

Physiologic variations of TLI (trypsin like immunoreactivity) in serum of German shepherd dog and correlation with gastrin levels

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Summary

The term trypsin-like immunoreactivity (TLI) defines the fraction of trypsin and trypsinogen which can be determined with immunochemical reactions. The physiological and pathological role of this parameters in the dog is reviewed. The aim of present study was to investigate the physiological variations of TLI in 40 healthy German shepherd dogs, 20 females and 20 males. The animals under study were divided in 5 age groups of 8 animals each (4 females and 4 males) ranging from 3 months to 10 years. A commercial, double-antibody, dog-specific, RIA kit has been employed to determine TLI. The reference range of TLI obtained for the German shepherd dog (5.7-19.3 ng/ml, SD 3.2) was comparable to that reported in literature for other breeds of dogs (5.2-35 ng/ml). No difference in the TLI concentration in relation to sex has been observed (females 12.6 ng/ml, SD 3.2; males 12.3 ng/ml, SD 4.6). Even though still within the normal limits, a higher TLI concentration was observed in the group of subjects aged 3-3.5 months (15.9 ng/ml, SD 3.7).

The TLI activity stabilized in the subsequent age groups from 7 months to 10 years, with no significant variations. The variations, in percentage terms, of TLI serum concentrations have been compared to those observed for gastrin in a previous study of the same authors. This comparison revealed a strict correlation between the variations observed in the different age groups of these hormones, both important in the digestion and absorption of proteins. This study supports the need for critical evaluation of pathological variations of TLI and gastrin in serum of the dog.

Introduction

The term TLI (Trypsin-like immunoreactivity) refers to the fraction of trypsinogen and trypsin present in the bloodstream and recognizable by immunochemical reactions (Braun and others, 1997). Trypsinogen is secreted by exocrine pancreas and, converted into trypsin, enables the digestion of proteins from food. In Figure 1 a simplified description of the mechanisms regulating the synthesis of TLI and the complex links known in literature with the other hormone systems is reported. In particular, it is important to consider the relationship with the regulation of the secretion of gastrin at the vagal level, as this has direct synergistic activity on pancreatic secretion (Agugini and others, 1996; Braun and others, 1997; Faglia, 1997; Ghatei and others, 1997; Inoue and others 1985; Johnson and others, 1986; Le Drean. and others, 1998; Nustede and others, 1993; Shulkes and Daldwin, 1997; Williams, 1989). TLI is strictly organ-specific and therefore abnormal variations in serum suggest pancreatic damage. Nevertheless, being excreted by the kidney, its serum concentration can rise also in the presence of nephrosis (Archer F.J. and others, 1997; Koop and others, 1980; Geokas and others, 1982; Simpson and other, 1991; Williams and Batt, 1983; Williams and Batt, 1986; Williams and Batt, 1988; Williams, 1987; Williams, 1989; Williams, 1990; Williams, 1994). Therefore, in routine clinical investigations it is necessary to combine TLI determination with parameters of kidney function. In the case of acute pancreatitis its increase in systemic circulation appears earlier than that of amylase and lipase, and decreases faster during the re-

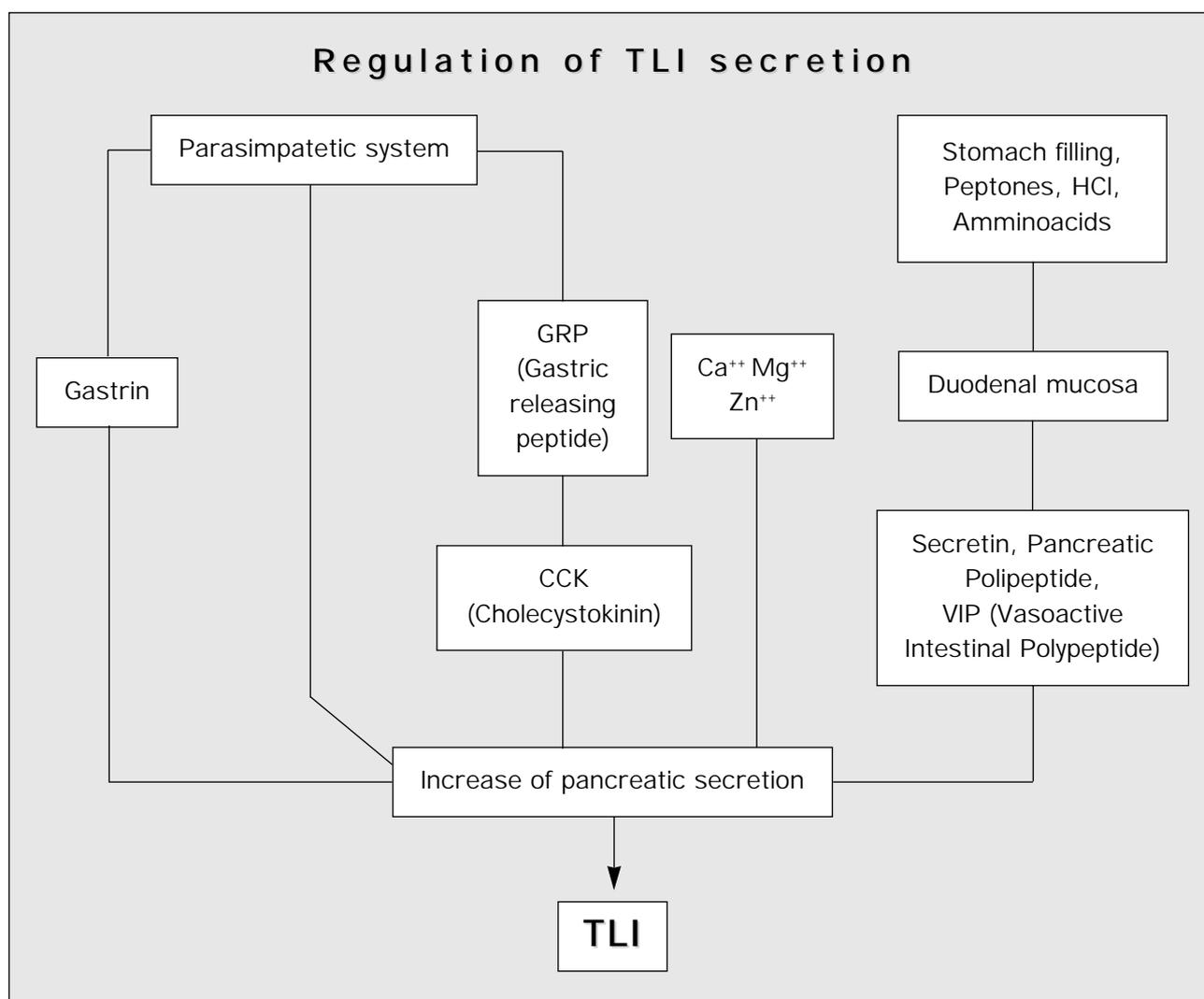


Figure 1. Regulation of TLI secretion.

mission of the disease. Important diagnostic and prognostic information deduced from this behaviour, has been discussed elsewhere (Archer, 1997; Braun, 1997; Williams, 1994). In pancreatic failure the TLI levels in serum decrease remarkably, and its determination furnishes a clear picture of the severity of the disease and is useful in monitoring of treatment (Boari and others, 1993; Braun, 1997; Dotta and others, 1985; Williams and Batt, 1983). Requiring just the analysis of a single serum sample, the TLI determination is easier and less time-consuming compared to other biochemical investigations. For example, the determination of faecal chymotrypsin, even though useful and meaningful, requires the analysis of many portions of the same sample (Abate and Massirio, 1985; Boari and others, 1993; Pozza and others, 1989; Williams and Batt, 1988). In any case, to follow the course of the disease and the efficacy of the therapy, serial determinations in the time are necessary.

The aim of present study has been to establish in German shepherd dog the reference range of TLI, and its eventual variations in relation to age and sex. In addition, a comparison with the variations of gastrin, in the same species and at different ages, has been performed.

Materials and methods

Blood samples have been drawn from 40 German shepherd dogs, 20 female and 20 male, of the Perugia Revenue Guard Corps breeding. The samples were taken in the course of routine health checks. The dogs were grouped according to the age in groups of 8 subjects, 4 female and 4 male, each.

The age ranges were:

1st group: 3-3.5 months

2nd group: 7-15 months

3rd group: 2-3 years

4th group: 4-6 years

5th group: 7-10 years

All the subjects have been submitted to clinical examination and routine laboratory tests (complete blood count, blood glucose, creatinine and urea). All the subjects had all the laboratory values within normal ranges. The blood samples, taken at 10 a.m. in animals fasting for 18 hours, were immediately centrifuged and serum was subdivided in aliquots and stored at -20°C until TLI determination, performed 72 hours after venepuncture. For the determination of TLI a polyclonal dog-specific double-antibody RIA kit, labeled with ^{125}I , was used (DPC, Diagnostic Products Corporation, Los Angeles CA, USA, distributed in Italy by Medical Systems, Genoa). In all the samples the TLI concentration was determined in the bound fraction, obtained by centrifugation at $+4^{\circ}\text{C}$ and after supernatant aspiration. The samples were read in a computerized gamma counter with NaI (T1) detector (Canberra Packard, Meriden, CT, USA).

Results

The reference range obtained in present study is reported in Table 1.

No variations related to sex were observed, except for a lightly higher standard deviation in males (Table 2).

Subjects number	Mean value	SD (standard deviation)	Reference range
40	12.5	3.4	5.7 - 19.3

males (mean value)	SD	females (mean value)	SD
12.3	4.6	12.6	3.2

Group	Mean value	Standard deviation
1° (3-3.5 months)	15.9	3.7
2° (7-17 months)	9.3	1.6
3° (2-3 years)	12.2	3.3
4° (4-6 years)	11.3	3.9
5° (7-10 years)	11.0	3.4

As regards variations related to the different age groups, a sharp decrease in the serum concentration of TLI was observed between the group 3-3.5 months to the group 7-15 months (Fig. 2). The values then stabilized, and no significant variations in the subsequent age groups was observed.

The values (mean and standard deviation) obtained in the different groups under study are reported in Table 3.

The values of gastrin in different age groups, determined in the same animals in a previous study of the same authors, are reported for a comparison in Fig.3 (Cavallone and others, 1999).

Given the importance of gastrin in regulating the synthesis of TLI, we compared the decrease of both parameters (TLI and gastrin) in percentage terms, calculated assuming the starting value as 100%. By this method a direct comparison of the trend of the two parameters in the time has been made possible, notwithstanding their different unit systems (Fig. 4).

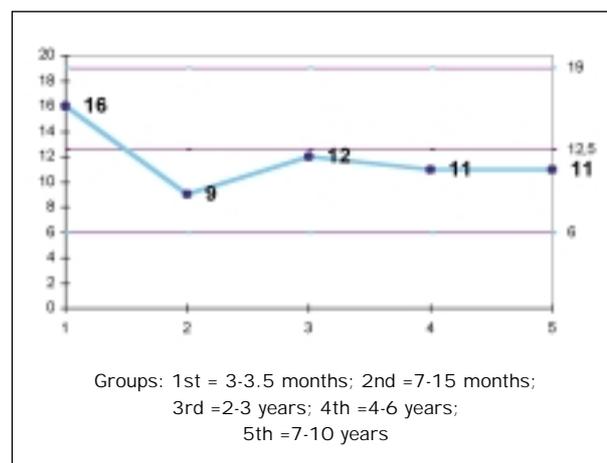


Figure 2. TLI values (ng/ml) in serum according to age.

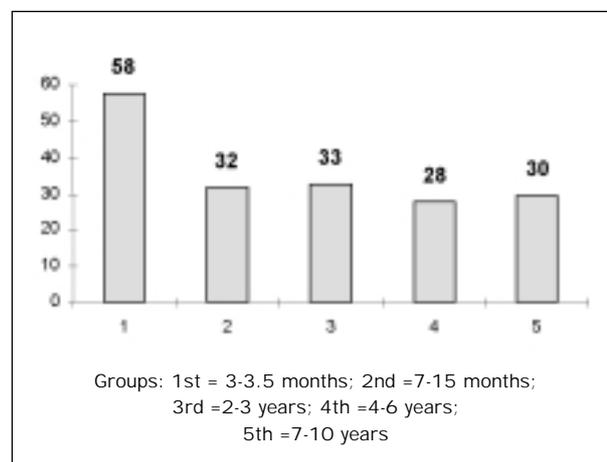


Figure 3. Gastrin values (pg/ml) in serum according to age.

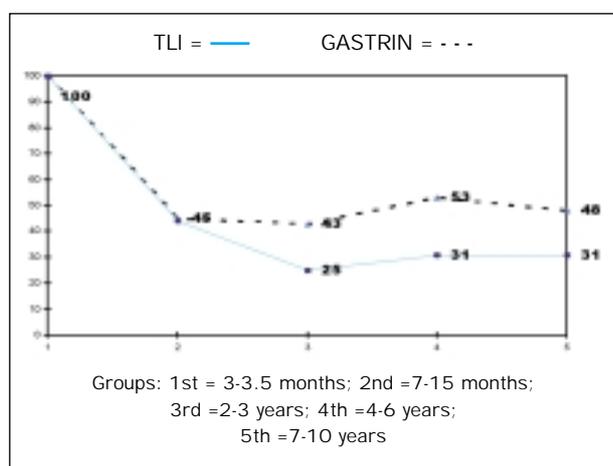


Figure 4. Percent decrease of Gastrin and TLI in serum in relation to age.

The decrease observed between the first (3-3.5 months) and the second group (7-15 months) was identical, and was similar in the subsequent age groups.

Discussion and conclusions

The determination of TLI in serum samples is highly reliable and dog-specific (Archer and others, 1997; Boari and others, 1993; Williams and Batt, 1988). The population sample studied was statistically significant, and therefore appropriate to establish the TLI reference values in German shepherd dog. Such reference values are comparable to those reported in literature (5,2 –35 ng/ml) also for other breeds, demonstrating that in the dog the breed does not influence the TLI serum concentration (Agugini and others, 1996; Archer and others, 1997; Boari and others, 1993; Braun, 1997; Williams, 1989). On the basis of present preliminary data we considered the TLI variations in the dog related to age, confirming trends already observed for PTH and gastrin (Cavallone and others, 1998; Cavallone and others, 1999). The growth of puppies is characterized by significant metabolic changes requiring by veterinary doctors to evaluate laboratory parameters carefully. The comparison between the proportional decrease of TLI and of gastrin in the time resulted was highly significant, confirming a suspected correlation of these two parameters in the dog. In fact also in this species the direct stimulatory activity of gastrin on the pancreatic secretion has been demonstrated (Le Drean and others, 1998; Nustede and others, 1993; Shulkes and Daldwin, 1997). The raised serum concentrations of both the parameters observed in the young subjects are probably related to the necessity to absorb proteins quickly, as they

have an important function in puppies' growth (Braun and others, 1997). With regard to gastrin, its main function is to stimulate the processes of digestion of proteins; the main function of TLI is the biochemical processing of proteins to allow absorption (Agugini and others, 1996; Faglia, 1997). The subsequent stabilization of these substances in the time, observed in adult animals, is probably related to the necessity to maintain a stable protein intake. As regards the sex of the dog, we did not observe any significant variation in healthy subjects. As the mechanisms regulating hormone secretions are very complex (e.g. their periodic secretion due to circadian cycles) the present study should be considered preliminary. Nevertheless we believe that it could be the basis for further investigations.

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High TLI levels are often present in dogs experiencing pancreatitis. However, that is not always the case. Some dogs with Pancreatitis have normal TLI levels. That may be due to the short period of time that this particular enzymes remain present in the blood stream after it is released. High TLI levels do not consistently occur in cats that have acute pancreatitis. So for them, the test is only helpful in the chronic pancreatic conditions that limit the amount of trypsin produced (exocrine pancreatic insufficiency or EPI). Reasons Why Your Dog or Cat Could Have Low TLI Levels : Both dogs and cats that have chronic pancreatic problems that have affected the amount of digestive enzymes the organ produces (exocrine pancreatic insufficiency or EPI) can be expected to have low TLI levels.