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**Citation for published version:**

Oikonomidis, IL & Milne, E 2023, 'Clinical enzymology of the dog and cat', *Australian veterinary journal*, vol. 101, no. 12, pp. 465-478. <https://doi.org/10.1111/avj.13291>

**Digital Object Identifier (DOI):**

[10.1111/avj.13291](https://doi.org/10.1111/avj.13291)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Publisher's PDF, also known as Version of record

**Published In:**

Australian veterinary journal

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## INVITED REVIEW

## Clinical enzymology of the dog and cat

IL Oikonomidis<sup>a,†,\*</sup> and E Milne<sup>a</sup>

Clinical enzymology studies the enzyme activity in serum or other body fluids for the diagnosis, prognosis or monitoring of a variety of diseases. Clinical enzymology has greatly benefited from advances in technology and is now an integral part of laboratory analysis. However, to maximise the clinical benefits of serum enzyme measurement, clinicians and clinical pathologists must have a good understanding of the pathophysiology behind serum enzyme alterations. They must also be aware of the preanalytical and analytical factors that can affect the accuracy of serum enzyme activity measurement. This review article first covers the basic concepts of clinical enzymology and the general mechanisms related to serum enzyme alterations. Then, the review discusses the potential effects of various preanalytical and analytical factors on enzyme activity measurement. Lastly, it explores the pathophysiology and clinical use of various serum enzymes in canine and feline medicine. The present review article aims to be a comprehensive one-stop source for clinical pathologists and small animal practitioners.

**Keywords** ALP; ALT; amylase; AST; CK; GGT; lipase; PLI; TLI

Aust Vet J 2023

doi: 10.1111/avj.13291

Clinical enzymology studies the enzyme activity in serum, plasma or other body fluids for diagnostic, prognostic or monitoring purposes. Since the first assay for measuring amylase was developed in the early 1900s, automated spectrophotometric analysers and commercially available reagents have greatly advanced the field of clinical enzymology. Clinical enzymology is now an integral part of laboratory analysis. However, a good knowledge of the pathophysiology of serum enzyme alterations is required for an appropriate interpretation. Additionally, clinicians must be aware of the potential effects of various preanalytical and analytical factors in enzyme measurement. This review covers the basic principles of clinical enzymology and the preanalytical and analytical factors that can affect enzyme activity measurement. It also explores the pathophysiology and clinical use of various serum enzymes in small animal practice. The enzymes that are covered in detail include alanine aminotransferase (ALT), aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), creatine kinase

(CK), lipase, amylase and trypsin-like immunoreactivity (TLI). Information on selected enzymes that are uncommonly used in small animal medicine is provided in Table 1.

**General principles**

Enzymes are proteins that catalyse chemical reactions and belong to one of the following classes based on the type of reaction they catalyse: (i) hydrolases, (ii) isomerases, (iii) ligases, (iv) lyases, (v) oxidoreductases and (vi) transferases.<sup>1</sup> Isoenzymes are enzymes that have different molecular structures but catalyse the same chemical reaction, whereas isoforms are enzymes that result from a posttranslational modification of an original gene product.<sup>2</sup> Many enzymes require cofactors, which convert the enzymes from apoenzymes to holoenzymes. The cofactors are often ions (e.g.  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ ) or related to vitamins (e.g. pyridoxal-5'-phosphate from pyridoxine or nicotinamide adenine dinucleotide from niacin).<sup>2</sup>

The enzymes are primarily located in the membrane, cytoplasm or mitochondria of cells. When the rate of an enzyme entering the blood exceeds the rate of inactivation or removal, the activity of the enzyme in serum increases.<sup>3</sup> Serum enzyme activity is typically increased by one or more of the following mechanisms: (i) increased release from damaged cells; (ii) induction of synthesis (and possibly cell proliferation); (iii) decreased clearance and (iv) increased ingestion and absorption (e.g. in colostrum).<sup>2</sup> The rate of the increase in serum activity of an enzyme generally reflects the severity of tissue damage, the intracellular concentration and location of the enzyme, the route of access to blood (e.g. direct release to blood, release to lymph, intestinal lumen or urine, blood-brain barrier) and the blood clearance rate.<sup>2</sup> The more extensive the tissue damage and the higher the concentration gradient between the tissue and blood, the greater will be the increase in serum activity of the enzyme. Not surprisingly, severe cell damage (e.g. necrosis) is required for a substantial release of mitochondrial enzymes, whereas cytoplasmic enzymes can be released more easily. The site of release is also very important, for example, the induction of ALP synthesis in enterocytes leads to its release into the intestinal lumen instead of blood.<sup>4</sup> The exact mechanism of enzyme removal from blood is not well established; some enzymes with small molecular weight, such as amylase and lipase, are filtered through the glomerulus and excreted in the urine, whereas others undergo endocytosis by hepatocytes or macrophages, or degradation by proteases and their activity is lost although they might continue to circulate in blood.<sup>4</sup>

Increased release from damaged or injured cells is the primary mechanism leading to an increase in the serum activity of cytoplasmic and mitochondrial enzymes. Cellular damage, whether reversible

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or irreversible, causes the release of cytoplasmic, and in some cases mitochondrial, enzymes into the extracellular fluid and subsequently into blood. This can occur through the rupture of cell membranes. An additional mechanism for cytoplasmic enzymes is the formation of cytoplasmic blebs secondary to cytoskeletal disruptions due to depletion of ATP, damage by proteases or lipases, binding of toxicants, and so forth. These blebs can rupture *in situ* or after release into blood.<sup>5–7</sup> If the concentration of the enzyme is higher in the cytoplasm of the cells compared to the extracellular fluid, this will result in an increase in serum activity, assuming that the enzyme is active in serum.

Induction of synthesis by cells is the major mechanism resulting in an increase in serum activity of the enzymes that are located in the cell membrane. This mechanism may also contribute to increased serum activity of cytoplasmic and mitochondrial enzymes. Induction refers to the increase in enzyme production via modifications in physiological processes like transcription and translation. This can be triggered by endogenous substances like bile acids or exogenous substances (e.g. specific drugs).<sup>8,9</sup> Cell proliferation can also be associated with increased serum enzyme activity, particularly those located in the cell membrane. This cell proliferation may result from a hyperplastic or neoplastic condition.<sup>2</sup>

The serum activity of enzymes can also be increased due to prolonged half-life (e.g. macroenzymes as occurs with amylase), decreased removal from blood (e.g. decreased renal excretion of amylase and lipase) and potentially decreased inactivation (e.g. due to a defect in binding to antiproteases, proteolysis or uptake by macrophages or hepatocytes).<sup>2</sup>

Decreased serum activity of routinely evaluated enzymes is typically regarded as clinically insignificant and may result from improper sample handling or the presence of anticoagulant. However, in certain instances, a decrease in serum activity of an enzyme may reflect a decline in the mass of the tissue of origin.

#### **Preanalytical and analytical concepts**

An enzyme (E) catalyses a chemical reaction by reacting with a specific substrate (S) to form a product (P) according to the following reaction:  $E + S \leftrightarrow ES \rightarrow P + E$ . Routine assays used for the measurement of serum enzyme activities determine the reaction rate by detecting how fast a substrate is consumed or a product is formed under conditions that are initially saturated with respect to substrate concentration. The reaction rate can be measured using both fixed-time reactions and continuous-monitoring methods.<sup>1</sup> The latter involve multiple readings during a specified time period and are preferred whenever possible.<sup>1</sup> Various analytical techniques (e.g. spectrophotometry, fluorometry and chemiluminescence) can be used to measure the amount of substrate transformed into the product during reaction catalysed by an enzyme.<sup>1</sup> Spectrophotometry is typically used for routine analysis.

The serum activity of enzymes is typically expressed in international units per litre (U/L). An international unit is the amount of enzyme that catalyses the conversion of 1  $\mu\text{mol}$  of substrate per minute under defined conditions.<sup>2</sup> Different assays can produce considerably different results; therefore, canine and feline patient results should always

be compared with the reference intervals generated for the specific assay.<sup>2</sup> Differences in reactions and substrates used, pH and incubation temperature, use of cofactors, activators or inhibitors and measurement times may all account for differences in serum enzyme activity results obtained.<sup>2</sup> Immunological assays have also been developed for specific enzymes and they provide a measurement of the concentration of the enzyme expressed in mass per unit of volume. This is the case with pancreatic lipase immunoreactivity (PLI) and TLI. These immunological assays may measure active and inactive forms or precursor forms of an enzyme.<sup>1</sup>

Serum is the preferred sample for measuring enzyme activity. The sample should be allowed to clot, and the serum should be separated within 30–60 min of blood collection to prevent the release of certain enzymes, such as AST or LDH, from erythrocytes.<sup>2</sup> Plasma can be used for some, but not all, enzyme measurements because most anticoagulants bind divalent cations, such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ , which are required as cofactors for certain enzymes, such as ALP, SDH, CK, amylase and lipase.<sup>10</sup> Haemolysis may affect the results of some enzyme assays.<sup>11</sup> A direct leakage or release of AST and LDH from erythrocytes may result in increased serum activity of these enzymes when haemolysed serum samples are examined. Other enzymes are not found in considerable concentrations in erythrocytes, but other molecules present in erythrocytes can participate in the assays used to measure the serum enzyme activity, as can happen with glucose-6-phosphate or adenylate cyclase and CK.<sup>10</sup> Finally, haemolysis as well as hyperbilirubinaemia and lipaemia can interfere with light transmission in spectrophotometric assays, leading to erroneous results.<sup>2</sup>

The stability of enzymes in serum varies depending on the enzyme and storage temperature, while species-dependent differences are also possible.<sup>12–14</sup> To the best of our knowledge, studies on the stability of enzymes in feline serum samples are currently lacking. In canine serum samples stored at room temperature (20°C), ALT, AST, GLDH, ALP, GGT, LDH, CK, lipase and amylase were found to be stable for 3 days.<sup>15</sup> Similarly, the enzymes ALT, AST, ALP, LDH and CK appear to remain stable in human serum samples stored at 4°C for 6 days.<sup>13</sup> Likewise, the stability of the same enzymes appears acceptable in rat serum samples when stored at 4°C for 7 days.<sup>14</sup> Regarding the stability of the enzymes when stored at -20°C, in a study using canine serum samples, the observed mean differences for ALT, AST, GLDH, ALP, GGT, CK, amylase and lipase between day 0 and day 90 are considered acceptable.<sup>12</sup> On the other hand, LDH was proven to be unstable.<sup>12</sup> ALT, AST, ALP, LDH and CK were also found to be stable in rat serum samples stored at -20°C for 3 months.<sup>14</sup> It is worth mentioning that, in one study using human serum samples stored at -20°C for a month, ALT was found to be unstable.<sup>13</sup> SDH is notorious for its instability and should be measured as soon as possible and certainly within 24 h, even if the serum is stored at -20°C.<sup>16</sup>

#### **Biological variation**

The results of serum biochemistry, including those of enzymes, are traditionally interpreted in relation to previously calculated population-based reference intervals. However, the concept of subject-based reference values, which was introduced in human medicine many years ago,<sup>17</sup> is increasingly discussed in veterinary medicine.<sup>18</sup>

**Table 1.** Selected less commonly used blood enzymes that have been studied in veterinary medicine

Enzyme	Reaction	Investigated diagnostic purpose	Selected references
5'-nucleotidase	Hydrolysis of various ribonucleoside-5'-monophosphates to form the corresponding ribonucleosides and inorganic phosphate	Hepatobiliary disease	41,153
Acid phosphatase	Hydrolysis of orthophosphate monoesters under acidic conditions	Benign prostatic hyperplasia and prostatic carcinoma	101,154–156
Aldolase	Cleavage of fructose 1-6-biphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in the Embden–Meyerhof pathway	Skeletal and cardiac myocyte injury	157–160
Arginase	Conversion of arginine to urea and ornithine in urea cycle	Hepatobiliary disease	161–164
Butyrylcholinesterase	Hydrolysis of choline and noncholine esters	Sepsis Metabolic diseases, such as obesity, diabetes mellitus and hyperadrenocorticism	165–168,168,169
Canine prostatic-specific esterase (CPSE)	Serine protease with chymotrypsin-like activity	Prostatic disease, including benign prostatic hyperplasia, prostatitis and prostatic carcinoma	154,156,170–172
Lysozyme (muramidase)	Hydrolysis of $\beta$ -1,4 linkages between N-acetylmuramic acid and 2-acetyl-amino-2-deoxy-D-glucose residues in bacterial cell walls	Neoplastic disease	173–175
Isocitrate dehydrogenase	Oxidative decarboxylation of isocitrate to $\alpha$ -ketoglutarate	Hepatobiliary disease	176
Ornithine carbamoyltransferase (OCT)	Reaction between ornithine and carbamoyl phosphate forming citrulline and inorganic phosphate	Hepatobiliary disease	4,177
Paraoxonase-1 (PON-1)	Hydrolysis of various organic esters and organophosphate compounds	Marker of inflammation and/or oxidative damage; studied in sepsis, pancreatitis, leishmaniosis and feline infectious peritonitis among other conditions.	178–183

According to this concept, for analytes that exhibit a high degree of inter-individual variation, the use of subject-based reference values is considered more appropriate compared to population-based reference intervals for interpreting results.<sup>17–19</sup> The determination of subject-based reference values requires knowledge of the biological variation, which refers to the variability in analyte concentration or activity around a homeostatic set point.<sup>18</sup> The decision whether to use population-based reference intervals or subject-based reference values for interpreting the results of each analyte is based on the index of individuality, which is a mathematical expression of individuality that incorporates the coefficient of variation (CV) of analytical performance, as well as the CV that occurs within and between individuals.<sup>18</sup> Biological variation studies on ALT, AST and ALP in dogs<sup>20</sup> and ALT, AST, ALP, GTT and CK in cats<sup>21</sup> have demonstrated that some of these enzymes are characterised by high individuality. Specifically, according to indices of individuality, the results of ALT and ALP in both species, along with AST in dogs, should be interpreted using subject-based reference values.<sup>20,21</sup> It's important to consider this when interpreting the results

of these enzymes, as values falling within the population-based reference intervals may be clinically important, whereas in other instances, values that fall outside of the reference intervals may not be clinically relevant.

#### **Alanine aminotransferase**

ALT, formerly known as glutamic pyruvate transaminase (SGPT), catalyses the reversible transamination of L-alanine and 2-oxoglutarate to pyruvate and L-glutamate. The formation of the active holoenzyme of ALT requires pyridoxal 5'-phosphate as a cofactor.<sup>4</sup> ALT is primarily a cytoplasmic enzyme found in various tissues, but its activity in hepatocytes is substantially higher compared to other tissues in dogs and cats, making it a specific indicator of hepatocellular injury in these species.<sup>22</sup> Although the ALT activity in skeletal and cardiac muscle is considerably lower than in the liver, generalised muscle injury (e.g. rhabdomyolysis) can increase ALT serum activity due to the large total body muscle mass.<sup>23</sup> The reported half-life of ALT varies considerably in the dog, ranging

from 3 to 60 h, with a half-life of 40–60 h most commonly cited.<sup>4,24,25</sup> In cats, the half-life of ALT is estimated to be around 3–4 h.<sup>26</sup>

ALT is used as a marker of hepatocellular injury in dogs and cats. The degree of increase in serum ALT activity reflects the degree of injury, but it cannot predict reversible versus irreversible hepatocellular injury and thus is not prognostic.<sup>2</sup> The highest serum activity of ALT is expected with hepatocellular necrosis and acute inflammation. After acute severe hepatocellular injury, ALT activity typically increases markedly (up to 100-fold normal) within 24–48 h, reaching its peak in the first 5 days postinjury.<sup>27–30</sup> If the injury resolves, serum ALT activity gradually decreases to normal over 2–3 weeks, while at the same time, serum ALP activity may increase due to the regenerative proliferative process.<sup>31</sup> However, in some instances, a declining ALT activity may also indicate a significant loss of hepatocytes. Importantly, chronic hepatitis is characterised by fluctuating serum ALT activity, and a declining trend may reflect improvement or loss of liver mass.<sup>2</sup> Interestingly, certain toxins, such as microcystin and aflatoxin B1, are potentially hepatotoxic but they may not be associated with increased ALT activity because they inhibit the hepatic biosynthesis of transaminases.<sup>31</sup>

The highest sensitivity of ALT in dogs is expected for the detection of necrosis (100%), followed by chronic hepatitis and extrahepatic bile duct obstruction (approximately 80%), whereas the lowest sensitivity is seen for conditions such as microvascular dysplasia (portal vein hypoplasia) and portosystemic vascular anomaly (less than 50%).<sup>31</sup> In cats, ALT is highly sensitive in detecting extrahepatic bile duct obstruction and cholangitis/cholangiohepatitis (sensitivity slightly higher than 90% and 80%, respectively), whereas its lowest sensitivity is noted for the detection of portosystemic vascular anomaly (close to 20%).<sup>31</sup> The sensitivity of ALT in detecting other hepatic diseases in dogs and cats is expected to be between 50% and 80%.<sup>31</sup> A recent study found that the sensitivity of ALT in detecting acute and chronic hepatitis in clinically healthy Labrador retrievers was 45% and 71%, respectively, with the specificity being high (93%).<sup>32</sup> This indicates that liver disease cannot be excluded in the absence of elevated serum ALT activity and its sensitivity in detecting subclinical hepatitis is lower. Nevertheless, it remains a specific marker of hepatocellular injury.

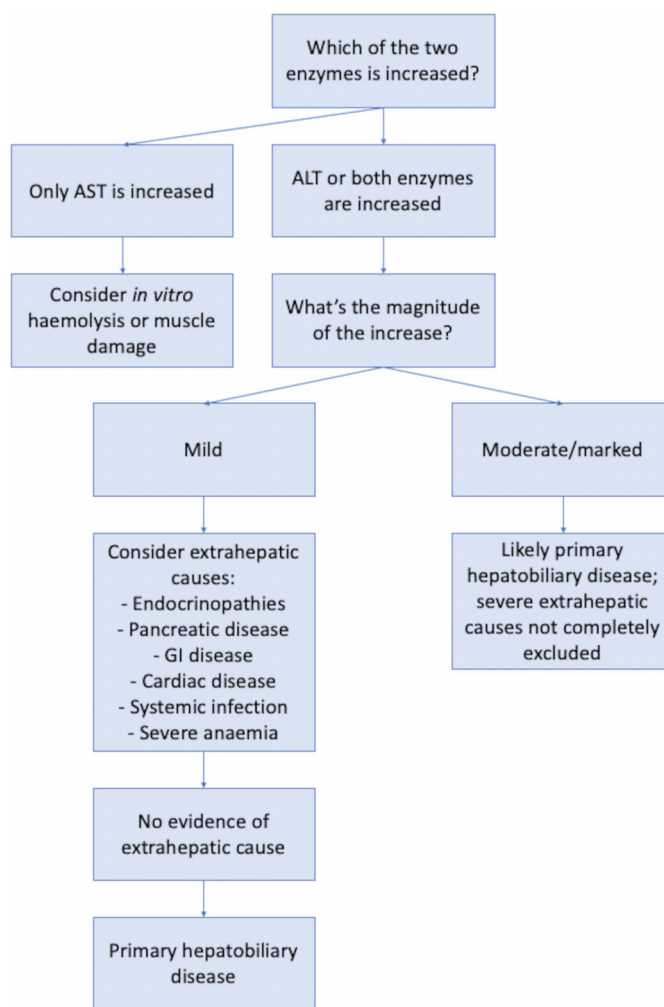
Certain conditions and medications can be associated with secondary changes to the liver (such as lipodosis, 'vacuolar' hepatopathy and reactive hepatitis), which can result in elevated serum ALT activity. Endocrinopathies (such as diabetes mellitus, hyperadrenocorticism and hyperthyroidism), pancreatic or gastrointestinal diseases, tissue hypoxia (e.g. secondary to cardiac disease or severe anaemia), metastatic neoplasia and systemic infections can all be associated with increased serum ALT activity.<sup>33–36</sup> Serum ALT activity can also increase with corticosteroid or phenobarbital therapy, and several weeks may be required before the normalisation of ALT activity after corticosteroid withdrawal.<sup>24,37</sup> Mild increases (up to 5-fold) in serum ALT activity are typically seen with these conditions and medications, but a moderate increase (up to 10-fold) may occur. Therefore, when mild increases in ALT activity are observed, it is important to consider extrahepatic conditions or drugs that may affect the liver before investigating the possibility of a primary liver disease. Conversely, a primary liver disease or hepatotoxicity secondary to drug administration (e.g. phenobarbital) should be considered

when the increase in serum ALT activity is marked (more than 10-fold the normal activity).<sup>33</sup> The suggested approach to increased ALT and AST activities in dogs and cats is summarised in Figure 1.

A decrease in serum ALT activity is generally considered to have no clinical significance. However, in certain cases of chronic liver disease or severe hepatotoxicity, it may indicate a significant loss of hepatocytes.<sup>31</sup> Theoretically, low systemic vitamin B6 concentration due to disease or drug administration (such as cephalosporins, cyclosporine or isoniazid) may also result in decreased serum ALT activity.<sup>38</sup>

### Aspartate aminotransferase

AST, formerly known as glutamic oxaloacetic transaminase (SGOT), catalyses the transamination of L-aspartate and 2-oxoglutarate to oxaloacetate and glutamate. Similar to ALT, pyridoxal 5'-phosphate is the cofactor of AST, forming the active



**Figure 1.** Suggested approach to increased serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in dogs and cats. An increase is considered mild if it is up to 5-fold the normal, moderate if it is 5- to 10-fold the normal and marked if it is more than 10-fold the normal. For more information, the reader is referred to the main text. GI, gastrointestinal.

holoenzyme.<sup>4</sup> AST is found in both cytoplasm and mitochondria and is present in several tissues, with the highest activity observed in the liver, and skeletal and cardiac muscles.<sup>22,23,26,39</sup> Additionally, a considerable amount of AST is found in erythrocytes, and thus, *in vitro* or *in vivo* haemolysis is expected to increase the serum AST activity.<sup>2</sup> The half-life of AST in dogs is somewhat controversial but it is most likely 12–22 h.<sup>24,38</sup> In cats, the half-life of AST is reported to be 1.5 h.<sup>24</sup>

Serum AST activity increases during hepatocellular injury and generally parallels the increase in serum ALT activity. However, the increase is typically milder (up to 30-fold normal in dogs and 50-fold normal in cats) and AST may normalise earlier as it has a shorter half-life than ALT.<sup>31</sup> Serum AST activity can increase due to both reversible and irreversible hepatocellular injury, but since mitochondria are a major site of AST, the increase during irreversible injury (e.g. necrosis) is expected to be greater. Like ALT, mild to occasionally moderate increases in serum AST activity may reflect secondary liver changes associated with certain conditions or medications, such as diabetes mellitus, hyperadrenocorticism, hyperthyroidism, pancreatic, gastrointestinal or cardiac diseases, severe anaemia, metastatic neoplasia, systemic infections and administration of corticosteroids or anticonvulsants.<sup>33–35</sup> It may take several weeks for the serum AST activity to normalise after corticosteroid withdrawal.<sup>37</sup> The suggested approach to increased AST and ALT activities in dogs and cats is summarised in Figure 1.

The sensitivity of AST for detecting hepatocellular damage in dogs is comparable to or maybe slightly lower than that of ALT. However, in cats, AST might be slightly more sensitive than ALT for detecting hepatobiliary diseases.<sup>31</sup> Despite this, AST is less specific for hepatocellular injury as it is found in considerable concentrations in both skeletal and cardiac muscles. Therefore, correlation with other enzymes, such as ALT and CK, is necessary when an increase in serum AST activity is noted. For instance, elevated serum activities of both ALT and AST with serum CK activity within the reference interval suggest hepatocellular injury, whereas elevated AST and CK serum activities with ALT within the reference interval suggest muscle injury. In addition, increased AST activity can also be caused by *in vitro* or *in vivo* haemolysis, so correlation with the haematology results and the colour of the serum is also advisable.

Like ALT, decreased serum AST activity is typically not considered clinically significant. Nonetheless, it may reflect significant loss of hepatocytes in certain cases of chronic liver disease or severe hepatotoxicity<sup>31</sup> or low systemic vitamin B6 concentration.<sup>38</sup>

#### **Sorbitol or iditol dehydrogenase**

Sorbitol or iditol dehydrogenase (SDH or ID) is a cytosolic enzyme that catalyses a reaction involving the conversion of sorbitol to fructose.<sup>4</sup> The highest activity of SDH is in the liver followed by the kidney while it is also present in small amounts in other tissues.<sup>22,26,39</sup> The half-life of SDH is as short as 5 h in dogs and 3–4 h in cats.<sup>26,40</sup> Serum SDH activity is an indicator of hepatocellular damage in all domestic animals and is superior to ALT and AST in detecting hepatocellular damage in ruminants and horses.<sup>2</sup> It does not offer a significant advantage over ALT in dogs and cats but it could be used as a sensitive indicator and monitoring tool for active hepatocellular

injury in dogs because of its short half-life. However, it is rarely used in small animal medicine due to its labile nature and the lack of availability of assays for routine measurement.<sup>41</sup>

#### **Glutamate dehydrogenase**

GLDH (also GMD, GLD or GDH) is a mitochondrial enzyme that catalyses the removal of hydrogen from L-glutamate to form the corresponding ketamine acid that then undergoes spontaneous hydrolysis to 2-oxoglutarate.<sup>4</sup> The liver has by far the highest activity of GLDH, followed by the kidneys and small intestine.<sup>22,39</sup> The half-life of GLDH has been estimated to be 8 h in dogs.<sup>40</sup> Like SDH, GLDH is a specific indicator of hepatocellular damage in all domestic animals.<sup>41,42</sup> Interestingly, in one old study, GLDH was found to be more sensitive than ALT in detecting liver disease in dogs.<sup>41</sup> As GLDH is a mitochondrial enzyme, this finding was unexpected, but it could be explained by the fact that the vast majority of the cases included in that study was histologically characterised by hepatocellular necrosis.<sup>41</sup> Despite its high specificity for detecting hepatocellular damage, GLDH is not routinely used in clinical practice, possibly because it is mostly available in referral laboratories. An increase in GLDH activity was observed in 15% of dogs receiving phenobarbital, but the underlying mechanism (induction vs hepatocellular injury) is unclear.<sup>43</sup>

#### **Lactate dehydrogenase**

LDH is a cytoplasmic enzyme that catalyses the reaction that converts pyruvate to lactate at the end of anaerobic glycolysis. There are five different LDH isoenzymes, which are tetramers composed of heart or muscle subunits.<sup>2</sup> The half-life of LDH in dogs is less than 6 h.<sup>44</sup> LDH is widely distributed in tissues, with the highest activity found in the liver, heart and skeletal muscles and kidneys.<sup>22</sup> Red blood cells also have a substantial LDH activity.<sup>2</sup> Consequently, increases in serum LDH activity are nonspecific because they can be associated with hepatocellular damage, cardiac or skeletal myocyte injury or haemolysis (*in vitro* or *in vivo*, even mild). As a result, LDH is less useful in clinical practice compared to other enzymes, such as ALT for hepatocellular damage and CK for muscle injury. The measurement of the different LDH isoenzymes is possible using electrophoretic assays and can improve the specificity of LDH, but this is rarely performed in clinical practice.<sup>45</sup> Serum LDH has been evaluated for the diagnosis and prognosis of canine lymphoma. Although it has been found to be increased in dogs with lymphoma, a substantial overlap was detected between these dogs and those with other neoplasms or nonneoplastic diseases.<sup>46</sup> High serum LDH activity was initially associated with shorter survival rates in dogs with lymphoma<sup>47</sup> but this was not confirmed in another study.<sup>48</sup> However, the results of the latter study suggested a prognostic role for the serial measurement of serum LDH, as dogs with elevated serum LDH immediately after or a month after chemotherapy were more likely to relapse within 1.5 months.<sup>48</sup> The mechanism of this increase in serum LDH activity is not clear, but the authors of this study speculated that accelerated turnover of one or more novel neoplastic clusters may be implicated.<sup>48</sup>

### Alkaline phosphatase

Alkaline phosphatases hydrolyse a range of monophosphates or pyrophosphates at alkaline pH. Alkaline phosphatases are attached to the cell membrane via a hydrophobic phosphatidylinositol-glycan anchor.<sup>4</sup> In the dog, the highest concentration of ALP is found in descending order in the intestinal mucosa, renal cortex, placenta, liver and bone.<sup>31</sup> In the cat, the highest concentration is found in the intestine or kidney (depending on the study), with lower concentrations in the liver and bone.<sup>49–51</sup> Two genes produce two ALP isoenzymes in domestic animals: the intestinal ALP (I-ALP) and tissue nonspecific ALP. The tissue nonspecific ALP is posttranslationally modified to form two isoforms found in the liver (L-ALP) and bones (B-ALP). In dogs, there is also a unique corticosteroid-associated ALP (C-ALP), which has the same amino acid sequence as I-ALP but is more highly glycosylated.<sup>2</sup> The half-life of ALP found in the placenta, kidney and intestine in dogs is less than 6 min and, similarly, the half-life of I-ALP in cats is 2–4 min.<sup>31,52</sup> Therefore, despite the high concentration of ALP in these tissues, this ALP does not contribute to serum ALP activity due to its extremely short half-life. Equally important is the location of ALP in the intestine and kidney. Specifically, ALP is located on the tips of villi of the enterocytes<sup>53</sup> and on the luminal surface of the proximal renal tubular epithelial cells<sup>54</sup> and is therefore lost in the intestinal lumen and urine, respectively. Consequently, only L-ALP and B-ALP (and C-ALP in dogs) significantly contribute to serum ALP activity. The half-life of L-ALP and C-ALP in dogs is approximately 3 days,<sup>55</sup> whereas the half-life of L-ALP in cats is approximately 6 h.<sup>52</sup> To our knowledge, the half-life of B-ALP has not been determined yet.

The contribution of different ALP isoforms to serum ALP activity can be determined by affinity electrophoresis.<sup>56</sup> C-ALP activity can be selectively inhibited by levamisole and is relatively heat stable at 56°C and 65°C, allowing the contribution of C-ALP to total ALP activity to be determined by adding levamisole to serum or incubating serum in a heated water bath. B-ALP and C-ALP can be selectively inhibited by wheat germ lectin; therefore, L-ALP activity can be determined and if combined with the previous tests, B-ALP activity can also be calculated.<sup>56,57</sup> However, the determination of serum activity of L-ALP, B-ALP and C-ALP has questionable clinical significance.

Serum ALP activity increases in both dogs and cats due to intrahepatic or extrahepatic cholestasis. In experimentally induced cholestasis in dogs, serum L-ALP activity started increasing after 24 h and reached a 30- to 40-fold increase at 4–7 days.<sup>58</sup> A similar response was observed in cats with experimentally induced cholestasis, although the increase was substantially milder and between fivefold and eightfold normal activity,<sup>51</sup> essentially reflecting the shorter half-life of feline L-ALP. The mechanism of increased serum L-ALP activity in cholestatic disorders was originally attributed to the regurgitation of ALP from bile into blood through tight junctions. Despite the presence of disruptive changes within tight junctions during cholestasis,<sup>3</sup> the passage of macromolecules like ALP is doubtful.<sup>59</sup> Instead, the primary underlying mechanism appears to be the accumulation of bile acids in hepatocytes, which stimulate the production of L-ALP and promotes the accumulation of L-ALP on sinusoidal hepatocyte membranes.<sup>2</sup> This location in hepatocytes makes it susceptible to release into blood or hepatic lymph.

The sensitivity of serum ALP activity in detecting hepatobiliary disorders has been reported around 70%–75% in dogs, with the highest sensitivity noted for necrosis, mucocele and extrahepatic bile duct obstruction (more than 80%).<sup>60</sup> However, the specificity of ALP is low for diagnosing hepatobiliary diseases in dogs.<sup>60</sup> In cats, the sensitivity of serum ALP in diagnosing hepatobiliary disorders is lower than in dogs (approximately 65%–70%), with the highest value reported for hepatic lipidosis (approximately 80%).<sup>31</sup> However, serum ALP is considered more specific for detecting cholestasis in cats than in dogs. Serum ALP can increase before icterus appears in dogs while cats are usually icteric before an increase in serum ALP is observed.<sup>2</sup> It should be noted that serum ALP activity can increase in dogs with nodular hyperplasia of the liver up to twofold, but more marked increases cannot be excluded.<sup>34</sup> However, in the authors experience, liver function, as assessed by bile acids, is not expected to be compromised in these cases. Therefore, mild elevations in serum ALP activity in older dogs without overt evidence of liver disease can be monitored initially. On the other hand, an increase in serum ALP activity in cats should always be investigated further.

There are probably several drugs with the potential to increase the production of L-ALP. The mechanism of this increase is believed to involve primarily the induction of synthesis of the enzyme, but other mechanisms (e.g. hepatotoxicity, secondary response of liver to drug-mediated cytokines) are also possible. Corticosteroids are known to induce ALP synthesis in dogs; they initially induce the synthesis of L-ALP, followed by the appearance of C-ALP in 7–10 days.<sup>61,62</sup> This increase in serum ALP activity can be very high, similar to that seen in cholestatic disorders.<sup>31</sup> Hence, serum ALP is an excellent screening test for hyperadrenocorticism in dogs, but it lacks specificity as C-ALP can also be elevated in chronically ill dogs due to increased endogenous glucocorticoids.<sup>31</sup> Anticonvulsants, such as phenobarbital, primidone and phenytoin, have also been associated with increased serum ALP activity in dogs,<sup>63,64</sup> thought to be due to induction and only occasionally due to true hepatotoxicity.<sup>50,65</sup> The increase is expected to be up to 5-fold the normal ALP activity, but increases as high as 30- to 40-fold the normal activity have been reported.<sup>31</sup> Interestingly, in cats, neither corticosteroid nor phenobarbital administration has been associated with elevated serum ALP activity.<sup>51</sup>

Increased osteoblastic activity is associated with increases in B-ALP and therefore can increase serum ALP activity. The increase is usually mild (less than 5-fold the normal activity) but it can reach as high as 12-fold normal activity in some cases.<sup>57</sup> B-ALP is expected to be increased during the healing phase of a fracture and in puppies and kittens until the age of 7 months due to normal growth.<sup>38,66</sup> Pathological conditions that can result in increased B-ALP include bone tumours, renal secondary hyperparathyroidism, osteomyelitis and feline hyperthyroidism, although in the latter L-ALP contributes substantially to increased serum ALP activity.<sup>38,67,68</sup> Importantly, elevated serum ALP activity has been associated with reduced survival time in patients with osteosarcoma.<sup>67</sup>

Almost half of dogs with mammary neoplasia exhibit elevated serum ALP activity, which does not typically exceed eightfold normal activity.<sup>69</sup> The source of the increased serum ALP activity is unknown; however, in the absence of detectable liver and bone metastases,

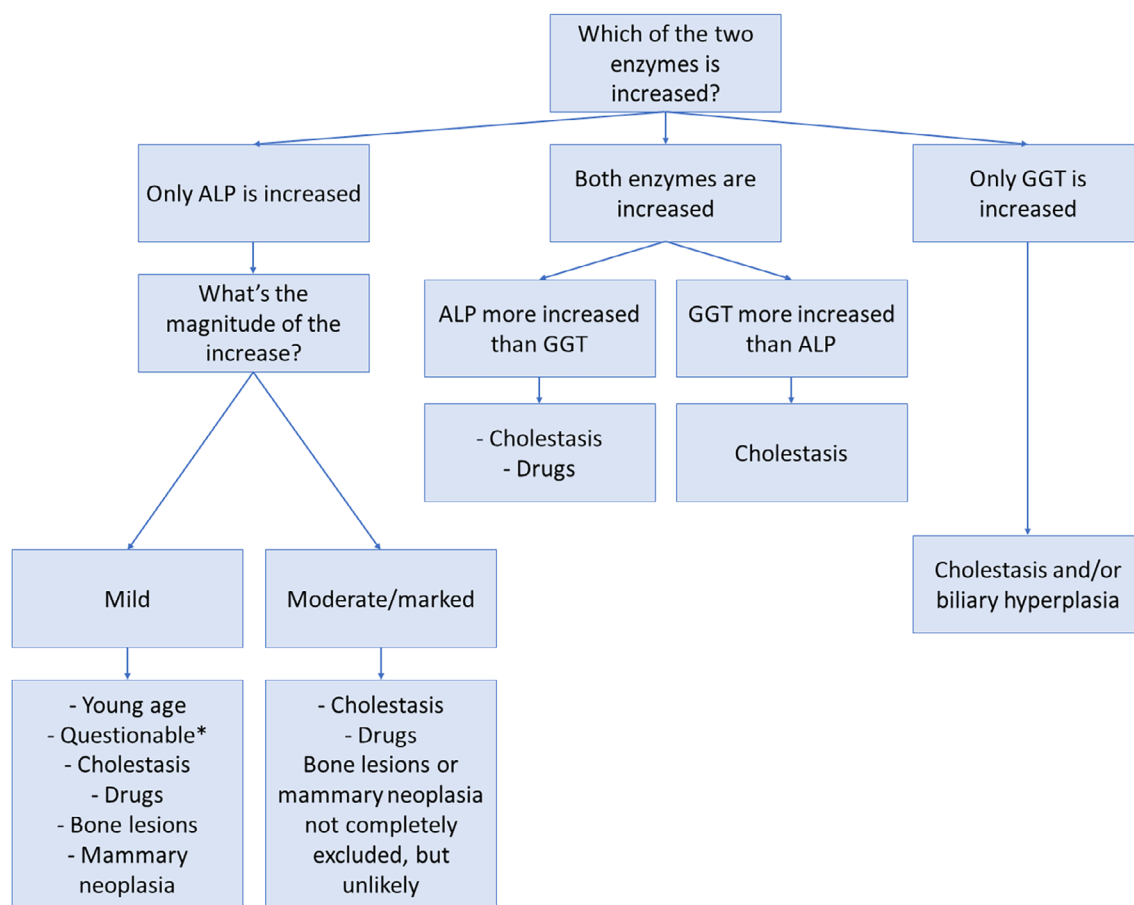
myoepithelial cells of mammary neoplasms appear a likely source of ALP.<sup>70</sup> Similar to pregnant women, serum ALP activity may be increased in cats in late-term pregnancy, but the increase noted in pregnant dogs is too minimal to affect the interpretation of serum ALP.<sup>2</sup> Marked increases in serum ALP activity have also been observed in 1- to 3-day-old puppies and 1- to 2-day-old kittens after ingestion of colostrum.<sup>71,72</sup> Finally, benign inherited hyperphosphatasemia has been reported in humans and also in Siberian huskies.<sup>73,74</sup> Known as benign familial hyperphosphatasemia, the condition was reported in related litters of Siberian husky puppies, which showed a mean ALP increase of fivefold that of unaffected Siberian huskies of the same age without any evidence of disease. B-ALP was found to be the isoform that was responsible for the observed increase.<sup>73</sup> The suggested approach to increased ALP activity in dogs is summarised in Figure 2. To our knowledge, decreased serum ALP activity has not been associated with an underlying pathological condition and is considered clinically insignificant.

### Gamma-glutamyltransferase

GGT functions in the gamma-glutamyl cycle where it catalyses the transfer of gamma-glutamyl groups from gamma-glutamyl peptides,

such as tripeptide glutathione to other peptides, amino acids and water.<sup>4</sup> GGT is located on the external surface of cells, where it is bound to the membrane via a hydrophobic transmembrane peptide.<sup>4</sup> The highest concentrations of GGT in dogs and cats are found in the kidneys and pancreas, with lower concentrations present in the liver, gall bladder, intestine, spleen, cardiac and skeletal muscles, lungs and erythrocytes.<sup>58,75</sup> Despite the high concentration of GGT in kidneys and pancreas, only GGT present in the liver contributes to increased serum GGT activity, according to the results of electrophoretic studies.<sup>76</sup> This is because GGT is located in the luminal surface of the proximal renal tubular cells in the kidney and in the cells lining the acini and pancreatic ducts, which restricts the release of the enzyme into the blood.<sup>4</sup> GGT is primarily found on biliary epithelial cells in the liver, but it has also been found on the canalicular and sinusoidal surface of hepatocytes in rats, albeit in considerably lower concentration.<sup>77</sup> To our knowledge, the half-life of GGT has not been studied, but in dogs, serum GGT and ALP activities change in parallel during cholestasis, indicating that the half-life of GGT is likely similar to that of ALP, which is 3 days in dogs.<sup>55</sup>

Serum GGT activity is typically an indicator of intrahepatic or extrahepatic cholestasis and/or biliary hyperplasia. The mechanism of



**Figure 2.** Suggested approach to increased serum activities of alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) in dogs. An increase is considered mild if it is up to 5-fold the normal, moderate if it is 5- to 10-fold the normal and marked if it is more than 10-fold the normal. The interpretation of increased ALP and GGT is more straightforward in cats. For more information, the reader is referred to the main text. \*In older dogs, nodular hyperplasia can be associated with increased ALP (typically up to twofold), but no evidence of liver disease.



elevated serum GGT activity during cholestasis is likely similar to that described previously for ALP and involves the induction of synthesis due to the accumulation of bile acids or other substances.<sup>9</sup> Interestingly, according to experimental studies on rats, the increase in serum GGT activity appears to be primarily dependent on the degree of biliary hyperplasia rather than induction of synthesis, cholestasis or hepatocellular injury.<sup>78</sup> Certainly, if the increase in serum GGT activity is persistent, biliary hyperplasia should be suspected. Experimentally induced hepatic necrosis and reversible hepatocellular damage are associated with no to minimal increase in serum GGT activity.<sup>58,79</sup> Serum GGT activity increases 1- to 4-fold within 4 days and up to 10- to 50-fold within 1–2 weeks after extrahepatic bile duct obstruction in dogs. After the first 2 weeks, the serum activity may plateau or increase further to as high as 100-fold normal activity.<sup>31,58</sup> In cats, serum GGT activity may increase up to 2-fold within 3 days, 3- to 12-fold within a week and 4- to 16-fold within 2 weeks.<sup>31,80</sup> In one study of 270 dogs with liver disease, serum ALP had a higher sensitivity (85%) but lower specificity (51%) compared to serum GGT (46% sensitivity and 87% specificity).<sup>60</sup> On the other hand, in a study of 69 cats, serum GGT was more sensitive for detecting liver disease than serum ALP (83% sensitivity vs 43% sensitivity).<sup>81</sup> However, in cats with hepatic lipidosis, the increase in serum ALP activity was often greater than that of serum GGT.<sup>81</sup> In summary, the concurrent measurement of both ALP and GGT activities improves the sensitivity and specificity for detecting cholestasis in both dogs and cats.

GGT synthesis appears to be induced in dogs on long-term phenobarbital treatment, but the increase is expected to be mild (up to threefold) unless hepatotoxicosis develops.<sup>65</sup> Although glucocorticoids did not induce GGT synthesis in cultured hepatocytes,<sup>78</sup> an increase in serum GGT activity can be observed in dogs receiving corticosteroid therapy. This increase may be due to induction or the result of 'steroid hepatopathy'.<sup>62</sup> Administration of corticosteroids has been associated with a 4- to 7-fold increase in serum GGT within a week and up to 10-fold increase within 2 weeks.<sup>82,83</sup> On the other hand, corticosteroids or anticonvulsants are not associated with a clinically significant induction of GGT synthesis in cats.<sup>31,84</sup>

It is also worth noting that 1- to 3-day-old puppies normally have elevated serum GGT (up to 100-fold increase) due to the ingestion of colostrum, which is rich in GGT, but GGT activity returns to normal levels within 10 days of suckling.<sup>71</sup> This phenomenon has not been reported in cats. A decrease in serum GGT activity is not considered clinically significant as it has not been associated with any underlying pathological condition.

### **Creatine kinase**

CK catalyses the exchange of a phosphate moiety between creatine phosphate and ATP. In myocardial and skeletal muscles, CK allows energy storage as creatine phosphate when demand is low and catalyses the transfer of the high-energy phosphate from creatine phosphate to ADP to form ATP when energy is needed for muscle contraction.<sup>4</sup> In both dogs and cats, by far the highest CK activity is found in skeletal and cardiac muscles, followed by the brain and smooth muscles.<sup>22</sup> In dogs, skeletal muscles contain more than twice the CK concentration of the cardiac muscle, while in cats, the

concentration between the skeletal and cardiac muscles is similar.<sup>22</sup> CK is primarily located in the cytoplasm and only a small percentage is found in the mitochondria.<sup>4</sup>

There are two distinct subunits of CK: the muscle (M) subunit and the brain (B) subunit. These subunits are randomly combined to form three different CK isoenzymes: CK-MM, CK-MB and CK-BB. In most species, skeletal muscles contain nearly 100% CK-MM.<sup>22</sup> The cardiac muscle also primarily contains CK-MM with a smaller amount of CK-MB. The amount of CK-MB in cardiac muscle varies substantially between the different species with dogs having a very small amount (98% CK-MM and 2% CK-MB).<sup>22</sup> The brain primarily to exclusively contains CK-BB.<sup>4,22</sup> Serum CK has a very short half-life reported to be around 2 h.<sup>2</sup> However, after intramuscular injection of muscle homogenate in dogs, the half-life of CK was approximately 6.5 h, with lymphatic absorption from the site being the rate-limiting factor.<sup>85</sup> This relatively longer half-life of CK might better reflect the source of CK in blood after a muscle injury. The mechanisms of CK clearance from blood are unknown, but inactivation is possible, as CK is not excreted in urine and arrest of hepatic blood flow did not affect the clearance rate in one study.<sup>86</sup>

CK is a marker of myofiber injury, which can be either reversible or irreversible. The degree of the increase in serum CK activity generally reflects the extent of muscle damage but it should be noted that serum CK rises and falls rapidly due to its short half-life.<sup>2</sup> In dogs and cats, elevated serum CK activity is used primarily as an indicator of skeletal muscle damage. Marked increases in serum CK activity (more than 20,000 U/L) are typically seen in dystrophic and necrotising myopathies, whereas moderate increases (between 2000 and 20,000 U/L) are usually seen with generalised inflammatory myopathies.<sup>87–89</sup> On the other hand, focal inflammatory myopathies, such as masticatory muscle myositis, endocrine myopathies and neuropathies, are usually associated with only mild (less than 2000 U/L) to no increase in serum CK activity.<sup>87</sup> Exercise-induced mild to moderate increases of serum CK activity have been reported in trained sled dogs,<sup>4</sup> but only minimal increases were noted in a study on untrained beagle dogs.<sup>90</sup> Nonetheless, it is reasonable to consider exercise as a potential cause of mild to moderate increase in serum CK activity. Injury to organs containing smooth muscle may potentially cause an increase in serum CK activity, but this is expected to be substantially lower compared to that seen with striated muscle injury.<sup>2</sup> Other enzymes such as AST and LDH may also increase with myofiber injury, but they lack the tissue specificity of CK. On the other hand, the short half-life of CK may reduce its diagnostic sensitivity but it also makes serum CK an excellent tool for monitoring response to therapy.

Electromyographic studies, surgical procedures, prolonged recumbency and intramuscular injections have also been associated with mild to moderate increases in serum CK activity; with intramuscular injections, the peak is noted at about 4 h and CK returns to baseline by day 3.<sup>91</sup> Therefore, a nonpersistent, mild to even moderate increase in serum CK activity is of questionable clinical significance as it can be associated with restraint, recumbency or minor trauma. Interestingly, anorexia in cats has been associated with mild to marked increases in serum CK, which is thought to result from muscle catabolism secondary to decreased caloric intake.<sup>92</sup> Refeeding these

feline patients results in a substantial decline in serum CK activity in 48 h; if serum activity of CK does not decline, an underlying neuromuscular disease should be considered assuming that external factors (e.g. restraint, trauma and intramuscular injection) have been excluded.<sup>92</sup> If serum CK activity is markedly or persistently elevated, a congenital or inherited muscle disease should be considered even if the animal appears asymptomatic.<sup>93</sup>

Serum CK activity is often elevated in dogs and cats with neurological disorders. This was traditionally thought to be a result of involuntary skeletal muscle contractions or recumbency.<sup>2,4</sup> However, a recent study found that various types of neurological diseases (degenerative, inflammatory, space-occupying and idiopathic epilepsy) were associated with increased CK-BB activity.<sup>94</sup> This increase may be due to the release of the isoenzyme from the cytoplasm of neurons or to changes in the blood–brain barrier.

Finally, it is important to note that puppies and young dogs have higher CK activity compared to adult dogs. Serum CK activity is approximately 4-times higher in puppies less than a month, 2- to 3-times higher in puppies aged 1–6 months and about 1.5-times higher in young dogs aged 6–12 months.<sup>95</sup> The reason for these age-specific differences is not known, but it has been assumed that the increase in serum CK activity can be related to normal development of musculature.<sup>96</sup>

### Amylase

$\alpha$ -Amylase is a low-molecular-weight enzyme that cleaves the  $\alpha$ -D-(1–4) glycan linkage of starch and glycogen.<sup>4</sup> Four different isoenzymes or isoforms (I–IV) of amylase have been identified using cellulose acetate and agarose gel electrophoresis.<sup>97,98</sup> Isoenzyme IV includes macroamylases, which are protein complexes composed of amylase bound to other proteins, such as immunoglobulins.<sup>2</sup> The pancreas has by far the highest activity of amylase in dogs and cats,<sup>99</sup> but serum amylase activity is decreased up to 50% after pancreatectomy, indicating that nonpancreatic sources contribute to serum amylase activity.<sup>100</sup> Nonpancreatic sources of serum amylase, at least in healthy animals, include intestinal amylase, macroamylase and possibly hepatic amylase.<sup>101,102</sup> The estimated half-life of serum amylase is 1–5 h.<sup>103–105</sup>

There are three major assays for measuring serum amylase activity. In dogs, the preferred method is the amyloclastic assay, as it is not affected by the presence of glycoamylase, which is normally found in canine serum.<sup>2</sup> Glycoamylase and maltase can interfere with the saccharogenic assay, resulting in falsely increased serum amylase activity.<sup>106</sup> The performance of the chromogenic assays can be acceptable but it depends on the specific assay used.<sup>107</sup>

In dogs, serum amylase increased 8- to 29-fold within 1–3 days in experimentally induced pancreatitis and returned to normal in 3–8 days.<sup>98,108,109</sup> However, the sensitivity and specificity of serum amylase in dogs are suboptimal and reported to be 13%–78% and 77%, respectively.<sup>97,110,111</sup> Hence, many dogs with pancreatitis have serum amylase activity within the reference interval. A cut-off value of threefold the upper reference limit has been suggested to increase specificity,<sup>112</sup> but serum amylase activity can reach this level in nonpancreatic conditions, such as renal, hepatic and intestinal

disease.<sup>113,114</sup> In cats, spontaneous pancreatitis has been associated with no to mild increases in serum amylase activity (less than threefold the upper reference limit).<sup>2</sup> Interestingly, in cats with experimentally induced pancreatitis serum amylase was not increased.<sup>115</sup> Therefore, serum amylase is not recommended for the diagnosis of feline pancreatitis.<sup>116</sup>

Decreased renal excretion and/or inactivation can result in elevated serum amylase activity, which is expected to be mild and typically less than threefold the upper reference limit.<sup>2</sup> Therefore, caution is advised when interpreting mild increases in serum amylase activity in dogs and cats with decreased glomerular filtration rate. Unlike serum lipase, two studies found that corticosteroid therapy does not affect serum amylase activity.<sup>117,118</sup>

### Lipase

Lipase is a low-molecular-weight protein that hydrolyses triglycerides at the 1 and 3 positions, resulting in the formation of a monoglyceride.<sup>4</sup> Several lipases are found in different tissues, including pancreas, stomach, liver, muscle and adipose tissue.<sup>2</sup> In dogs, the highest lipase activity is found in the pancreas, followed by the intestine, and then considerably less activity is noted in other organs.<sup>22</sup> The blood half-life of lipase from pancreatic extracts or juice has been calculated between 1 and 3 h in dogs<sup>103,104</sup> and has been found to increase after nephrectomy, indicating excretion and/or inactivation in the kidneys.<sup>104</sup>

Lipase is used to detect pancreatic disease in clinical practice. In dogs, serum lipase activity showed an approximately 25-fold increase within 2–5 days after experimental induction of pancreatitis. Serum amylase activity tends to follow this increase but returns to reference interval sooner.<sup>98,108,109</sup> In cats, serum lipase activity showed a two-fold to fourfold increase within 4 days after experimental induction of pancreatitis.<sup>115</sup>

The original pancreatic lipase assay used an incubation medium of an emulsion of long-chain triglycerides in a buffer containing glycocholic acid and colipase in an effort to minimise nonpancreatic lipase and esterase activity. However, this method does not completely inhibit the activity of these enzymes, which leads to a wider reference interval for lipase and a decreased sensitivity in detecting pancreatic disease.<sup>4</sup> The specificity is also reduced due to an increase in false-positive results (e.g. due to corticosteroid administration).<sup>4</sup> Studies have shown that nonpancreatic lipase significantly contributes to serum lipase activity as measured with this assay. Specifically, in dogs that underwent pancreatectomy, serum lipase activity remained approximately at half of the presurgery activity.<sup>100</sup> Another study of dogs with exocrine pancreatic insufficiency (EPI) found that their mean serum lipase activity was not significantly different from that of control dogs.<sup>119</sup>

A radioimmunoassay for the measurement of canine PLI was introduced 20 years ago<sup>120</sup> and later replaced by a quantitative ELISA, known as the specific pancreatic lipase.<sup>112</sup> This assay measures the actual concentration of pancreatic lipase instead of its activity. Studies have investigated the diagnostic performance of the PLI and found to be superior to lipase. The sensitivity of PLI for diagnosing histologically confirmed pancreatitis in dogs was reported to be between 26% and 71% when the cut-off value for pancreatitis (>400  $\mu\text{g/L}$ ) was applied.<sup>121–124</sup> As expected, the highest sensitivities

were observed for moderate to severe pancreatitis and the lowest for mild pancreatitis. The specificity was reported to be between 90% and 100%.<sup>121–123</sup> In the same studies, when the upper reference limit of PLI (200 µg/L) was used as a cut-off value, the sensitivity was 43%–71% and the specificity was 80%–95%.<sup>121–123</sup> In studies that used clinical criteria to diagnose pancreatitis, the sensitivity of PLI was 72%–91% and the specificity was 74%–88% when the cut-off value for pancreatitis was applied.<sup>125–127</sup> When the upper reference limit of PLI was used as a cut-off value, the sensitivity and specificity were 87%–94% and 66%–77%, respectively.<sup>127</sup> On the other hand, the sensitivity of serum lipase activity for diagnosing canine pancreatitis varies widely from 14% to 71%, depending on the cut-off value (upper reference limit vs threefold the upper reference limit) and the gold standard used to diagnose pancreatitis (pathological findings, histopathology or clinical criteria).<sup>111,122–124,127</sup> The specificity of serum lipase ranges from 41% to 81%.<sup>123,127</sup> In cats, the sensitivity of PLI for diagnosing pancreatitis ranges from 54% to 74% for mild inflammation and 63% to 100% for moderate to severe inflammation, depending on the type of inflammation and the cut-off value used.<sup>128,129</sup> The specificity of PLI was reported to be 74% and 91%.<sup>128,129</sup> Another study of cats with histologically confirmed pancreatitis reported a sensitivity of 53%–61% and specificity of 55%–100% when the upper reference limit was used as a cut-off value and a sensitivity of 42%–61% and specificity of 69%–100% when the cut-off value for pancreatitis was applied.<sup>130</sup> PLI is considered the gold standard laboratory test for the diagnosis of pancreatitis in both dogs and cats and animals with clinically significant pancreatitis typically have an increased PLI. A low PLI is expected in EPI, but TLI is considered more accurate.<sup>119</sup>

An assay using 1,2-o-dilaryl-rac-glycero-3-glutaric acid-(6'-methylresorufin) ester (DGGR) as a substrate has been validated for use in dogs and cats and showed a better performance compared to the traditional assay for measuring serum lipase activity. The sensitivity and specificity of the DGGR assay in diagnosing pancreatitis in dogs were reported to be 63%–91% and 64%–92%, respectively.<sup>125,131,132</sup> In cats, the sensitivity and specificity of the DGGR assay were reported to be 37%–67% and 79%–100%, respectively.<sup>130</sup> While the DGGR assay appears to be more accurate for diagnosing pancreatitis compared to traditional lipase assays, a recent study found that DGGR can also be hydrolysed by hepatic and lipoprotein lipases, suggesting that it may not be as specific as originally believed.<sup>133</sup>

Pancreatic and extrapancreatic neoplasia (such as liver neoplasms) have been associated with dramatic increases in serum lipase activity (up to 93-fold the upper reference limit) in the absence of increased serum amylase activity. This suggests that the proliferation and possible damage of neoplastic cells containing lipase can cause a rise in serum lipase activity.<sup>134</sup>

Elevated serum lipase activity has been associated with decreased renal excretion and/or inactivation.<sup>104</sup> This increase is generally mild (up to 4-fold normal) but can be as high as 10-fold normal in cases of renal failure without evidence of pancreatitis.<sup>114</sup> Increases in PLI and lipase measured with DGGR assay have also been reported, albeit inconsistently, in cats with naturally occurring kidney disease<sup>135</sup> and in dogs with experimentally induced acute kidney injury.<sup>136</sup> Therefore, mild increases in serum activity of lipase should be interpreted cautiously in dogs and cats with evidence of decreased glomerular filtration rate.

It is important to note that corticosteroid therapy has been associated with increases in both lipase and PLI. Specifically, dogs receiving corticosteroids can show up to a fourfold increase in serum lipase activity without histological evidence of pancreatitis.<sup>117,118</sup> Two recent studies also found significant increases in PLI in 17% and 50% of dogs treated with corticosteroids.<sup>137,138</sup> The effect of corticosteroids on the pancreas is uncertain in both canine and human medicine, but the administration of corticosteroids does not appear to be a risk factor for pancreatitis.<sup>138</sup> The underlying mechanism of the increase in serum lipase and PLI might involve increased synthesis of lipase by acinar cells or increased permeability of acinar cells to lipase.<sup>137</sup>

### Trypsin-like immunoreactivity

Trypsin is a serine proteinase produced by the pancreas as proenzyme called trypsinogen. Trypsinogen is secreted into the intestine where it is converted to trypsin by enterokinase. Early attempts to measure tryptic activity in serum failed, possibly because trypsinogen, instead of trypsin, is released into the circulation.<sup>4</sup> To overcome this, species-specific immunoassays were developed to measure the concentration of cationic trypsinogen, trypsin and trypsin bound to protease inhibitors, referred to as TLI.<sup>2</sup> The pancreas is the primary contributor to serum TLI, as demonstrated by a great reduction in TLI after pancreatectomy in dogs, though nonpancreatic sources of TLI also exist as TLI was still detectable.<sup>100</sup> To our knowledge, the half-life of serum TLI has not been established but it is likely short as the enzymes that are included under TLI are rapidly scavenged by endopeptidases.<sup>4</sup>

TLI is considered the gold standard for the diagnosis of EPI in dogs with its sensitivity and specificity reaching 100%.<sup>119,139</sup> However, it should be noted that active pancreatitis or conditions associated with decreased glomerular filtration rate can elevate TLI concentration, potentially masking an EPI.<sup>140</sup> Although TLI has not been studied as extensively for the diagnosis of EPI in cats, results from one study of 20 cats with low TLI suggest it is a useful tool. Out of the 20 cats, 17 had compelling evidence of EPI and 3 had supportive evidence of EPI, indicating that TLI is a specific test for EPI in cats.<sup>141</sup>

Serum TLI can be elevated in dogs suffering from acute pancreatitis. In an experimental study, it was noted that TLI concentration began to rise within 1 day and remained elevated until day 5 before, returning to near baseline values at 2 weeks. This trend was also observed for serum amylase and lipase activities, but the peak in TLI occurred 1–2 days earlier than the peaks in amylase and lipase.<sup>98</sup> In a study of spontaneous pancreatitis, 6 of 10 dogs with severe pancreatitis and 2 of 5 dogs with mild pancreatitis were found to have elevated TLI concentrations.<sup>142</sup>

The accuracy of TLI in diagnosing pancreatitis in cats has been inconsistent, with sensitivity ranging from 8% (for mild pancreatitis) to 86% depending on the threshold used.<sup>128,143</sup> This sensitivity is substantially lower than that of PLI. TLI also appears to be less specific in cats compared to PLI with a reported specificity of 82%.<sup>128</sup> Elevated TLI concentrations have been seen in cats with decreased glomerular filtration rate and chronic intestinal diseases (such as inflammatory bowel disease and gastrointestinal lymphoma), even in the absence of histologically confirmed pancreatitis.<sup>144–147</sup>

Since trypsinogen is cleared by the kidneys, decreased glomerular filtration rate can result in elevated serum TLI concentration.<sup>148</sup> In an experimental study on acute kidney injury, TLI was increased in 3 of 5 dogs and in 34% of samples collected during the study.<sup>136</sup> Additionally, experimentally induced renal failure was also associated with elevated TLI concentration in 13 of 20 cats.<sup>149</sup> Therefore, it is important to highlight that decreased glomerular filtration rate can potentially obscure an EPI diagnosis.

Dexamethasone has also been associated with increased TLI in dogs by day 7 of treatment, returning to baseline levels 7 days after withdrawal. Nonetheless, only 2 of 12 dogs had TLI concentrations above the reference interval.<sup>150</sup> TLI can also be increased due to stimulation by food intake or administration of cholecystokinin.<sup>151</sup> Dogs on high-protein diets also had higher TLI concentrations according to one study, but the mean value remained within the reference interval.<sup>152</sup>

### Conclusion

Clinical enzymology plays a critical role in the diagnosis, monitoring and potential prognosis of a variety of diseases in dogs and cats. Therefore, serum enzyme testing has become an essential part of routine diagnostic testing in small animal medicine. However, to maximise the clinical benefits of enzyme testing, clinicians and clinical pathologists must be aware of the preanalytical and analytical factors that can affect the accuracy of the measurement. Importantly, understanding the pathophysiology of serum enzyme alterations is crucial for the diagnosis and management of canine and feline patients. With continuing advances in technology and research, many more enzymes may potentially be of clinical use in the future.

### Conflicts of interest and sources of funding

The authors declare no conflicts of interest or sources of funding for the work presented here.

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(Accepted for publication 10 September 2023)