leads to minimal variability in time-action profile from one day to the next and a better safety profile (minimal frequency of hypoglycemic events). Insulin detemir has other advantages that are likely related to its tendency to bind to albumin. After adjusting for its high potency and comparing at equivalent units of action in terms of glucose lowering effects, insulin detemir decreases endogenous glucose output and NEFA more than other insulin formulations. This means that SQ administration of insulin detemir resembles the physiological effect of insulin more than other insulin formulations do. Insulin secreted from the pancreas and into the portal system reaches the liver in high concentrations. It is then degraded by the liver and eventually reaches peripheral target tissues in much lower concentrations (about 3 fold difference in dogs) so that overall endogenous insulin has more effect on shutting down endogenous glucose production than on peripheral glucose uptake. By mimicking this differential effect insulin detemir causes less weight gain while maintaining the same degree of glycemic control.

Insulin degludec
Recombinant human insulin analog in which B30 is replaced by a fatty acid (hexadecandioic acid) that is bound to B29 via a glutamic spacer. These changes allow for multi-hexamers to form in subcutaneous tissues and a long acting and completely peakless time-action profile.
Species source
Amino acid sequence in traditional formulations depends on the animal source (porcine, bovine, etc.). To date, the basic molecule of synthetic analogs is human insulin and that molecule is engineered (with varying modifications of sequence) to achieve desired PK/PD profiles. The importance of the amino acid sequence is questionable: While anti-insulin antibodies can potentially form, they do not seem to affect glycemic control.

Insulin formulations: Practical considerations
Most available insulin formulations that are considered intermediate-long acting are most consistently used as twice daily injections in dogs and cats. On average, some might be longer-acting than others but inter-patient variability (and perhaps intra-patient variability as well) precludes a safe prediction for the average patient. The average time-action profile of a formulation should be considered but may not be relevant for a specific patient on a specific day. Variability in time-action profiles limits our ability to balance safety (avoiding hypoglycemia) and efficacy (normalizing blood glucose). Day-to-day variability is crucial to consider when monitoring blood/tissue glucose concentration.6, 7 When performing blood glucose curves, the result of a single curve may be helpful when hypoglycemia is detected but otherwise, repeated curves from multiple days might be required to appreciate long-term patterns of insulin action in the individual animal.8

Factors contributing to apparent variability in time-action profile
1. Injection site (vasculature, temperature)
2. Injection technique
3. Dose inaccuracies (dependent on syringe type, insulin formulation, and operator proficiency)
4. Insulin absorption (dependent on the above but also inherent to each insulin formulation)
5. External factors: Meal composition and size, physical and emotional stress and activity level

Minimizing day-to-day-variability of insulin action
Consider use of synthetic formulations over traditional formulations. Synthetic analogs have more predictable time-action profiles because: 1. They are supplied as solutions (and not suspensions): increased accuracy in dosing. 2. They have more predictable absorption from SQ depot. 3. Detemir and degludec are buffered by albumin.

Synthetic analogs are U100 while traditional formulations that are used in veterinary medicine are supplied as U40. U40 syringes are generally more convenient and more accurate than U100 but at low doses they are not as precise.9 Injection pens increase precision and accuracy regardless of the insulin formulation being used.10

What do we know about the pharmacology of different insulin formulations in veterinary medicine?
The time-action profiles of different insulin formulations have been tested in cats and dogs mostly using glucose serial glucose monitoring in client-owned patients. On a population level, these are useful in estimating dose and frequency of administration in the naïve patient (Table 1). However, the inter-subject variability and day-to-day intra-subject variability have rarely been reported in veterinary medicine. In a study comparing inter-subject variability of lente, NPH and PZI in dogs it was concluded that “individual idiosyncracies in the absorption of SQ administered insulin of any form may be as important in determining the individual’s glucose response as the type of insulin that is used.” This was particularly true for PZI and NPH in that study.11 Interestingly, NPH showed lesser inter-subject variability than insulin glargine in a later study that used the is-glycemic clamp technique.12 The same group later reported on the inter-subject variability of insulin detemir in dogs using the same technique and although the value of this the comparison is very limited, it seems that insulin detemir had lesser inter-subject variability than NPH or glargine.13 In a clinical study on PZI in dogs, the day-to-day variability partially reported by comparing the time to minimum blood glucose concentrations between glucose curves of individual dogs.14 This study reported an unpredictable time to minimum blood glucose in dogs on PZI, with lowest blood glucose detected 54% of the time at either time zero or at 10h post insulin injection (in 10h long glucose curves).

Another problem with understanding time-action profiles in veterinary medicine is that most studies report means and do not report patient specific profiles which could result in under or overestimating some parameters. For example, an insulin formulation with a peak of action that vary greatly between individuals, when averaged would appear as “peakless”.11, 12, 15 This might become important when choosing insulin formulations in cats where a “peakless” insulin formulation might be advantageous in patients that tend to “graze” throughout the day (in contrast to consistent twice daily meals). Insulin glargine has been reported as “peakless” in people but in cats, using the iso-glycemic clamp method, the time-action profiles of insulin glargine varies between “peakless” to having a very pronounced peak.15
Table 1: Insulin formulations in cats and dogs: species source and type, syringe type, and typical dosing frequency.

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Species source/Type</th>
<th>Syringe</th>
<th>Dog</th>
<th>Cat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humulin N</td>
<td>Human/NPH U-100</td>
<td>q 8-12h</td>
<td>q 8h</td>
<td></td>
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<tr>
<td>Vetsulin</td>
<td>Porcine/Lente U-40</td>
<td>q 12-24h</td>
<td>q 8-12h</td>
<td></td>
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<tr>
<td>Prozinc</td>
<td>Human/PZI U-40</td>
<td>q 12-24h</td>
<td>q 12-24h</td>
<td></td>
</tr>
<tr>
<td>Lantus</td>
<td>Human recombinant /Glargine U-100</td>
<td>q 12h</td>
<td>q 12-24h</td>
<td></td>
</tr>
<tr>
<td>Levemir</td>
<td>Human recombinant /Detemir U-100</td>
<td>q 12-24h Starting dose 0.1U/kg</td>
<td>q 12-24h</td>
<td></td>
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</tbody>
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References
Diagnosing Addison’s Disease: New Ideas, New Challenges
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I. Highlights
A. Hypoadrenocorticism (HA, Addison’s disease): Two different syndromes:
   1. Glucocorticoid deficiency (“atypical” Addison’s, G-HA)
   2. Glucocorticoid + mineralocorticoid deficiency (“classic” Addison’s disease, GM-HA)
B. HA is an uncommon condition (1:200 to 1:2,000 dogs). The prevalence of Atypical Addison’s is unknown but it is underdiagnosed.
C. The diagnosis is straightforward if you maintain a high index of suspicion. Especially for Atypical Addison’s however, the overlap of clinical signs with other diseases makes it really easy to miss!!!
D. Key to diagnosis is maintaining a high index of suspicion (especially in dogs that do not have the classic electrolyte abnormalities)
E. A lack of a stress leukogram is neither sensitive nor specific for the diagnosis of HA
F. In theory, corticosteroid-induced ALP should not be increased in HA dogs. In practice, it is increased in about 20% of cases, making it an unreliable screening test.
G. Screening for HA is added by measuring basal cortisol: Basal cortisol should be low in HA and if it is not low, HA can be ruled out. A low cortisol, however, cannot replace the ACTH stimulation test in confirmation of HA.
H. Consider screening for HA in: chronic GI disease (including protein losing enteropathy), megaesophagus, non-specific illness, recurrent “renal failure” and many others…

II. Classification of hypoadrenocorticism
A. Primary hypoadrenocorticism (ACTH concentration high) (> 95% of cases)
   1. Mineralocorticoid- and glucocorticoid-dependent primary hypoadrenocorticism (Addison’s disease)
      a. Idiopathic destruction and collapse of adrenal cortex (Suspected to be immune-mediated based on lymphoplasmacytic infiltration of the adrenal glands): Most common form of primary hypoadrenocorticism
      b. Other causes of destruction: Drug-related (mitotane, trilostane), bilateral adrenalectomy, infectious (fungal) and neoplastic (lymphoma).
   2. “Atypical” or glucocorticoid-dependent primary hypoadrenocorticism
      a. Normal sodium and potassium on presentation
      b. Not presented for “Addisonian crisis”: Signs related to hypovolemic shock and hyperkalemia (bradycardia) are absent
      c. Usually more chronic with history of GI signs
      d. Most affected dogs remain only glucocorticoid-dependent for a prolonged period of time (months to years)
      e. Some affected dogs progress to glucocorticoid and mineralocorticoid dependent disease (i.e. classical primary hypoadrenocorticism) in the months after diagnosis of “atypical” Addison’s and consequently long-term monitoring including serum electrolytes is necessary.
      f. Difficult to diagnose because clinical signs are vague and serum electrolyte concentrations normal (see later)
      g. In old literature it was reported as the less common form of primary hypoadrenocorticism (5 to 25% of cases of primary hypoadrenocorticism) however it is recognized that this form is underdiagnosed (can easily be misdiagnosed as Inflammatory Bowel Disease or just be missed all together)
B. Secondary hypoadrenocorticism (ACTH concentration low) (< 5% of cases)
   1. Glucocorticoid deficiency only (serum electrolyte concentrations normal)
   2. Caused by congenital or acquired hypopituitarism (neoplasia, trauma, immune-mediated diseases, etc.)
   3. Because this form of the disease is rare and because the clinical signs, laboratory findings, and treatment are identical to “atypical” Addison’s, the 2 will mostly be discussed together.

III. Presentation
A. Duration of illness: few days to several months
B. Some have a history of a chronic disorder that follows a waxing and waning course (25-40% of cases)
C. Others present in crisis with acute collapse (10% of cases)
D. History of previous treatment with and favorable response to fluids and/or glucocorticoids (25-35% of cases) should increase your index of suspicion for hypoadrenocorticism
E. Chronic recurrent non-specific signs (lethargy, decreased appetite, weight loss, dehydration). GI signs are common.
   Vomiting is less common in G-HA than in GM-HA. Polyuria, polydipsia and bradycardic shock are a consequence of electrolyte disturbances (seen in GM-HA)

IV. Laboratory findings
A. Hemogram
   1. Anemia (25-35% of cases)
      a. Anemia of chronic disease and/or gastrointestinal blood loss
      b. Anemia may be masked by dehydration
2. Leukogram: Absolute eosinophilia (10-20% of cases) and absolute lymphocytosis (10-15% of cases) or a lack of a stress leukogram (i.e. normal lymphocyte and neutrophil counts) are all helpful clues BUT: A significant number of non-Addisonian sick dogs present without a stress-leukogram and a significant number of Addisonian dogs present WITH a stress leukogram. Thus, lack of a stress leukogram is neither sensitive or specific for diagnosis of HA.

B. Biochemistry
1. Mineralocorticoid deficiency leads to sodium wasting, potassium retention, and acidosis. These lead to severe volume depletion, dehydration and eventually decreased GFR (leading to increased urea, creatinine, phosphorus). Although chloride is lost in the urine with sodium, its loss is less significant than sodium’s, resulting in a hyperchloremic (relative) metabolic acidosis with normal anion gap. Decreased GFR and renal failure may lead to accumulation of organic acids and a shift towards high anion gap metabolic acidosis.

2. Hypercalcemia (total calcium) is due to hyperproteinemia caused by dehydration and hemoconcentration as well as decreased renal excretion of calcium (30% of cases). Increased ionized calcium is observed in about 20% of HA dog and the degree of elevation is inversely proportional to the decrease in blood pH.

3. Increased liver enzyme activity (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP]) occurs in 20-30% of cases possibly due to decreased hepatic perfusion. Corticosteroid-induced ALP is increased in 20% (although only mildly and probably representing the cross reactivity of the assay with total ALP).

4. Although glucocorticoids increase hepatic gluconeogenesis and decrease glucose uptake and utilization in peripheral tissues, hypoglycemia is only observed in 15-20% of dogs with classical hypoadrenocorticism.

5. Hypocholesterolemia is more common in those with glucocorticoid-dependent hypoadrenocorticism.

6. Serum proteins may be decreased (GI loss) or normal-increased due to dehydration. Hypoalbuminemia is found in about 45% of cases and is more common in those with glucocorticoid-dependent hypoadrenocorticism.

C. Urinalysis
1. In general, high urine specific gravity (USG) indicates normal renal concentrating capacity and typically rules out primary renal disease. However, low urine specific gravity often is found in dogs with hypoadrenocorticism (60% of cases have USG < 1.030) making primary renal disease a confusing differential diagnosis.

2. The low USG in dogs with hypoadrenocorticism results from renal medullary washout of solute (i.e., urea and NaCl are the primary solutes of the renal medullary interstitium responsible for maintaining normal renal concentrating capacity). The low USG in combination with pre-renal azotemia (secondary to volume depletion) may cause confusion with acute renal failure.

3. Renal function returns to normal after re-hydration and re-establishment of normal renal medullary solute concentrations.

D. Abdominal ultrasound examination:
1. In cases of idiopathic or immune-mediated destruction of the adrenal gland or secondary to ACTH deficiency, the adrenal glands are expected to be smaller than normal.

2. In rare cases of hypoadrenocorticism caused by infiltrative disease (lymphosarcoma, fungal infections) the adrenal gland will appear normal or enlarged on ultrasound but show decreased response to ACTH.

3. Reference intervals for adrenal gland size vary based on dog size and body weight but these are not routinely used. When evaluating the sensitivity and specificity of adrenal gland caudal pole thickness for diagnosis of HA, groups of varying body sizes were used and therefore the degree of overlap between diseased and controls was probably greater than it would have been if dogs were grouped into body weight categories.

   a. Left: <2.8 mm is 90% sensitive, 100% specific
   b. Right <3.05 mm is 82% sensitive and 90% specific
   c. Sensitivity and specificity might be improved if using body-size appropriate reference intervals

5. Although the accuracy of ultrasound in diagnosis of HA is limited, it may be helpful in cases of chronic GI disease to increase suspicion and justify further testing for HA.

E. Screening test: Some advocate use of resting plasma cortisol concentration to “rule out” a diagnosis of hypoadrenocorticism
1. Lennon et al. JAVMA 2007:
   a. A resting cortisol concentration of ≤ 1 μg/dL had a sensitivity of 100% and a specificity of 98%
   b. A resting cortisol concentration of ≤ 2 μg/dL had a sensitivity of 100% and a specificity of 78%

2. These 1 and 2 μg/dL cutoffs will vary to some degree between laboratories.

3. Remember “sPin” and “sNout” – “a specific test, if positive, rules a disease in” whereas “a sensitive test, if negative, rules a disease out”
   a. It is not unusual for sick dogs (with non-adrenal illness) to have an occasional low resting cortisol (about 22% of the time…) but these dogs will respond to ACTH adequately (post ACTH cortisol > 5 ug/dL).
   b. You may want to use a resting plasma cortisol concentration to “rule out” hypoadrenocorticism but you should NOT use this test to try and “rule in” hypoadrenocorticism – you must rely on the ACTH stimulation test for that purpose (i.e. the “gold” standard to “rule in” hypoadrenocorticism)
   c. Assuming a disease prevalence of 0.5% (1:200), the positive predictive value (i.e. percentage of those patients with a positive test result that indeed have the disease) of a resting plasma cortisol is approximately 2% whereas its negative predictive value (i.e. the percentage of those with a negative test result that do not have the disease) is 100%. Thus, you can see that this test must only be used to “rule out” hypoadrenocorticism.

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d. Consider measurement of basal cortisol in the following instances (and many more – these are just a few examples):
   i. Acute Renal Failure, especially if sodium is low
   ii. Increased liver enzymes with “synthetic liver failure” (decreased albumin, cholesterol and glucose) but normal bilirubin, especially if the lymphocytes are not low and before performing biopsies.
   iii. Megaesophagus: Addison’s is not a common cause but one of the only treatable ones
   iv. Worked up cases suspected of IBD/lymphangiectasia or chronic GI signs with hypoglycemia, (especially if the lymphocytes are not low), before performing biopsies

F. Confirmatory test: ACTH stimulation is the gold standard test and must be used to “rule in” a diagnosis of hypoadrenocorticism

1. ACTH stimulation test (the “gold standard” for diagnosis)
   a. Method
      i. Plasma sample for resting cortisol concentration is not needed for the interpretation and should definitely not be repeated if a basal cortisol was already measured before and was low.
      ii. Administer 5 μg/kg (or more) synthetic ACTH (Cortrosyn) intravenously
      iii. After 1 hour, collect plasma sample for post-ACTH cortisol concentration
      iv. Normal reference range in dogs (varies with laboratory)
         (A) Resting cortisol concentration: 1.0-5.0 μg/dL
         (B) 1-hour post-ACTH cortisol concentration: 5.0-17.0 μg/dL
      v. The test is positive for hypoadrenocorticism: no response to ACTH (post-ACTH cortisol concentrations < 2.0 μg/dL in nearly 100% of cases)
      vi. In cases of hypoadrenocorticism due to chronic exogenous glucocorticoid drug administration (iatrogenic): low to normal resting cortisol concentration (≤ 5.0 μg/dL) with subnormal response to ACTH (≤ 5.0 μg/dL)
      vii. Perform before administering glucocorticoids that may interfere with test results.
         (A) Assay cross reactivity: Most glucocorticoid cross react in the cortisol assay. Dexamethasone does not and can be given if necessary
         (B) Biological interference over time: Dexamethasone, like other glucocorticoids, will suppress ACTH secretion, leading to atrophy of the adrenal gland and decreased response in an ACTH stimulation test. If suspecting primary hypoadrenocorticism in a dog that is already receiving glucocorticoids, a long (usually weeks, depending on the drug) wash-out period is needed before and ACTH stimulation can be done. In a dog that was previously receiving glucocorticoids (that have been discontinued long enough before the testing and are not cross reacting in the assay), normal response to ACTH (high cortisol concentration) rules out hypoadrenocorticism but a suppressed response should be interpreted cautiously.

b. Endogenous ACTH
   i. Why measure eACTH: In atypical disease, differentiate between primary and secondary
      (A) Progression to mineralocorticoid deficiency has been reported with Atypical Addison’s: Primary atypical HA requires periodic testing of electrolytes for prevention of an Addisonian crisis (life threatening)
      (B) Secondary HA does not require further monitoring of electrolytes: Big money saver!
   ii. ACTH is high in primary hypoadrenocorticism
   iii. ACTH is normal to low (often undetectable) in secondary hypoadrenocorticism
   iv. Normal reference range in dogs (varies with laboratory): 6.7 – 25 pmol/L
   v. ACTH is fragile and sample must be handled and shipped appropriately (avoid contact with glass during collection, separation and storage; store and ship frozen). Sending a sample from a normal dog as a control will facilitate interpretation of results (if both return low, problems likely occurred in sample handling)
A. Diagnosis of hyperthyroidism in cats
   1. Signalment: No sex predisposition, older cats (< 5% are < 8 years of age at diagnosis)
   2. History: Weight loss (~90% of hyperthyroid cats) often concurrently with polyphagia (60% of hyperthyroid cats), polydipsia and polyuria (45-50% of hyperthyroid cats), increased activity or restlessness (40% of hyperthyroid cats), gastrointestinal signs (vomiting, diarrhea), heat intolerance (5% of hyperthyroid cats)
   3. Physical findings: Palpable enlargement of thyroid gland (91% of hyperthyroid cats), thin (only 71% of hyperthyroid cats!), cardiovascular abnormalities (Tachycardia, systolic murmur, gallop rhythm), unkempt coat, hyperactivity, easily stressed, mild hyperthermia (14% of hyperthyroid cats), systemic hypertension
   4. Laboratory findings
      a. Hemogram (aka complete blood count or CBC): Erythrocytosis (40-50% of cases), increased MCV (20-25% of cases), stress leukogram (but eosinophilia and lymphocytosis have also been reported)
      b. Biochemical profile: Mild to moderate increase in liver enzyme (ALT, ALP, AST) activities (60-90% of cases). Increased BUN and phosphorus (10-30% of cases)
      c. Urinalysis: Wide variability in urine specific gravity (USG): Isosthenuria doesn’t indicate renal failure necessarily, even if the BUN is increased.
      d. Tests of thyroid function:
         i. Serum total T4 concentration (TT4):
            (A) Increased in > 90% of hyperthyroid cats.
            (B) TT4 is normal in ≤ 10% of hyperthyroid cats:
               1. In early disease, daily fluctuations in T4 can result in an isolated normal result. Often, repeating the test on another day (or week) is useful. Alternatively, you can measure free T4 (see below).
               2. Non-thyroidal illness (especially chronic renal disease) can result in a normal serum total T4 concentration in a hyperthyroid cat.
         ii. Serum free T4 (fT4) concentration by equilibrium dialysis: More sensitive but less specific than total T4 (fT4 is sometimes increased in diabetes, GI disease, and other conditions)

V. Hyperthyroidism and the kidneys
   A. Hyperthyroidism and renal disease are both common in older cats.
   B. Hyperthyroidism leads to increased cardiac output and increased renal perfusion resulting in a relative increase in GFR.
      1. Increased GFR leads to a decrease in creatinine (potentially masking an underlying renal disease).
      2. Increased renal perfusion leads to increased glomerular capillary pressure \( \rightarrow \) proteinuria \( \rightarrow \) progression of renal disease.
   C. About 30% of cats will develop azotemia within 30 days after treatment of hyperthyroidism, but the azotemia tends to remain stable in the majority of cats.
   D. In hyperthyroid cats, prior to treatment, there is no practical way to predict accurately:
      1. In which cat kidney disease will be unmasked by treatment
      2. In which cat kidney disease will develop after treatment
   E. A combination of GFR, crea, T4, and USG might be somewhat predictive but cannot be used conclusively to predict response to treatment
   F. Medical therapy is recommended in cats with pre-existing kidney disease because of the risk of worsening azotemia.
   G. A Methimazole trial is the most reliable way to assess kidney function in euthyroidism
      1. Confirm euthyroidism for a minimum of 4 weeks
   H. There is some evidence that hypothyroidism might cause a decrease in GFR.
      1. Any treatment of hyperthyroidism might result in hypothyroidism (but severity and reversibility differ)
   I. Definitive treatment of hyperthyroidism in a cat with CKD might be detrimental, beneficial or neither…

VI. Treatment of hyperthyroid cats:
   A. Medical, surgical, dietary and I131 therapy can all be effective. Side-by-side comparison of these various treatment modalities have not been performed in cats. Based on mostly retrospective, often uncontrolled studies, as a whole, I131 seems to be associated with the longest survival time. Usually, the choice between these treatments is made based on convenience, cost, and side effects.
   B. With all treatment modalities, effective reduction of thyroxine concentration could lead to significant reduction in GFR. The magnitude of reduction in GFR is depends on how effective treatment is (as well as on intrinsic factors).
   C. In cats with kidney disease.
   D. Oral methimazole: Predictable response, quickly reversible
      1. significant side effects, and not uncommon:
a. Anorexia, vomiting, lethargy (10-15%)
b. Cutaneous excoriations (2-3%), often peri-auricular
c. Hematologic changes (3-9%): ↑eos, ↑lymphs, ↓neuts, bleeding tendencies
d. Liver toxicity (↑ liver enzymes, rarely ↑Tbil)

E. Topical methimazole: Inconsistent absorption? Owners must wear gloves. quickly reversible
1. Side effects: Similar to oral methimazole except for reduced frequency of anorexia vomiting

F. Thyroidectomy: Predictable response, irreversible (except with oral supplementation of thyroxine). Need to verify that no ectopic tissues are present prior to surgery. High cost up front.
1. Risks:
   a. Anesthesia (should ideally stabilize first with methimazole)
   b. Hypocalcemia (potentially prolonged hospitalization)
   c. Neurological damage
   d. Vagosympatheic trunk -> Horner’s syndrome
   e. Recurrent laryngeal -> change in meow/purr
   f. Recurrence in contralateral lobe (with unilateral)
   g. Hypothyroidism (with bilateral)

1. 95% of cats are euthyroid within 3 months.
2. Serum TT4 is low in 16% of cats after treatment but only 2% develop clinical hypothyroidism.
3. <2% of cats fail to respond by 6m and require second treatment
4. 2.5% of cats relapse within 1-6 years
5. High cost upfront
6. Isolation of a potentially unstable patient (should ideally stabilize first with methimazole)

H. Iodine deficient diets (Hill’s a/d): Significant reduction in T4 might take weeks. Quickly reversible (by feeding any non-iodine deficient diet). Palatability is sometimes an issue. Efficacy seems lower than other treatment modalities (inconsistent feeding?). Long-term survival studies of treated hyperthyroid cats are not available.
Diagnosing Hypercortisolism in Dogs
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VII. Etiology of HC (general term: Cushing’s syndrome):
   o Most common: Pituitary adenoma (AKA pituitary-dependent HC or PDH = Cushing’s disease) secreting excess ACTH which leads to bilateral adrenocortical hyperplasia and excessive production of cortisol (80-85% cases of naturally occurring HC)
   o Adrenocortical neoplasia (15-20% of naturally occurring HC cases), AKA adrenal-dependent HC or ADH: May be benign (adenoma) or malignant (carcinoma)
     ▪ Benign and malignant tumors are not easily distinguished clinically, but:
       - Carcinomas tend to be bigger
       - Carcinomas tend to invade adjacent structures (e.g. caudal vena cava)
     ▪ Tumors usually are unilateral and, although presumably atrophied, the contralateral gland typically does not always appear smaller on abdominal ultrasound examination
   o Iatrogenic: Long-term administration of (or exposure to) glucocorticoids
   o Food-dependent HC (ACTH-independent):
     ▪ Rare
     ▪ Likely the result of aberrant expression of receptors to GI hormones on cortisol-secreting cells

VIII. Signalment
   o A disease of middle-aged and older dogs: Almost all are > 6 years of age; rarely reported in young dogs
   o No strong sex predilection
   o Can occur in any breed or in mixed breed dogs
     ▪ Commonly affected breeds include Poodle, Dachshund, Boston terrier, Boxer, and German shepherd
     ▪ Of all dogs with PDH, 75% are <20 kgs. Of all dogs with ADH, “only” 50% are < 20kgs. In small dogs PDH is about 10 times more likely than ADH. In big dogs, PDH is “only” about 4 times more likely than ADH. This difference is the root of the misconception that ADH is more common in large breed dogs (it is more common than in small breeds but it is not more common than PDH regardless of the breed).

IX. History and Physical Findings
   o Generally considered “healthy” (“just getting older”) by their owners
   o Clinical signs are due to the gluconeogenic, lipolytic, protein catabolic, anti-inflammatory, and immunosuppressive effects of excess glucocorticoids
   o Polyuria and polydipsia (> 80% of cases)
   o Polyphagia (> 60% of cases)
   o Abdominal enlargement due to weak abdominal muscles and hepatomegaly (> 70% of cases)
   o Decreased exercise tolerance
   o Muscle weakness
   o Lethargy
   o Hepatomegaly (hepatocyte vacuolation due to glycogen accumulation)
   o Panting
   o Cutaneous problems (almost all cases have some dermatologic signs, BUT… it is rare to have just dermatologic signs without at least Pu/Pd or PP)
     ▪ Bilaterally symmetrical truncal alopecia
     ▪ Thin, dry, scaling skin
     ▪ Hyperpigmentation
     ▪ Easy bruising (e.g. after venipuncture)
     ▪ Comedones (especially around teats)
     ▪ Calciosclerosis cutis (calcium deposition in the dermis; uncommon but very suggestive of hyperadrenocorticism)
     ▪ Pyoderma (increased susceptibility to infection)
   o Hypertension (> 50% of cases)
   o Increased susceptibility to infections
   o Poor wound healing
   o Pulmonary thromboembolism (see complications)

Some clinical signs are very inconsistent with HC and should prompt you to think of other differentials for the above problems (or at least prompt you to delay testing for HC until they resolve): Decreased appetite, vomiting, diarrhea, sneezing, coughing, pruritus, icterus, pain, seizures, bleeding.

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X. Laboratory findings
   o Hemogram (“stress” leukogram)
     - Lymphopenia (cortisol causes lymphocytolysis) and eosinopenia (cortisol causes increased sequestration of eosinophils in the bone marrow)
     - Leukocytosis due to neutrophilia and monocytes (cortisol causes capillary demargination of these cell types)
     - Lymphopenia and eosinopenia are observed most consistently (~80% of cases)
     - Mild to moderate erythrocytosis (increased PCV) occasionally
     - Thrombocytosis (cause unknown)
   o Biochemistry
     - Mild hyperglycemia - ~35% of cases. Severe hyperglycemia, glycosuria and clinical signs of DM are unusual (<10% of cases).
     - Hyperlipidemia (high cholesterol and triglycerides) (~75% of cases)
     - Mildly to moderately increased alanine aminotransferase (ALT) (~50% of cases)
     - High bile acids - Indicating liver dysfunction
       - HC is associated with gall bladder mucocoeles in dogs
     - Increased alkaline phosphatase (ALP) including total ALP and corticosteroid-induced isoenzyme of cALP (cALP is unique to the dog).
       - Approximately 90% of cases have increased ALP (and in 10% ALP is normal!)
       - cALP is very sensitive but not specific: normal cALP tends to rule out hyperadrenocorticism but a high value does not rule it in
       - No correlation between magnitude of increase in ALP and…
         o The likelihood of having HC
         o The severity of HC
   o Urinalysis
     - Low urine specific gravity (1.001-1.020 in approximately 80% of cases)
     - UTI is common (approximately 50% of cases) but pyuria (i.e. white cells in the urine sediment) is not (~20% of cases) because increased concentrations of glucocorticoids impair migration of white blood cells into urine. The anti-inflammatory effects of cortisol also may explain why few of these dogs have clinical signs of cystitis.
     - Avoid catheterization for urine collection because of the increased risk of infection; use cystocentesis instead
     - Proteinuria (UPC > 1.0 in 46% of HC)
   o Serum total thyroxine (T4) and free thyroxine (fT4) concentrations
     - Often decreased (~50% of cases) due to decreased thyroid-binding globulin, increased metabolism of thyroid hormones and decreased peripheral conversion of T4 to T3. Glucocorticoids also suppress TSH secretion and cause secondary hypothyroidism.
     - Don’t mistake for primary hypothyroidism!!!
     - Treatment with T4 is not indicated!!!
   o Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion also are inhibited by cortisol and can contribute to failure to cycle in females or testicular atrophy in males
   o Abdominal ultrasound examination
     - Useful mostly for differentiation of PDH (symmetric) vs. ADH (asymmetry)
     - Adrenal gland size is correlated to body weight: Most of the variation is in the length of the adrenal gland, less so in the width/thickness (or diameter, terminology varies between sources)
     - Normal adrenal gland thickness varies less and a cutoff of 0.75 mm was used until recently to differentiate normal from enlarged
     - Using this cutoff, especially for small dogs, adrenomegaly would only be present in about 80% of HC dogs
     - Recent data suggest different cutoffs based on breed/size might be useful in refining the accuracy of adrenal width measurement in the diagnosis of HC but more studies are required to assess the validity of these new cutoffs.
     - Hyperplastic nodules, non-functional tumors, and non-cortisol secreting tumors can all cause adrenomegaly
     - Could non-adrenal illness cause enlarged adrenal glands?
       - Yes: In cats with hypersomatotropism
       - Other diseases???
     - Alone or combined with the endogenous ACTH assay, identification of adrenal glands on abdominal ultrasound examination can be used to successfully discriminate between PDH, ADH and iatrogenic causes of HC
       - Bilateral symmetrical enlargement (i.e., adrenal glands retain their normal “peanut” shape) with normal echogenicity in a dog with confirmed HC suggests PDH
       - Non-symmetrical abnormalities (Unilateral enlargement, distortion of shape, invasion into adjacent structures, and a small contralateral gland) in a dog with confirmed HC suggests ADH
       - In a dog with signs of HC, small adrenal glands are suggestive of iatrogenic HC
     - Carcinomas usually are bigger than adenomas and may invade local structures (e.g. caudal vena cava)
   o Screening tests
     - Used to decide if an animal has hypercortisolism or not (i.e., answers the question, “Cushing’s or not?”)
Resting cortisol concentrations are not valuable because many dogs with HC have normal cortisol concentrations at any given moment due to the episodic secretion of ACTH, and dogs with stress due to non-adrenal illness can have higher than normal resting cortisol concentrations.

The first and most important screening test is taking a thorough history and doing a thorough physical exam!!!

From the American College of Veterinary Internal Medicine (2012) consensus statement on diagnosis of HC:
- Avoid testing if other serious illness is present – any serious illness will cause false positive results for HC
- Postponing testing for HC is recommended until concurrent illness is resolved or at least well controlled
- Reference ranges for cortisol-related tests are outdated and all cutoff values should be interpreted cautiously (Below, cutoff values are approximate for normal, positive and negative results).

From the American College of Veterinary Internal Medicine (2012) consensus statement on diagnosis of HC: “The LDDST is the screening test of choice unless iatrogenic HAC is suspected”

- Low dose dexamethasone suppression test (LDDST)
  - Best used as a screening test but sometimes can provide discriminatory information (see below)
  - Obtain resting plasma cortisol concentration
  - Give 0.01 mg/kg dexamethasone IV
  - Obtain 4- and 8-hour post-dexamethasone plasma cortisol concentrations
  - The 8 hour sample is used for diagnosis of HC. The zero and 4 hour samples are only useful as part of the discriminatory test (see below)
  - In normal dogs, plasma cortisol concentration is suppressed to \( \leq 1.0 \mu g/dL \) at 4 and 8 hours post-dexamethasone. Failure to suppress at the 8 hour time point, (i.e. 8 hour cortisol > 1.0 \( \mu g/dL \)) suggests hypercortisolism but does not differentiate between etiologies (PDH, ADH, etc.)
  - Using 8 hour cortisol concentrations as a screening test (at a cutoff of 1 \( \mu g/dL \)) the LDDST is very sensitive (approximately 95%) but not very specific (approximately 70%).

For use of LDDST as a discriminatory test: See below.

- ACTH stimulation test
  - Old protocol: Obtain resting plasma cortisol concentration, give 0.25 mg synthetic ACTH IV or IM, obtain a 60-minute post-ACTH plasma cortisol concentration
  - Normal resting cortisol concentration: 1-5 \( \mu g/dL \) but fluctuates considerably, especially in ill animals and has no diagnostic value.
  - Up-to-date protocol: No need to obtain a resting plasma cortisol concentration!
    - Give 5 \( \mu g/kg \) cosyntropin (synthetic ACTH) IV or IM
    - 5 \( \mu g/kg \) is sufficient; higher doses increase the cost unnecessarily. Cosyntropin is expensive. It is supplied as a 250 \( \mu g \) vial. To decrease cost, divide the vial into 5 aliquots of 50 \( \mu g \) and use as many aliquots as needed to reach a minimum dose of 5 \( \mu g/kg \)
  - Obtain a 60-minute post-ACTH plasma cortisol concentration
  - normal post-ACTH cortisol concentration: 8-20 \( \mu g/dL \) (reference range will vary among laboratories)
  - Interpretation
    - An exaggerated response to ACTH (i.e. post-ACTH cortisol > 20 \( \mu g/dL \)) is consistent with PDH or ADH but also can be observed in animals with non-adrenal illness
    - A post-ACTH cortisol below the normal range (<5 \( \mu g/dL \)) is the result of atrophied/suppressed adrenals caused by iatrogenic HC, treated HC or Addison’s disease (Usually <2 \( \mu g/dL \) in Addison’s)
    - For diagnosis of HC: The ACTH stimulation is a reasonably specific test (approximately 90%) but NOT a sensitive one (60-95%). The sensitivity is reasonably high (95%) when testing PDH but abysmally low (60%) when testing ADH. Normally though, the screening test is done BEFORE it is clear if the dog has PDH or ADH…
      - Because of its low sensitivity: If negative, cannot rule out HC (especially if ADH is suspected)
    - Chronic stress of non-adrenal illness can cause abnormal results on ACTH stimulation (but not as much as it would in the LDDST and UCCR)
    - Acute stress has no effect on the test (the test mimics maximal acute stress – overdose of ACTH)
    - Advantage of ACTH stimulation test (over LDDST): Takes less time than LDDST and is less affected by non-adrenal illness
    - The ACTH stimulation test is the only diagnostic test available to identify dogs with iatrogenic hypercortisolism
    - The ACTH stimulation test is the test of choice to monitor dogs being treated for hypercortisolism with mitotane or trilostane (see later)
    - 17-hydroxyprogesterone (17-OH Prog) measurement: Can be measured before and after ACTH stimulation.
      - Used for diagnosis of “atypical Cushing’s syndrome”, i.e. Cushing’s like signs that are caused by an excess in sex hormones

Sex-hormone secreting adrenal tumors causing signs of Cushing’s have been described rarely but increased sex hormones concentrations (including 17-OH Prog) are frequently found in “regular” HC. Overall, measuring 17OH Prog is less sensitive and less specific than measuring post-ACTH cortisol (“regular ACTH stim”)

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Urine cortisol/creatinine ratio

- In this test, urine cortisol (and some of its degradation products) and urine creatinine are measured on the same urine sample. Dividing the cortisol concentration by the creatinine concentration corrects for the effect of concentrated or dilute urine on the urine cortisol concentration.
- The main advantage of this test is its simplicity: the owner need only bring in a morning urine sample from the dog.
- The main disadvantage is that, although the test is very sensitive (> 90%), it has very low specificity (20-40%) and is easily affected by stress (of any kind).
- Thus, it is useful to rule out a diagnosis of hyperadrenocorticism, but it is not helpful in ruling in the diagnosis. Remember sPin and sNout: a specific test, if positive, rules the diagnosis in; a sensitive test, if negative, rules the diagnosis out.
- Clinicians sometimes use this test if they don’t think hypercortisolism is very likely and want to remove it from their differential diagnosis list. However, if the test comes back positive, the diagnosis must be pursued by other screening tests (e.g., ACTH stimulation test, LDDST).
- The urine cortisol/creatinine ratio is affected by non-adrenal illnesses more so than the LDDST and the ACTH stimulation test.

Discriminatory tests: used to differentiate PDH from ADH

- Abdominal ultrasound: Identification of adrenal glands on abdominal ultrasound examination can be used to successfully discriminate between PDH, ADH and iatrogenic causes of HC.
  - Bilateral symmetrical enlargement (i.e., adrenal glands retain their normal “peanut” shape) with normal echogenicity in a dog with confirmed HC suggests PDH.
  - Non-symmetrical abnormalities (Unilateral enlargement, distortion of shape, invasion into adjacent structures, and a small contralateral gland) in a dog with confirmed HC suggests ADH.

In one study of dogs with inconclusive asymmetry between glands, adrenal width < 5 mm on the contralateral gland was highly sensitive and specific for the diagnosis of ADH. (Benchekroun et al. JVIM 2010)

- In a dog with signs of HC, small adrenal glands are suggestive of iatrogenic HC (combine with ACTH stimulation).

Dexamethasone suppression test (DST)

- Low dose [LDDST]: 0.01 mg/kg (used primarily as a screening test).
- High dose [HDDST]: 0.1 mg/kg (used primarily as a discriminatory test).
- The discriminatory aspect of the test is based on the principle that a dose of dexamethasone would sometimes suppress cortisol concentrations in dogs with PDH but never in dogs with ADH.
- Thus, if dog with suspected hypercortisolism suppresses on the DST that result is consistent with PDH, but if the dog fails to suppress you cannot draw any conclusions and must use other discriminatory tests.

Four criteria for suppression:
- 50% decrease in [cortisol] (from time 0) at 4h (LDDST, HDDST).
- 50% decrease in [cortisol] (from time 0) at 8h (LDDST, HDDST).
- [Cortisol] < cutoff value (1.0 ug/ml?) at 4h (LDDST, HDDST).
- [Cortisol] < cutoff value (1.0 ug/ml?) at 8h (HDDST only).
- These 4 criteria apply to HDDST. In the LDDST, only criteria 1-3 apply for differentiation of PDH from ADH. The 8h sample in the LDDST is used for diagnosis (see above) and by definition, a cortisol concentration below the cutoff at that time point rules out HC and therefore cannot be used to differentiate PDH from ADH.
- Using these criteria, dogs with PDH will have suppressed cortisol 63% on LDDST and 75% on HDDST. Hypothetically, higher doses of dexamethasone will lead to suppression of more pituitary tumors (thus increasing the sensitivity of the test), however, it is not practical to repeat the test with higher doses (diminishing return…).

Endogenous ACTH concentrations

- Normal: 6.7-25 pmol/L.
- eACTH is completely suppressed in ADH but is measurable (and frequently high) in PDH.
- Adrenocortical tumor (ADH): < 5 pmol/L (assay detection limit).
- Pituitary-dependent hypercortisolism (PDH): > 6 - 1250 pmol/L.
- ACTH is fragile and sample must be handled and shipped appropriately (avoid contact with glass during collection, separation and storage; store and ship frozen). Sending a sample from a normal dog as a control will facilitate interpretation of results (if both return low, problems likely occurred in sample handling).
- Out-dated assays had low sensitivity and were frequently unable to detect ACTH in cases of PDH, misclassifying PDA as ADH.
- A recent study (using a new ACTH assay) reported that for the diagnosis of ADH, the eACTH has a sensitivity of 85-100% and a specificity of 97-100% (Rodríguez Piñeiro et al. JVIM 2009).
- Performed correctly, both ultrasound and eACTH are highly accurate as discriminatory tests however:
  - eACTH is not widely available.
  - eACTH is VERY fragile (sample must be collected, handled, and shipped very carefully, constantly frozen).
- Abdominal U/S is more expensive
- Abdominal U/S provides additional information on other organs and on the presence of metastasis and local invasion of adrenal tumors and

- Other imaging modalities (e.g. magnetic resonance imaging [MRI])
  - Normal pituitary gland height is approximately 5 mm
  - Approximately 50% of dogs with untreated PDH and no neurologic signs have normal MRI findings and 50% have identifiable pituitary tumors (4-13 mm in height)
  - Neurologic signs typically are associated with tumors > 10 mm (so-called “macrotumors”) that expand dorsally beyond the sella turcica
  - Abdominal CT has no advantage over ultrasound for differentiation of PDH from ADH.
Overview of calcium homeostasis

Parathyroid hormone (PTH) is the principal hormone involved in the minute-to-minute fine regulation of blood calcium concentration through effects on tubular reabsorption of calcium, intestinal absorption of calcium mediated indirectly via calcitriol, and bone resorption of calcium. PTH is secreted by chief cells in the parathyroid glands. **Overall, PTH increases plasma calcium concentrations. The parathyroid glands are exquisitely sensitive to fluctuations in ionized calcium (iCa),** especially when iCa is low. If the parathyroid glands are responding appropriately, PTH secretion will dramatically increase when iCa is low; when iCa is high, PTH secretion will decrease. Parathyroid hormone has direct effects in the kidneys and bones and indirect effects in the intestines:

- In bone, PTH induces osteoclastic bone resorption, which increases calcium and phosphorus resorption.
- In the kidney, PTH increases calcium reabsorption and phosphorus excretion. It also increases the activity of 1α-hydroxylase, the enzyme responsible for converting 25-hydroxyvitamin D (25OHD) to the active form of vitamin D: Calcitriol (1,25-dihydroxyvitamin D3).
- Calcitriol increases calcium and phosphorus absorption in the gastrointestinal tract.

Tissues involved in calcium regulation (parathyroid gland, thyroid gland, kidney) express a Ca²⁺-sensing receptor (CaSR). In the parathyroid gland, the CaSR insures that the increase in secretion of PTH is proportional to the decrease in plasma iCa in the physiologic range of iCa concentrations. In that range, a small change in iCa causes large changes in PTH concentrations. However, outside of that normal range the response of the parathyroid gland to changes in iCa is remarkably different: Above the normal iCa concentrations, PTH secretion is quickly and mostly inhibited. Below the normal iCa concentrations, PTH secretion quickly reaches maximum:

The set-point for PTH secretion is defined as the concentration of circulating calcium that results in half the maximal PTH secretion that can be achieved. In some diseases, abnormalities in iCa concentrations are caused by shift in this set point. For example, in people with Familial Benign Hypocalcuric Hypercalcemia (FBHH), a genetic defect in the CaSR causes decreased sensitivity to iCa so that the set point is increased: PTH secretion is “normal” at abnormally high concentrations of iCa. This genetic disturbance may be a good model for Feline IHC.

**Figure 1. Measurement and interpretation of PTH concentrations**

**Differential diagnoses for hypercalcemia in cats**

H = Hyperparathyroidism (primary)
A = Addison’s disease
R = Renal disease (acute or chronic kidney disease)
D = vitamin D toxicity
I = Idiopathic
O = Osteolysis
N = Neoplasia or Nutritional
S = Spurious or Systemic granulomatous disease

From the most common to least common: Idiopathic, renal disease, neoplasia… all the rest…

**Diagnostic tools for calcium-related disorders: Major challenges**

PTH is released from the PTGs as an 84 amino acid single-chain peptide. This intact active form of the hormone (PTH1–84) is inactivated by hepatic and renal metabolism (plasma half-life approximately 2–4 mins). The 1–34 N-terminal region is essential for the biological activity of PTH and cleavage at that site by endoproteases renders the hormone inactive. In this process, fragments of various lengths are produced and released into the blood. These fragments are then cleared from the circulation by renal excretion. The analysis of ‘true’ PTH1–84 is not straightforward – there exists a surprisingly heterogeneous range of PTH fragments. Both the exact composition and possible biological functions of PTH fragments remain to be fully elucidated as does the variable influence of these
fragments on currently available analytical methods for PTH testing. Accumulation of PTH fragments in patients with renal failure for example can be so pronounced that the intact 1-84 hormone accounts for only 5-20% of the measured PTH depending on calcemia status and stage of renal disease.

Second-generation (or ‘intact’), and third-generation (or ‘whole’) two-site PTH immunoassays use 2 antibodies: one directed at the C-terminal region (amino acids 39-84) and one directed at the N-terminal region of the intact 1-84 PTH molecule. The use of a two-site assay eliminates detection of inactive PTH partially (in ‘intact’ 2nd generation assays) or entirely (in ‘whole’ 3rd generation assays). Third generation assays are directed against the well-conserved region of amino acids 1-4. This is also a region that is necessary for activation of the PTH receptor, making these assays specific to the active PTH molecule. In contrast, second generation assays detect amino acids 12-32. With these second generation (‘intact’) assays the specificity to the active PTH is incomplete. Another important issue in cats is that commercial PTH assays use antibodies raised against human PTH. Feline PTH however differs in amino acids 16, 18 and 26 which are detected by second-generation assays. Thus, the affinity of these human PTH assays to feline PTH is decreased, rendering these assays insensitive. To date, most studies in which PTH was measured in cats used second generation human PTH assays with sensitivities so low that the “reference range” of PTH concentrations overlapped with the low limit of detection of the assay. This reduces the ability of these assays to detect PTH concentrations that have been suppressed below normal by hypercalcemia. This is crucial in conditions in which a low PTH concentration is the key finding for diagnosis (e.g., differentiating primary hyperparathyroidism from idiopathic hypercalcemia).

Feline Idiopathic Hypercalcemia (IHC)
An “emerging” disease, first described in a retrospective study by Midkiff et al (JVIM 2000). Defined as persistent hypercalcemia in a cat in which no diagnosis (from the list of differentials above) can be made.

- Mostly middle age to older cats but has been reported in cats as early as 2 years of age.
- Male ≈ female, various breeds
- Unclear relationship to commercial diets
- Mild-to-moderate hypercalcemia (total and ionized calcium)
- Hypercalcemia may be detected incidentally: about ~50% of cats present with no clinical signs
- Reported clinical signs might be incidental. In a recent retrospective study comparing cats with IHC to a control population of cats referred to a teaching hospital for a variety of conditions, no clinical sign emerged as more likely to occur in IHC. In that study, IHC cats were more likely to present with lethargy and hypercalcemic cats in general were less likely than controls to present with signs of lower urinary tract disease.
- Previously reported association between urolithiasis and IHC was based on anecdotal evidence. CaOx are frequently found in cats with IHC but their frequency in this disease is not higher than the frequency in a control population.
- Gastrointestinal signs (including vomiting, weight loss, decreased appetite, constipation) have been reported with IHC but they do not occur more frequently in IHC than in a control population.
- IHC is likely an umbrella diagnosis: The lack of obvious association between certain clinical signs and other potential disease consequences (as explained above) may be representative for IHC as a group but not necessarily for an individual cat.
- To date, there is no evidence that treatment of idiopathic hypercalcemia has any benefits in cats.
- In people with Familial Benign Hypocalciuric Hypercalcemia (a disease that resembles feline IHC in many ways), treatment of the hypercalcemia is generally not indicated. In FBHH, mutations in the CaSR cause decreased sensitivity of the target tissue to the effect of calcium. In the parathyroid gland this leads to the “perception” that iCa is not as high as it “should be” which leads to increased secretion of PTH. Similarly, the renal tubules do not sense calcium normally in this disease, leading to increased calcium reabsorption, increased plasma calcium concentration and decreased urinary calcium excretion.
Insulin Resistance in Cats
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Insulin resistance
• In the treated diabetic patient in practice, insulin resistance is defined by response to treatment (i.e. high insulin dose required to achieve glycemic control):
  i. >3U/inj/Cat
  ii. >1.5 – 2.0 U/kg/inj in dogs
  iii. In the non-diabetic patient: Insulin/glucose ratio
  iv. In the research settings: hyperinsulinemic-euglycemic clamps
• Pathophysiology of insulin resistance:
  1. Circulating insulin antagonists (related to stress, inflammation, obesity)
     • ↑ Counterregulatory hormones (e.g. glucagon, cortisol, GH)
     • ↑ Cytokines (TNF-α, IL-6)
     • Insulin antibodies
  2. Target tissue defects (obesity, congenital, others)
     • Insulin-receptor defects
     • Post-receptor defects
• Common causes of insulin resistance:
  a. Bacterial infections (periodontal disease, UTI)
  b. Major organ failure (heart, liver, kidney)
  c. Pancreatitis
  d. Concurrent endocrinopathies (obesity, hypercortisolism, acromegaly, hyperthyroidism)
  e. Administration of any drugs which antagonize insulin (eye, ear Rx common culprits)
  f. Heat cycles
  g. Insulin-induced hyperglycemia?
     1. Result of too much insulin
     2. Response to either hypoglycemia
     3. (BG < 60 mg/dl) or too rapid a decline in BG
     4. Secretion of counter-regulatory stress hormones
     5. Can results in an overall increase of BG with worsening of Pu/Pd and increased fructosamine
• Diagnosis of insulin resistance:
  1. Correlate results of glucose curves with clinical signs
  2. Is it really insulin resistance, or are owners not giving insulin properly?
     a. Handling of insulin & syringe, administration technique (Watch owners administer injection!!!)
     b. Check type, dose and expiration date
     c. If using a diluted insulin, switch to non-diluted
     d. Storage of insulin
  3. Is there any concurrent disease?
  4. If none of the above applies, consider insulin-induced hypoglycemia and decrease insulin dose

Hypercortisolism in cats
• Uncommon
• Females > males?
• Mostly similar to the syndrome in dogs
• Valentin et al. JVIM 2014: multicenter retrospective (1990 – 2011), N = 30
  a. Females = males
  b. Age range: 4 – 17y (median 13y)
  c. 90% PDH, 10% AT
  d. Dermatological signs (100%) including Skin Fragility Syndrome
     i. Thin skin (70%)
     ii. Alopecia (60%)
     iii. Skin lacerations (57%)
     iv. Dull coat/scaling/seborrheic (13%)
  • Mellett JVIM 2013: N = 15, Dermatological signs (73%)
  e. Polyuria/Polydipsia (87%)
  f. Polyphagia (70%)
  g. Abdominal distention (67%)
  h. Muscle wasting (67%)

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i. Lethargy (47%)

j. Weight loss (47%), weight gain (23%)

k. Unregulated DM (90%)

l. Other concurrent diseases:
   i. Bacterial infections (56%), including UTI’s, skin abscesses, rhinitis
   ii. Pancreatitis (30%)

m. CKD (36%)

n. Laboratory tests:
   i. Anemia (48%)
   ii. Hypochloremia (41%)
   iii. Hypertriglyceridemia (71%)
   iv. Normal TT4 (84%), low TT4 (16%)
   v. Normal ALP (80%)!!!

   o. ACTH stim: Variety of protocols
      i. Sensitivity: 56% (46% at 60 min)
      ii. All AT cases negative
      iii. Specificity??
      iv. 89% but not in diabetics

p. Dexamethasone Suppression test:
   i. 0.1 mg/kg
   ii. Sensitivity: 96%
   iii. Specificity?? No disease free group

   • Abdominal ultrasound:
     ▪ Valentin et al. JVIM 2014: 90% sensitivity (3/30 normal)
     ▪ Combes et al. JFMS 2013: PDH, N = 4, 2/4 normal, 2/4 slightly enlarged
     ▪ Combes et al. Vet Rad Ult 2012: The adrenal glands were significantly larger in hyperthyroid cats compared to normal.

   • Other tests ACTH precursors for diagnosis of PDH (Benchekroun et al. JVIM 2012)
     a. 229 pmol/L cut off: 89% sensitivity (8/9); 100% specificity in DM and in DM+acromegaly
     b. Not commercially available

   • Medical management:
     o Valentin et al. JVIM 2014:
       ▪ Mitotane is generally considered ineffective in cats but one cat survived > 5 years
       ▪ Trilostane 0.5 – 12 mg/kg, q12-24h, survival: < 1 – > 21 m
     o Neiger et al. JVIM 2004: Trilostane reduced clinical signs and improved endocrine test results in all cats, but insulin requirements did not change and all continued to have some signs of hypercortisolemia.
     o Mellett JVIM 2013: N = 15
       ▪ Trilostane 10 – 30 mg/cat q24h or 10 – 20 mg/cat q12h
       ▪ Clinical signs and ACTH stims improved in 13/15 cats.
       ▪ Insulin requirements decreased by 36% within 2 months in 6/9 diabetic cats.
       ▪ Median survival time: 617 d (range 80–1,278).
       ▪ Hypocortisolemia was documented in 1 case.

   • Surgical/radiation therapy
     o Adrenalectomy (uni or bilateral)
       ▪ Smith et al. JAVMA 2012: Laparoscopic adrenalectomy for AT in a cat
     o Hypophysectomy (Utrecht)
     o Radiation Therapy (Sellon JVIM 2009): Variable response. DM remission is uncommon. Low complication rate

Hypersomatotropism (Formerly known as: Acromegaly) in cats

• Cause: GH-secreting pituitary adenomas
• Much more common than previously recognized
• Often causes diabetes without causing over signs of acromegaly
• Excessive GH secretion leads to:
  o Insulin resistance
  o Increased IGF-1 secretion from the liver → Bony (growth of extremities) and soft tissue overgrowth (causing HCM, renomegaly, hepatomegaly, enlarged endocrine glands, DJD, Spondylosis and more…)
  o Signs develop slowly

• Signalment:
  o Males and females are equally represented
  o No breed predisposition
  o Middle age and older
• Clinical signs:
Signs of uncontrolled DM often appear first

- The “typical” physical manifestations of acromegaly develop slowly and are often not present at all or at least not obvious when diabetes is already present (Renomegaly, hepatomegaly, heart murmur, plantigrade stance, procanthia inferior, broad face, stridor/stertor)
- Weight loss is sometimes seen but often not and many cats experience weight gain despite uncontrolled DM.
- Insulin resistance that could be extreme (insulin dose: median = 7U/cat BID [range 1-35])
- PuPdPP: 100%

**Diagnosis**

- IGF-1 in uncontrolled diabetics is often high (low specificity) but above a certain cutoff, the positive predictive value of IGF-1 is high (95% PPV for IGF-1 > 1000ng/ml)
- IGF-1 in an untreated diabetic secondary to hypersomatotropism can be low (low sensitivity) and increases with insulin treatment
- IGF-1 is a good screening test and should be performed in all diabetic cats that have not experienced diabetic remission (assuming appropriate treatment of DM with insulin and low carb diet for at least one month).
- Confirm diagnosis with brain imaging

**Treatment**

- Radiation therapy (Sellon JVIM 2009, Dunning JVIM 2009)
  - Variable response. DM remission is uncommon.
  - Low complication rate
  - IGF-1 levels are not useful for monitoring
- Hypophysectomy
  - Most often curative and leads to diabetic remission
  - Available in a handful of centers around the world (WSU, OSU, RVC, Utrecht)
- Somatostatin-analogs (Niessen, JVIM 2013 abstract):
  - Pasireotide, once monthly injections.
  - Improved glycemic control and decreased IGF-1. No side effects except for hypoglycemia in one cat!
  - EXPENSIVE!
Diabetes mellitus: Pathophysiology
When hyperglycemia is persistent and severe enough to exceed the renal capacity for glucose reabsorption (180 – 200 mg/dl in dogs, 260 – 280 mg/dL in cats) glycosuria ensues leading to osmotic diuresis with free water, electrolytes and energy losses. Hyperglycemia and free water loss cause an increase in serum osmolality which in turn stimulates thirst. Insulin is an important anabolic hormone. A relative or absolute insulin deficiency will lead to breakdown of fat and muscles and weight loss. Lipolysis in peripheral adipose tissue leads to hyperlipidemia, mobilization of free fatty acids to the liver and hepatic lipidosis.

Glucose toxicity
- Is the result of prolonged and persistent hyperglycemia
- Causes impaired insulin secretion and down-regulation of glucose transport systems
- Leads to overt diabetes
- Resolves with insulin therapy
- May explain transient diabetes “honeymoon period” in cats

Diabetes remission
In cats, Type II DM is considered the most common type and although most cats require insulin therapy on initial presentation, periods of insulin independence (“remission”) may occur with appropriate dietary and insulin therapy. Remission in dogs is very unlikely, even in cases that seem secondary to steroid administration or in gestational diabetes although remission has been documented in dogs (gestational paper). In cats, remission occurs in approx. 50% of cases. Most studies did not find a correlation between remission rates and insulin type, duration of disease prior to starting treatment etc. One study found 100% remission rates with insulin glargine but these results were not reproducible and in other studies insulin type was not a significant factor. A canned, low fiber-low carbohydrate diet has been associated with higher remission rates (68%) when compared to a canned, moderate carbohydrate-high fiber diet (41%). Some studies suggest that longer duration of illness prior to starting insulin therapy decreases likelihood of remission. In 2 studies, an intensive treatment protocol (3-5 measurements of blood glucose daily and adjustment of insulin dose with the goal of reaching a blood glucose of 50 – 100 mg/dL) was associated with remission rates of almost 70% but all cats were fed a canned, low fiber-low carbohydrate diet. Importantly, it is unknown currently whether any protocol decreases the rate of complications of DM or prolongs survival.

Diagnosis of glycemic dysregulation
1. Based on appropriate clinical signs (polydipsia, polyuria, polyphagia and weight loss), persistent hyperglycemia, and glycosuria. A complete blood count (CBC), biochemistry panel, a urinalysis and a urine culture are recommended to rule out concurrent disease for any newly diagnosed diabetic and for the unregulated-treated diabetic. Serum total thyroxine concentrations should be included for cats over 9 years of age.
2. Serum fructosamine concentrations are useful for diagnosis of persistent DM and ruling out stress-related hyperglycemia/glycosuria.
3. For the treated, unregulated diabetic, an important component in the diagnosis of the cause of dysregulation is evaluation of therapy-related causes:
   a. Handling of insulin & syringe, administration technique (observe owners administer the injection?)
   b. Check type, dose and expiration date of insulin
   c. If using a diluted insulin, switch to non-diluted
   d. Appropriate storage of insulin vial

Monitoring of DM
Goals
- Avoid hypoglycemia
- Prevent DKA
- Resolve the clinical signs of diabetes
- Euglycemia is not a treatment goal in dogs (Maybe it should be in cats?)

Observation of changes in clinical signs is the corner stone of DM monitoring
- Consider changes in clinical signs since the last time therapy was altered

Serum fructosamine concentrations (SF)
- Reflect glycemic control over the course of 2 – 3 weeks.
• Indicated for routine monitoring of the treated diabetic. Small changes in glycemic control may not be apparent clinically but could still be reflected by changes in SF. Thus, it is recommended to obtain SF on diagnosis and every time a change is made in therapy: before (for baseline) and 2-3 weeks after.
• Trends in SF are more useful than absolute numbers (the latter don’t always fit the patient)
• SF are affected by the half-life of serum proteins. Thus, when protein turn-over is increased (e.g. hyperthyroidism, protein-losing enteropathy) SF can be falsely decreased.
• Hemolysis (either in-vivo or in-vitro) affects the laboratory’s ability to analyze SF and should be avoided.

Blood glucose curves (BGC)
• AAHA Diabetes Management Guidelines 2010: For a stable diabetic, advise owners to perform a blood glucose curve at home once a month.
• Results of BGC vary significantly from day to day even when factors like diet, meal size, insulin (formulation and dose) and site of injection are unchanged
• Day-to-day variability in insulin action precludes reliance on a single BGC for decision making unless hypoglycemia is documented.
• Day-to-day variability in BGC’s has been documented in studies of dogs in a hospital environment but also in cats when BGC’s are done at home with minimal stress.
• Factors associated with day-to-day variability in insulin action:
  o Meal composition and size
  o Stress
  o Activity level
  o Injection site (vasculature, temperature)
  o Injection technique
  o Dosing inaccuracies (especially with low doses)
  o Insulin absorption
• Dosing inaccuracies and variability in absorption from SQ depot are less of a problem with novel insulin formulations that are supplied as suspension (e.g. detemir and glargine) and more of a problem with traditional formulations that are supplied as suspensions (NPH, Lente, PZI)
• Measure BG at least q 2hr for at least as long as the insulin should last (12-24h depending on the insulin type)
• To determine the nadir: Identify at least 2 points on the upswing
• Interpret BGC’s carefully! Always correlate results with clinical signs and SF
• A diagnosis of hypoglycemia is useful: Reduce dose or start over with a different insulin formulation
• Diagnosis of duration, nadir, efficacy and “insulin resistance” based on a single BGC is problematic: Consider these diagnoses only if repeatable in multiple BGC’s
• More data points = decreased effect of day-to-day variability (consider home monitoring and continuous glucose monitoring).

Causes of inadequate glycemic regulation in the treated diabetic
• Insulin formulation and administration:
  o Check type and dose of insulin
  o Handling of insulin & syringe
  o Injection technique (observe owners administer injection!!!)
  o Storage, and expiration date of insulin
  o If using a diluted insulin, switch to non-diluted
• Concurrent diseases and causes of insulin resistance:
  o Bacterial infections (periodontal disease, UTI)
  o Major organ failure (heart, liver, kidney)
  o Pancreatitis
  o Concurrent endocrinopathies (Hypercortisolism, Acromegaly, hyperthyroidism, hypothyroidism)
  o Administration of any drugs which antagonizes insulin (eye, ear Rx are common culprits)
  o Heat cycles
  o Insulin-induced hyperglycemia
    ▪ Result of too much insulin
    ▪ Response to either hypoglycemia (BG < 60 mg/dl) or too rapid a decline in BG
    ▪ Hypoglycemia leads to secretion of counter-regulatory stress hormones
    ▪ Can results in an overall increase of BG with worsening of Pu/Pd and increased fructosamine
Incretin-based therapies are revolutionizing the field of diabetes therapy by replacing insulin therapy with safer and more convenient, long-acting drugs. Incretin hormones (GLP-1 and GIP) are secreted from the intestinal tract in response to the presence of food in the intestinal lumen. GLP-1 augments insulin secretion and suppresses glucagon secretion during hyperglycemia in a glucose-dependent manner. Clinical data have revealed that incretin-based drugs are as effective as insulin in improving glycemic control while reducing body weight (GLP-1 analogs, specifically) in patients with type 2 diabetes. Furthermore, incidence of hypoglycemia is relatively low with these drugs because of their glucose-dependent mechanism of action. Another significant advantage of these drugs is their duration of action. While insulin injections are administered at least once daily, long-acting GLP-1 analogs have been developed as once-a-week injections and could potentially be administered even less frequently than that in diabetic cats. This review considers the physiology of incretin hormones, and the pharmacology and use of GLP-1 analogs in people. We will also review up-to-date research data on GLP-1 analogs in veterinary medicine.

Incretin physiology
Incretin-secreting cells (K and L cells) are interspersed in the epithelium of the gut. Their physiological role is to sense the type and quantity of digested nutrients in the gut. They then secrete incretin hormones as preparatory signals to other remote organs (e.g. brain, pancreas etc.). In the pancreas, the incretin signal is translated to increased sensitivity to the stimulatory effect of glucose. This effect is responsible for the observed difference in insulin secretion between oral and intravenous glucose and is defined as the “incretin effect”; oral glucose leads to much greater insulin secretion compared to intravenous glucose even when blood glucose concentrations are equal.1,2 The incretin effect is thought to be exclusively mediated by 2 peptide hormones: Glucagon-like peptide-1 (GLP-1) which is secreted from L cells, and glucose-dependent insulinotropic polypeptide (GIP) which is secreted from K cells. Incretin hormones also promote expansion of pancreatic beta-cell mass: They promote differentiation of pancreatic ductal cells into beta cells and increase beta cell proliferation. Importantly, incretin hormones protect beta cells from apoptosis induced by various cytotoxic agents or dexamethasone. They also prevent apoptosis secondary to glucotoxicity and lipotoxicity.3

Incretin hormones affect glucagon secretion (GLP-1 inhibits, GIP stimulates), slow the rate of gastric emptying (GLP-1), increase satiety (GLP-1), and increase the sensitivity of adipose tissue to insulin (GIP). Thus, they play a major role in glucose homeostasis.3,4 Active GIP and active GLP-1 are degraded by the enzymes dipeptidyl peptidase-4 (DPP-4, also known as CD26) and neutral endopeptidase 24.11 (NEP-24.11) into inactive forms, thereby modifying or inhibiting their activity.5 DPP-4 and NEP are ubiquitous in tissues, and DPP-4 is also present in a soluble form in the blood. Consequently, when injected intravenously, active GLP-1 and GIP are quickly degraded into inactive forms. When injected intravenously, the half-life of GLP-1 is only about 1-2 minutes and the half-life of GIP is only about 5 minutes. The inactive GLP-1 and GIP are quickly cleared by the kidneys.

Incretin hormones in diabetes and obesity
The incretin effect is severely reduced or absent in people with type 2 DM, contributing to glucose intolerance and post-prandial hyperglycemia. In diabetics, the secretion of GIP is normal or slightly reduced but its effect on the pancreas is markedly decreased. In contrast, GLP-1 retains its insulinotropic effects in type 2 DM (at least in supraphysiologic concentrations), but its secretion is decreased.4 Whether a blunted incretin response is a primary process in type 2 DM or a secondary process caused by diabetes, is debatable. There is some evidence that obesity contributes to attenuation of the incretin effect independently of diabetes. In a recent study, obesity was associated with decreased incretin effect including decreased beta-cell response to GLP-1 but also decreased GLP-1 secretion.3 In some studies, GLP-1 secretion was normal in obese people but GIP secretion was increased during fasting and early after a meal.6 Insulin resistance in itself, regardless of obesity or diabetes, has been associated with abnormal secretion of GIP and GLP-1.7 Increased insulin concentrations in the insulin-resistant subjects might down-regulate incretin secretion.

Traditional treatments for diabetes are either just partially effective (diet change, oral drugs) or associated with significant side effects (insulin therapy). Owner compliance is a major problem because twice-a-day injections are required with most commonly used insulin preparations. Weight gain may indicate a good response to therapy initially but can eventually become a problem. Hypoglycemia is a common complication of insulin therapy and can be life-threatening. These side effects have been significantly reduced in human medicine with the introduction of incretin-based treatments. GLP-1 stimulates glucose-dependent insulin secretion. Its effect is blunted in euglycemia and therefore they are less likely to cause hypoglycemia. GLP-1 also promotes weight loss. GLP-1 analogs are not only effective in controlling blood glucose in diabetics but they are also associated with weight loss in diabetic and non-diabetic obese people. One major advantage of incretin-based therapies is their potential to reverse the course of the disease. Incretin-based treatments in diabetic people correct not only hyperglycemia but also all the major markers of beta-cell dysfunction.
The very short half-life of GLP-1 limits its clinical use. There are numerous treatment strategies that overcome this problem and take advantage of incretin-hormone physiology. Novel GLP-1 analogs have a prolonged half-life and are used as SQ injections at intervals of up to one week.

Incretin-based therapy strategies
There are several treatment modalities that overcome the problems associated with direct administration of incretin hormones. Long-acting, DPP-4-resistant, synthetic GLP-1 mimetic peptides are most commonly used. Available formulations are all for SQ injections but other delivery systems are being developed (including enteral, pulmonary, or sublingual). Also under development are non-peptidic GLP-1 receptor agonists. Oral DPP-4 inhibitors that prolong the half-life of incretin hormones are also commercially available.

Non-peptidic drugs that activate free fatty acid receptors in L cells and stimulate GLP-1 secretion are being developed (GRP120 and GRP119 agonists). Diet manipulation should also be investigated.

Drugs that are commercially available or that are currently in advanced clinical trials are described below.

GLP-1 mimetics and analogs

Exenatide
The peptide exendin-4 was first isolated from the poisonous venom of the Gila Monster (Heloderma suspectum). Exendin-4 is a 39-amino acid peptide that shares only a 53% sequence homology with GLP-1 but its affinity for the GLP-1 receptor is 1000 times greater than the affinity of GLP-1. Unlike GLP-1, exendin-4 is not a substrate for DPP-4 and NEP. Exenatide is a synthetic exendin-4. Resistant to degradation, exenatide is eliminated by the kidneys and has a half-life of 3-4 hours in people. Its biological effect lasts about 8 hours after subcutaneous injection and it can be detected in the plasma for up to 15 hours. Multiple studies, both in vitro and in vivo, have shown that, in general, exendin-4 has the same physiologic effects as GLP-1 in the pancreas, GI tract, and brain.

Exenatide is associated with improvement in some of the earliest and most fundamental abnormalities of type 2 diabetes: diminished "first-phase insulin response" and proinsulin/insulin ratio. Acute administration of exenatide in type 2 diabetic patients corrects the abnormal insulin secretion pattern after an IV glucose bolus (first phase and second phase insulin responses) and restores the ability of beta cells to respond to rapid changes in blood glucose concentrations. Exenatide also improves proinsulin/insulin ratio after 30 weeks of treatment.

Exenatide has been shown to be as effective as insulin glargine in the treatment of DM but with less side effects (e.g. hypoglycemia and weight gain). In a 2-year follow-up of patients receiving exenatide, patients achieved sustained and significant reductions in glycosylated hemoglobin, accompanied by significant weight loss (instead of weight gain commonly seen in diabetics receiving insulin) and improvement in serum liver enzyme activity and blood pressure. Most importantly, treatment with exenatide improved beta cell function as measured by homeostasis model assessment of beta cell function (HOMA-B). Exenatide has minimal side effects in people. It is mostly associated with nausea and less frequently with vomiting. Infrequently, it might cause hypoglycemia. Severe hypoglycemia (requiring assistance) was reported rarely (only 5 of 2781 patients) and only in patients who also received sulfonylurea drugs. Antibodies to exenatide developed in 67% of patients but this did not affect outcome and was not associated with side effects.

When first discovered, exendin-4 was shown to potentiate amylase release from rat acinar pancreatic cells in response to other hormones such as cholecystokinin. This was shown ex vivo and in high doses. A possible association between GLP-1 analogs and pancreatitis as well as medullary thyroid cancer has been suggested and lead to issuing of a warning by FDA. However, multiple studies have refuted these concerns and it is widely accepted that the proven enormous benefits of GLP-1 analogs outweigh its hypothetical risks.

Exenatide is commercially available in the USA under the trade name Byetta®.

Exenatide in cats
Exenatide was quickly absorbed after a SQ injection and caused glucose-dependent insulin secretion. Increased glucose tolerance, however, was not observed after a single SQ injection. At a dose of 1.0 mcg/kg SQ (about 10 times the dose that is used in diabetic people), exenatide injection did not cause any side effects in healthy cats, except for hypoglycemia in 1 out of 9 cats. Exenatide has led to significant weight loss in healthy cats of 7.0 ± 4.9% (from 4.78 ± 1.5 kg to 4.48 ± 1.5 kg) with a dose of 1.0 mcg/kg SQ BID for 28 days.

Exenatide extended-release
A long-acting sustained-release formulation of exenatide (Bydureon®) has recently been approved by the FDA as the first once-weekly subcutaneous injection for treatment of type 2 diabetes people. It consists of injectable microspheres of exenatide and poly(D,L-lactic-co-glycolic acid), a common biodegradable medical polymer with established use in absorbable sutures and extended-release pharmaceuticals, that allows gradual drug delivery at controlled rates. In people, Exenatide plasma concentrations are sustained at an effective concentration (50 pg/mL) for longer than 60 days after a single injection at doses of 5mg, 7mg or 10mg. It has been shown in a recent clinical study to be more effective than once-a-day insulin glargine in achieving glycemic control with decreased risk of hypoglycemia and with reduction (instead of gain) in body weight. This extended release formulation was also

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more effective than regular exenatide (Byetta) in achieving glycemic control with no increased risk of hypoglycemia, decreased side effects like nausea, and with similar reductions in body weight.23

**Exenatide extended-release in cats**

We have studied Exenatide Extended-Release in healthy cats at a dose of 0.13 mg/kg. Three weeks after a single subcutaneous injection fasting BG was decreased and during a hyperglycemic clamp at that time glucose tolerance improved, insulin concentrations increased and glucagon concentrations decreased. No side effects were observed throughout the study.24 Further evaluation is needed to determine its efficacy and duration of action in diabetic cats.

**Liraglutide**

Liraglutide is a GLP-1 synthetic analog with 2 amino acid substitutions and a fatty acid acyl group that enables noncovalent binding to albumin, thereby extending the pharmacokinetic profile of the GLP-1 molecule. Liraglutide exhibits a prolonged pharmacokinetic profile after a single injection, and exhibits all of the actions of native GLP-1.4 Liraglutide (once-a-day) was recently compared to exenatide (twice-a-day). Liraglutide provided significantly greater improvements in glycemic control than did exenatide and was generally better tolerated.25 Liraglutide once-daily was also more effective than Exenatide-ER once-weekly in achieving glycemic controlling but nausea, vomiting and diarrhea occurred less frequently with Exenatide-ER.26 Liraglutide has been used successfully to treat obesity in non-diabetic patients.27

Liraglutide is commercially available in the USA and Europe under the trade name Victoza®.

**Liraglutide in cats**

We have studied liraglutide in healthy cats at a dose of 0.6 mg/cat once daily for 7 days. Liraglutide caused significant weight loss in all cats at day (9% ± 3). Appetite was subjectively decreased in all cats and one cat was withdrawn on day 4 because of 48 hours of anorexia. During a hyperglycemic clamp, liraglutide was associated with a trend towards improved glucose tolerance, higher insulin concentrations and lower glucagon concentrations. Fasting glucose concentrations were not affected.28

**Albiglutide**

Albiglutide is a recombinant GLP-1-albumin fusion protein that exhibits a reduced affinity for the GLP-1R, but displays a broad spectrum of GLP-1R-dependent actions in preclinical studies, including inhibition of food intake and gastric emptying and reduction of glycemia excursion after meal ingestion. Importantly, it has the potential to be used as a very long-acting drug: It has been investigated in people for use as a weekly, biweekly and monthly injection.29 Albiglutide was approved by FDA in 2014.

**DPP-4 inhibitors**

DPP-4 inhibitors (e.g. sitagliptin, vildagliptin) are administered orally. In people, vildagliptin and sitagliptin are well-tolerated and not associated with hypoglycemia when used alone. Both agents increase plasma concentrations of GLP-1 and GIP after meal ingestion, enhance glucose-stimulated insulin secretion and reduce ratios of proinsulin:insulin, consistent with an improvement in beta cell function. They are, however, less potent than other oral hypoglycemic drugs.3 In contrast to GLP-1 analogs, DPP-4 inhibitors are not associated with nausea or vomiting in people, but they are associated with weight gain. DPP-4 inhibitors are also associated with increased risk of nasopharyngitis, urinary tract infections and headaches.16 Increased risk of infections might be related to the action of DPP-4 in T-cells as a co-stimulatory molecule (CD26).

A DPP-4 inhibitor has been used experimentally in cats and was effective in enhancing insulin secretion and inhibiting glucagon secretion after an intravenous glucose challenge. Glucagon inhibition was also observed after a meal challenge.30 In that study, the drug was administered subcutaneously and not orally.

References