The intravenous insulin tolerance test in the dog: experimental study

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SUMMARY

Objective: Resistance to insulin (hypercorticism, acromegaly, obesity) is the most frequent cause of canine diabetes. Thus, testing for insulin sensitivity is a prerequisite for the diagnosis and the treatment of this illness. This study evaluates the Intravenous Insulin Tolerance Test (IVITT: measure of the glycemia following intravenous administration of insulin).

Material and Methods: The role of endogenous insulin and cortisol during the test was examined, in 20 dogs, by measuring insulin and cortisol levels concomitantly with administration of an IVITT. The evaluation of the IVITT in 38 healthy dogs and the relationship between IVITT and weight status was then investigated. The sensitivity of the test was determined by subjecting a group of six dogs to IV-induced hyperglycaemia, and to the IVITT 24 hours later. The reproducibility of the IVITT was assessed by administering the test to 5 dogs twice, with a minimal interval of 24 hours. At the end, 12 dogs afflicted with conditions known to potentially cause resistance to insulin were subjected to an IVITT.

Results and Conclusion: This test gives information similar to that of other tests in veterinary medicine and offers a major advantage by allowing the assessment of a subject’s insulin resistance without involving insulin production. Other benefits are evident as well: simple implementation (the test requires 15 minutes only, and 3 blood samples), lower cost (3 measurements of glycemia), easy performance by the majority of veterinarians in their own clinics and easy analysis.

KEY-WORDS: dog - intravenous insulin tolerance test (IVITT) - diabetes.

Introduction

Resistance to insulin (hypercorticism, acromegaly, obesity) is the most frequent cause of canine diabetes. Thus, testing for insulin sensitivity is a prerequisite both for an accurate diagnosis and for the implementation of a planned-out treatment through hormone substitution therapy.

A classic way of testing for resistance to insulin consists in either establishing a glycemia curve after a week of trial treatment, or determining insulin-sensitivity by the euglycemic clamp technique (ref: rate of glucose perfusion needed to maintain a euglycemic state concurrently with a codified insulin infusion AKINMOKUN [1]. Both these techniques are complex and significantly delay the onset of treatment.

In human medicine, BONORA [2] and GRULET [4] compared estimations of insulin sensitivity obtained through the Intravenous Insulin Tolerance Test (IVITT) on one hand, and by the euglycemic clamp technique on the other. Both studies show that for humans:

- the insulin tolerance test, thus correlated with the clamp method, appears to be a reliable tool for measuring sensitivity
to insulin, and gives better results than measuring insulinemia,
- exogenous insulin is the sole factor responsible for the
decrease of glycemia during the test : endogenous insulin and
counter-regulatory hormones do not intervene during the test,
- reproducibility is acceptable.
The purpose of this study was to validate a test for insulin
tolerance in dogs, through five precise aims :
- examine the role played by endogenous insulin and corti-
sol (counter-regulatory hormones) while the test is in pro-
gress,
- evaluate the IVITT in healthy dogs,
- establish the sensitivity and reproducibility of the test,
- evaluate the relationship between obesity and IVITT,
- assess the test on animals that are either ill or have been
administered drugs liable to induce resistance to insulin,

1. Material and methods

A) ANIMALS

The selected dogs were classified into three categories :
- 38 healthy dogs selected on the basis of a clinical and bio-
 logical investigation,
- 14 dogs, which were hospitalised for medical reasons or
 had been administered drugs liable to interfere with glycore-
gulation.

Preliminary selection took into account the breed, age, gen-
der, medical history, reasons for hospitalisations, ongoing
 treatments and the degree of obesity as determined from the
 skin fold on the rib cage (weight index).

B) EXPERIMENTAL PROTOCOL

1) Approach
- the role of endogenous insulin and cortisol during the test
was examined in 20 dogs, randomly selected among the 38,
by measuring insulin and cortisol levels concomitantly with
administration of an IVITT,
- the evaluation of the IVITT in healthy dogs and the rela-
tionship between IVITT and weight status was investigated
through an epidemiological study using the 38 apparently
healthy dogs,
- the sensitivity of the test was determined by subjecting a
 group of six dogs to IV-induced hyperglycemia and to the
IVITT 24 hours later. The reproducibility of the IVITT was
assessed by administering the test to 5 dogs twice, with a
minimal interval of 24 hours. Results were compared,
- 12 dogs afflicted with conditions known to potentially
cause resistance to insulin were subjected to an IVITT.

2) Implementation of the tests
a) the Intravenous Insulin Tolerance Test (IVITT)

This method was directly taken from BONORA [2] and
GRULET [4] but was different from that of KANEKO [5]
who used IM administration ; the following protocol was
adopted :
- the animal had been fasting for a minimum of 12 hours,
- a first blood sample was drawn at time 0 (T0) to establish
initial glycemia levels. Afterwards a dose of 0.1 U/kg crys-
tallised insulin (Actrapid, 40 U/mL) was administered via
intravenous bolus,
- subsequently, blood samples were obtained via jugular
drive puncture at the times 3 min, 5 min, 7 min,
10 min and 15 min.

b) Intravenously induced hyperglycemia (Intravenous
Glucose Tolerance Test, IVGTT)

The method used is derived from that described in
FELDMAN [3], KANEKO [5] and SILIART [7]. Test dura-
tion was one hour ; the animal was fasting, and had been tes-
ted with an IVITT at least 24 hours previously. After per-
forming the initial blood test at time 0 (T0), 30 % glucose was
injected via intravenous bolus using the dose of 500 mg/kg.
Subsequent blood samples (1 mL) were taken via endovei-
nous jugular puncture at times 5 min, 10 min, 15 min, 30 min,
45 min and 60 min. Glycemia and insulinemia levels were
measured in all samples.

3) Sample analyses

The blood was collected onto an anticoagulant (Lithium
Heparinate)

Glycemia : the samples of heparinated blood were imme-
diately centrifuged and blood glucose levels were measured
immediately on all the collected samples upon completion of
the test with an analyser (Kodak Ektachem DT 60) using an
enzymatic method. Results are given in mmol/L.

Insulinemia and cortisolemia : from 20 dogs among the 38,
plasma was collected into dry tubes, then kept frozen at - 20°C
until analysis (between 15 days and 1 month later). Insulin
assays were performed by radioimmunology using a kit ref.
INSIK 5 supplied by DIASORIN, 92182 - ANTONY
(France). Results are given in mU/L. The sensitivity of the method
is below 4 mU/L. The intra-assay coefficient of
variation ranges between 5.5 % and 10.6 %, the inter-assay
coefficient of variation ranges between 6.2 % and 10.8 %. Cortisol
assays were performed by radioimmunology using the
AMERLEX Cortisol RIA KIT supplied by ORTHO CLIN-
ICAL DIAGNOSTICS, 92787, Issy-les-Moulineaux
Cedex 9 (France). Results are given in nmol/L. The sensitiv-
ity of the method is less than 2.8 nmol/L, the intra-assay
coefficient of variation ranges from 4.3 % to 5.8 % and the
inter-assay coefficient of variation ranges from 7.7 % to
8.9 %.

4) Data analysis/parameter calculation

A curve representing glycemia as a function of time was
drawn for each animal tested.

Insulin sensitivity is expressed by an index, Kivitt, calcula-
ted as in BONORA [2] and GRULET [4]. Kivitt represents
changes in glycemia relative to initial glycemia (Go), and
measured at the time of minimal glycemia level (Gmin)
during the test :
A curve showing insulinemia as a function of time was established for the 20 dogs in which insulin levels were measured during the test. Initial insulinemia and time of maximal insulinemia were noted as Ins.max and Ins. T0.

The statistical analysis was performed using a one or two factor analysis of variance (ANOVA, Statview software, SYSTAT company) followed if significant by a Fisher PLSD.

2. Results

A) ROLE OF ENDOGENOUS HORMONES DURING THE IVITT

1) IVITT and insulin

Usual values of insulinemia found in normal fasting dogs vary between 6 and 16 mU/mL [3]. The mean value of insulin peaks measured on the 20 dogs during the test was 109 mU/mL (SD : 36mU/L) [3]. This value far exceeds the average endogenous production, although marked variations were in evidence. Analysis of Kivitt as a function of the insulin peak during the course of the test showed the absence of any relationship ($R^2 = 0.02$) between Kivitt and the insulin peak (Figure 1).

2) IVITT and cortisol

Mean cortisolemia increased by 30 % from the third minute and remained at this level until completion of the test. Analysis of Kivitt as a function of the variation in cortisol levels for each subject showed that there is no relationship ($R^2 = 0.02$) between Kivitt and increases in cortisol (Figure 2).

B) STUDY OF THE INSULIN TOLERANCE TEST IN HEALTHY DOGS

Only transient and reversible weakness was observed as a clinical manifestation of hypoglycemia during the tests. The results, showing a mean Kivitt of 0.52 with variations ranging from 0.17 to 0.73, are presented in figure 3 and table I. Three distinctive sets of points are in evidence :

- group 1 : Kivitt from 0.56 to 0.73,
- group 2 : Kivitt from 0.37 to 0.52,
- group 3 : Kivitt inferior to 0.35.

The mean and standard deviation of age, weight index and initial glycemia by groups are also presented in table I. Kivitt diminishes as mean age and weight status increase.

C) COMPARISON OF THE RESULTS FROM THE INSULIN TOLERANCE TEST (IVITT) AND THE IV INDUCED HYPERGLYCEMIA METHOD (IVGTT)

In order to interpret the degree of insulin-sensitivity from Kivitt, three arbitrary thresholds were established to distinguish :

- a range of normal or high insulin sensitivity (Kivitt above 0.5),
- a range of low insulin sensitivity (Kivitt below 0.4),
- an intermediary zone in which insulin sensitivity needs analysis in a clinical context (Kivitt between 0.4 and 0.5).

The following results were analysed for each dog :

- results from the IVGTT test, as expressed by the curves established for both glycemia and insulinemia as functions of time,
- results of the IVITT, as shown by the Kivitt index.

These results are given in Table II. Both tests lead to the same conclusions, aside from dog n° 2 whose levels of initial glycemia were relatively high.

D) REPRODUCIBILITY OF THE IV INSULIN TOLERANCE TEST

Five dogs were subjected to two tests at a few days' interval. Results are given in Table III

The test exhibits a good qualitative reproducibility for all dogs tested.

E) STUDY OF THE INSULIN TOLERANCE TEST RELATIVE TO THE WEIGHT STATUS OF THE DOGS

Table IV shows the classification of 38 healthy, IVITT-tested dogs, according to degree of obesity. Three groups were defined :

- group 1 (18 dogs) : weight index inferior or equal to 0,
- group 2 (9 dogs) : weight index between 0 and 1 (overweight),
- group 3 (11 dogs) : weight index between 1 and 2 (obese).

For each group, mean results were calculated for age, degree of obesity, initial glycemia level and Kivitt. Group 2 shows a lowered mean Kivitt (0.50, SD : 0.10), with initial glycemia comparable to that of group 1 (0.60, SD : 0.10). In group 3, mean Kivitt is lower (0.40, SD : 0.15), with slightly raised initial glycemia levels.

The statistical analysis of the results (one factor (weight index) analysis of variance) indicates a statistically very highly significant difference ($p = 0.0004$) between the mean results observed in the three groups. A Fisher's PLSD analysis exhibits a statistically significant decrease ($p < 0.05$) between the mean results observed in groups 2 and 3 versus the results observed in group 1.

Thus we conclude that Kivitt decreases when the degree of obesity increases.

F) STUDY OF THE INSULIN TOLERANCE TEST IN PARTICULAR CASES

The IVITT was performed on 12 dogs hospitalised for medical reasons. Results were as follows :

- 2 dogs suffering from advanced chronic kidney malfunction showed a low Kivitt (0.24 ; 0.39),
FIGURE 1. — Kivitt as a function of insulinenia (mU/L) during the course of the test. (Kivitt = 0.625 - 0.001 * Insuline (mU/L) ; R² = 0.02).

FIGURE 2. — Kivitt as a function of the variation in cortisol levels (nmol/L) during the course of the test. (Kivitt = 0.537 + 0.001 * Cortisol ; R² = 0.02).

FIGURE 3. — Distribution of Kivitt in 38 healthy dogs.

TABLE I. — Classification of the 38 healthy dogs (group 1 : Kivitt from 0.56 to 0.73, group 2 : Kivitt from 0.37 to 0.52, group 3 : Kivitt inferior to 0.35).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dogs</th>
<th>Age</th>
<th>Weight</th>
<th>Weight index</th>
<th>Initial Glycemia</th>
<th>Kivitt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>5.5</td>
<td>18.9</td>
<td>0.2</td>
<td>5.8</td>
<td>0.64</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>5.9</td>
<td>15.0</td>
<td>1.0</td>
<td>5.7</td>
<td>0.44</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>9.0</td>
<td>14.3</td>
<td>1.7</td>
<td>6.1</td>
<td>0.23</td>
</tr>
</tbody>
</table>

TABLE II. — Comparison of IVGTT curve parameters with the Kivitt index.

<table>
<thead>
<tr>
<th>Dog n°</th>
<th>Glucose tolerance</th>
<th>Insulin production</th>
<th>Conclusion</th>
<th>Initial Glycemia</th>
<th>Kivitt</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
<td>5.3</td>
<td>0.63</td>
<td>normal</td>
</tr>
<tr>
<td>2</td>
<td>intolerant</td>
<td>very low</td>
<td>insulin dependant</td>
<td>7.5</td>
<td>0.45</td>
<td>borderline</td>
</tr>
<tr>
<td>3</td>
<td>normal</td>
<td>high</td>
<td>borderline</td>
<td>5.8</td>
<td>0.46</td>
<td>borderline</td>
</tr>
<tr>
<td>4</td>
<td>normal</td>
<td>very high</td>
<td>insulin resistant</td>
<td>6.4</td>
<td>0.36</td>
<td>insulin resistant</td>
</tr>
<tr>
<td>5</td>
<td>normal</td>
<td>rather weak</td>
<td>normal</td>
<td>6.4</td>
<td>0.62</td>
<td>normal</td>
</tr>
<tr>
<td>6</td>
<td>intolerant</td>
<td>very high</td>
<td>insulin resistant</td>
<td>6.3</td>
<td>0.17</td>
<td>insulin resistant</td>
</tr>
</tbody>
</table>
3 dogs afflicted with hypercorticism showed a low Kivitt (0.37; 0.37 and 0.34), while a fourth dog in which the condition was controlled through op'DDD treatment exhibited a standard Kivitt (0.42),

- 1 female with recurring pseudocyeses showed a high Kivitt (0.62),

- 1 female suffering from an ovarian tumor displayed a standard Kivitt value (0.46),

- 1 acromegalic female had a low Kivitt (0.4), but raised glycemia levels (Go = 6.7 mmol/L),

- 1 male suffering from hypertrophic osteodystrophy showed a standard Kivitt value (0.42), and raised glycemia levels (Go = 6.9 mmol/L),

- 1 dog with controlled diabetes displayed a standard Kivitt (0.42) and normal glycemia levels (Go = 6.0 mmol/L).

Two healthy dogs, hospitalised for minor procedures, were sedated and administered an IVITT:

- in the first dog, sedated with medetomidin, Kivitt was quite low (0.27), and there was a marked hyperglycemia (Go = 11.2 mmol/L),

- the second dog, which had been sedated with acepromazine, exhibited a very low Kivitt (0.22), with hyperglycemia (Go = 7.2 mmol/L).

### 3. Analysis and discussion

#### A) INSULIN TOLERANCE TEST AND ENDOGENOUS HORMONE PRODUCTION

The endogenous hormone production does not intervene while the test is in progress. In fact, insulinemia values are nearly 5 times higher than the usual levels of average endogenous insulinemia. This confirms the results obtained by BONORA [2] in human medicine. The dose of insulin administered appears to be sufficient to saturate the insulin receptors, as there is no relationship between maximal insulinemia and Kivitt. Moreover, for dogs subjected to 2 IVITTS, maximal insulinemia values differ but the two Kivitt are identical.

Similarly, endogenous secretion of cortisol plays no part during the test. According to BONORA [2], concentrations of this hormone begin to rise, in humans, 15 to 20 minutes after the beginning of the test. In our study, cortisol concentration increases slightly as of the third minute of the test, and remains stable until test completion. However, this concentration does not reach levels associated with hypercortisolemia (43 nmol/L). Had the increase in cortisolemia been due to the relative hypoglycemia induced by the insulin injection, a regular decrease in cortisolemia would have been observed during the test, which was not the case. Thus this phenomenon probably reflects stress. In any case, the increase in cortisolemia does not affect the test, as shown by the absence of a relationship between this increase and Kivitt.

#### B) RELIABILITY OF THE INSULIN TOLERANCE TEST

1) IVITT versus IVGTT

The results obtained by graphic analysis of IVGTT and those from the IVITT are comparable as long as initial glycemia is taken into account in the interpretation of Kivitt. In fact, a normal Kivitt (> 0.5) with high initial glycemia suggests that the dog is insulin dependent (dog 2).

For feasibility reasons, the two tests could only be administered to 6 dogs. The availability of a larger sample base would have been desirable in order to ascertain the existence of a strong correlation between the IVITT and a reference test. This was done in human medicine by BONORA [2] as well as by GRULET [4]. The results from our study suggested that the IVITT was reliable in dogs. To further verify this reliability, a comparison of our results with those from MATTHEEUWS [6] in obese dogs was made.

2) Reproducibility

The IVITT is qualitatively reproducible for all dogs tested twice in order to establish reproducibility. We conclude from these results, and from those obtained in human medicine [1, 3], that this test can be considered as reproducible in dogs.

3) IVITT and weight status

In 1982, MATTHEEUWS et al. [6] investigated resistance to insulin as a function of obesity, using IVGTT. 35 obese dogs with normal initial glycemia levels (mean = 6.00, SD = 0.40 mmol/L) and 20 dogs of ideal weight were tested by the means of intravenously induced hyperglycaemia. After analysing the results of glucose tolerance tests, initial insulinema and insulinic response to glucose, the authors concluded that just as with humans (SIR [8]), increases in insulinema response to glucose are related to the degree of obesity of the dog. Both studies show that insulin dependence is a function of the degree of obesity in the animal. Nonetheless, results diverge in one aspect. MATTHEEUWS [6] distinguished

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**Table III. — Reproducibility of the IVITT.**

<table>
<thead>
<tr>
<th>Dog n°</th>
<th>1</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitt 1</td>
<td>0.70</td>
<td>0.68</td>
<td>0.62</td>
<td>0.17</td>
<td>0.49</td>
</tr>
<tr>
<td>Kitt 2</td>
<td>0.60</td>
<td>0.62</td>
<td>0.62</td>
<td>0.18</td>
<td>0.53</td>
</tr>
</tbody>
</table>

**Table IV. — Classification of 38 healthy dogs according to degree of obesity.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Weight index</th>
<th>G₀</th>
<th>Kivitt</th>
</tr>
</thead>
<tbody>
<tr>
<td>group 1 m</td>
<td>5.3 - 0.16</td>
<td>5.7</td>
<td>0.60</td>
</tr>
<tr>
<td>(18 dogs) SD</td>
<td>3.4</td>
<td>0.34</td>
<td>0.6</td>
</tr>
<tr>
<td>group 2 m</td>
<td>5.6 - 0.88</td>
<td>5.6</td>
<td>0.50</td>
</tr>
<tr>
<td>(9 dogs) SD</td>
<td>2.5</td>
<td>0.22</td>
<td>1.2</td>
</tr>
<tr>
<td>group 3 m</td>
<td>7.5 - 1.86</td>
<td>6.0</td>
<td>0.40</td>
</tr>
<tr>
<td>(11 dogs) SD</td>
<td>2.8</td>
<td>0.23</td>
<td>1.0</td>
</tr>
</tbody>
</table>
three categories of obese dogs, whereas only two groups were isolated in the present study. This difference is essentially attributable to the limits of the IVITT and to its greater specificity with respect to peripheral insulin resistance. Data obtained from the IVITT are a unique function of insulin resistance, whereas data obtained from IVGTT derive from insulin resistance and insulin production. Potential glucose tolerance is not detected by the IVITT. Thus, in this study, group III of MATTHEEUWS et al., which exhibited increased insulinic response and intolerance to glucose, is not in evidence.

4) Analysis for some particular cases

An IVITT was administered to a number of dogs suffering from conditions known to be liable to induce insulin resistance. For a few of these dogs, notably those with kidney malfunctions, hypocorticism or acromegalia, Kivitt was particularly low. In the cases of other dogs, especially the two females afflicted respectively with recurrent pseudocyeses and an ovarian tumor, Kivitt remained normal; however, note that insulin resistance is only potentially induced by these conditions. It would therefore be desirable to use the IVITT on larger numbers of dogs with these illnesses, in order to quantify this susceptibility.

Finally, it should be noted that medetomidin and acepromazine trigger a marked resistance to insulin. Thus the test should not be performed on anaesthetised dogs.

4. Proposal for standardising the insulin tolerance test

Given the results of this study, it is tempting to consider that the IVITT, as administered in this study, is a reliable test for assessing insulin resistance in dogs. The hypotheses underlying the implementation of the IVITT, the calculation method for Kivitt and the analysis of this constant, following BONORA and GRULET, appear to be valid. However we would suggest a slight modification in the protocol. Kivitt is calculated as a function of initial glycemia and minimal glycemia. In all tests, it was noted that minimal glycemia was attained between 10 and 15 minutes after start of the test. Therefore, a simplified protocol is proposed here with blood sampling at times 0 min, 10 min and 15 min and calculation of the Kivitt using the lower glycemia level obtained.

Conclusion

This study shows that the Intravenous Insulin Tolerance Test appears to be a valid test for evaluating insulin sensitivity. The results obtained are comparable with those reached by the method of intravenously-induced hyperglycemia, as well as those found in MATTHEEUWS [6]. IVITT gives information similar to that of other tests in veterinary medicine (notably intravenously induced hyperglycemia, a standard in veterinary diabetes tests). However, it offers a major advantage by allowing the assessment of a subject’s insulin resistance without involving insulin production. Other benefits are evident as well:

- simpler implementation: an IVGTT test necessitates 60 to 90 minutes and a minimum of 7 blood samples. The Insulin Tolerance Test requires only 15 minutes and 3 blood samples,
- lower cost: the analysis of an IVGTT test necessitates accompanying measurements of insulin. The IVITT requires only measurement of glycemia,
- convenience: glucose measurement is a common test, which may be home performed by the majority of veterinarians, whereas insulin measurement can only be carried out in a specialised laboratory,
- easier analysis.

One asset of this test is that it provides an inexpensive tool in the various studies concerned with diabetes and the elaboration of effective treatments to lower blood sugar in dogs. A second benefit is the new possibility of testing obese and diabetic dogs, in which treatment has failed, for insulin resistance. The IVITT then permits the association of substitute insulin therapy with administration of substances designed to decrease blood sugar levels through their peripheral action/effect upon insulin receptors. In the context of preventive veterinary medicine, the test allows diagnosis of potential insulin resistance in dogs “at risk” (obese and so on), and evaluation of the success of preventive treatments (low calory diet). This easy evaluation could provide an added motivation for owners concerned about the health of their pet.

References