ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

Warburg effect in tumor bearing dogs

Anaëlle Marquier

Coimbra, julho de 2022

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Aluna do Mestrado Integrado em Medicina Veterinária

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Professor Doutor Joaquim Henriques
(Hospital Veterinário Fregis)

Orientadores externos

Professor Doutor Joaquim Henriques
(Hospital Veterinário Fregis)

Professora Doutora Helene Kolb
(Hospital Veterinário Atlantia)

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ATP: Adenosine triphosphate
DLBCL: Diffuse large B-cell lymphoma
GLUT: Glucose transporters
GSH: Reduced glutathione
GSSG: Oxidised glutathione
HIF-1: Hypoxia inducible factor 1
LDH: Lactate dehydrogenase
MCT4: Lactate exit transporter monocarboxylate transporter 4
NAD+/NADH: Oxidized and reduced nicotinamide adenine dinucleotide
OXPHOS: Oxidative phosphorylation
ROS: Reactive oxygen species
VEGF: Vascular endothelial growth factor

Warburg effect in tumor bearing dogs

Anaëlle Marquier[a], Damian Tortosa[b] , Joaquim Henriques[c], Hugo Vilhena[d,e,f,g]

[a] Departament of Veterinary Sciences, Vasco da Gama University School, Av. José R. Sousa Fernandes 197, Campus Universitário- Bloco B, Lordemão, 3020-210, Coimbra, Portugal (ana.marquier@gmail.com)

[b] Interdisciplinary Laboratory of Clinical Analysis (Interlab-UMU), University of Murcia, Campus de Espinardo Ed. 16, 30100 Espinardo, Murica, Spain (det20165@um.es)

[c] Department of Oncology, Veterinary Hospital Center of Fregis, Av. Aristide Briand 43, 94110 Arcueil, France (oncovet@gmail.com)

[d] Center for Investigation Vasco da Gama (CIVG), Departament of Veterinary Sciences, Vasco da Gama, University School, Av. José R. Sousa Fernandes 197, Campus Universitário- Bloco B, Lordemão, 3020-210, Coimbra, Portugal, (hugo.vilhena@euvg.pt)

[e] Animal and Veterinary Research Center (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), Quinta de Prados, Apartado 1013, 5001-801, Vila Real, Portugal

[f] Onevetgroup University Veterinary Hospital of Coimbra (HVUC), Av. José R. Sousa Fernandes, 197, 3020-210, Coimbra, Portugal

[g] Associated Laboratory of Animal and Veterinary Science – AL4Animals

RESUMO

Tal como em medicina humana, a frequência de cancros apresenta uma tendência crescente nos animais de companhia, e apesar dos recentes avanços no diagnóstico e tratamento, continua a ser uma das principais causas de morbilidade e mortalidade em cães e gatos. A descoberta de novos biomarcadores clinicamente úteis para triagem, diagnóstico, detecção precoce da progressão e prognóstico de doenças oncológicas, bem como a descrição de vias metabólicas que poderão ser potenciais alvos terapêuticos são de extrema importância em oncologia humana e veterinária.

O efeito Warburg descreve a síntese de ATP por células tumorais através de uma via metabólica alternativa à fosforilação oxidativa, a glicólise aeróbica, mesmo em condições de normoxemia. A glicólise aeróbia poderá produzir alterações bioquímicas que podem ser utilizadas como biomarcadores clínicos.

Na medicina humana, este fenómeno tem sido documentado em numerosos estudos em oncologia. No entanto, a informação relacionada com o efeito Warburg em oncologia veterinária é escassa. Por este motivo, este estudo visou investigar as alterações no metabolismo energético tumoral através do estudo das variações metabólicas associadas ao efeito Warburg, através da determinação da concentração sérica de glucose, fructosamina, lactato e lactato desidrogenase (LDH) em cães com diferentes neoplasias malignas.

Foram avaliados neste estudo 36 cães diagnosticados com diferentes tumores malignos e em diferentes estadios clínicos (grupo de animais doentes) e 23 cães considerados saudáveis, que constituíram o grupo controlo. Os animais doentes apresentaram concentrações séricas de lactato e LDH significativamente superiores ($P=0.009$ e $P=0.023$, respetivamente), e de fructosamina significativamente inferiores aos animais do grupo controlo ($P=0.007$). As concentrações séricas de glucose foram também inferiores em cães com neoplasias do que nos cães saudáveis, mas as diferenças não foram significativas ($P=0.174$). Os cães doentes foram divididos em grupos de acordo com o tipo de tumor que apresentaram, nomeadamente nos grupos de cães com carcinoma (n=13), sarcoma (n=10), linfoma (n=8) e adenocarcinoma mamário (n=8). Não foram encontradas diferenças significativas nas concentrações séricas de glucose, fructosamina, lactato e lactato desidrogenase entre estes grupos, ou entre estes grupos e os animais saudáveis ($P>0.05$ em todos os casos).

Os resultados obtidos neste estudo mostram a ocorrência de alterações metabólicas que poderão estar associadas ao efeito Warburg em cães com diferentes tumores malignos, sugerindo que a glicólise aeróbica pode estar implicada na carcinogénese de diferentes neoplasias caninas. Os resultados sugerem também que os metabolitos analisados, nomeadamente a fructosamina, o lactato e a LDH, poderão ser biomarcadores úteis na prática clínica para triagem, diagnóstico, detecção precoce da progressão e prognóstico de doenças oncológicas caninas. Sugerem ainda que as vias metabólicas implicadas no efeito Warburg poderão ser potenciais alvos terapêuticos de diferentes neoplasias caninas. No entanto, neste estudo foi analisado um grupo pequeno de animais, e com tumores diferentes e em diferentes estadios clínicos. Serão necessários estudos futuros, com um maior número de animais e com uma população mais homogénea, para avaliar a função biológica do efeito Warburg na carcinogénese e a sua aplicação clínica em cada tipo tumoral.

Palavras-chave: Canino; Efeito Warburg; Fructosamina; Glucose; Lactato; Lactato desidrogenase; Neoplasia

ABSTRACT

As in human medicine, the frequency of cancer presents an increasing trend in companion animals, and despite recent advances in diagnosis and treatment, remains a major cause of morbidity and mortality in dogs and cats. The discovery of new clinically useful biomarkers for screening, diagnosis, early detection of progression and prognosis of oncological diseases, as well as the description of metabolic pathways that could be potential therapeutic targets are of extreme importance in human and veterinary oncology.

The Warburg effect describes the synthesis of ATP by tumor cells through an alternative metabolic pathway to oxidative phosphorylation, the aerobic glycolysis, even under normoxemia conditions. Aerobic glycolysis may produce biochemical changes that can be used as clinical biomarkers.

In human medicine, this phenomenon has been documented in numerous studies in oncology. However, information related to the Warburg effect in veterinary oncology is scarce. For this reason, this study aimed to investigate changes in tumor energy metabolism through the study of metabolic variations associated with the Warburg effect, by determining the serum concentration of glucose, fructosamine, lactate and lactate dehydrogenase (LDH) in dogs with different malignant neoplasms.

In this study, 36 dogs diagnosed with different malignant tumors and in different clinical stages (group of diseased animals) and 23 dogs considered healthy, which constituted the control group, were evaluated. Diseased animals (n=36) had significantly higher serum lactate and LDH concentrations (P=0.009 and P=0.023, respectively), and significantly lower serum fructosamine than animals in the control group (P=0.007). Serum glucose concentrations were also lower in dogs with cancer than in healthy dogs, but the differences were not significant (P=0.174). The diseased dogs were divided into groups according to the type of tumor, namely in the groups of dogs with carcinoma (n=13), sarcoma (n=10), lymphoma (n=8) and mammary adenocarcinoma (n=8). No significant differences were found in the serum concentrations of glucose, fructosamine, lactate and LDH between these groups, or between these groups and healthy animals (P>0.05 in all cases).

The results obtained in this study show the occurrence of metabolic alterations that may be associated with the Warburg effect in dogs with different malignant tumors, suggesting that aerobic glycolysis may be involved in the carcinogenesis of different canine neoplasms. The results also suggest that the analyzed metabolites, namely fructosamine, lactate and LDH, might be useful biomarkers in clinical practice for screening, diagnosis, early detection of progression and prognosis of canine oncological diseases. They also suggest that the metabolic pathways involved in the Warburg effect might be potential therapeutic targets for different canine neoplasms. However, this study analyzed a small group of animals, with different tumors and at different clinical stages. Future studies, with a greater number of animals and with a more homogeneous population, will be necessary to evaluate the biological role of the Warburg effect in carcinogenesis and its clinical application in specific tumor types.

Key words: Canine; Fructosamine; Glucose; Lactate; Lactate dehydrogenase; Neoplasia; Warburg effect

INTRODUCTION

The Warburg effect, discovered in 1924 by Otto Warburg, describes the production of ATP by other metabolic pathways than the oxidative phosphorylation observed in healthy cells. Indeed, it was shown that tumor cells preferentially use aerobic glycolysis occuring in the cytosol for ATP synthesis, even under normoxemia conditions, instead of the oxidative phosphorylation used by healthy cells (Levine and Puzio-kuter, 2010; Lunt and Heiden, 2011; Gallo *et al*., 2015; Luc *et al*., 2015; Xu *et al*., 2015; Bose and le, 2018). The initial assumption was that this metabolic shift was due to damage in mitochondrias, impeding cellular respiration (Warburg, 1956), and this phenomenon was considered as a hallmark of cancer. The Warburg effect is considered to be due to different factors, including HIF-1 overexpression and subsequent normoxia/hypoxia, activation of oncogenes, loss of function of tumor suppressor genes, altered signaling pathways, interaction with components of the tumor microenvironment and epigenetic mechanisms (Vaupel *et al*. 2021).

Aerobic glycolysis is a less efficient metabolic pathway for obtaining ATP than oxidative phosphorylation, with only 2 moles of ATP per mole of glucose being produced; whereas 36 moles ATP from oxidation of 1 mole of glucose are obtained with oxidative phosphorylation (OXPHOS) (Gallo *et al*., 2015; Luc *et al*., 2015; Mookerjee *et al*.,2017; Vaupel et al.,2019). Nevertheless, aerobic glycolisis constitutes a faster metabolic process, where ATP is obtained from glucose at a rate 10 to 100 times higher than ATP generated in mitochondria, providing an abundant energy supply (Lu *et al*., 2015; Gallo *et al*.,2015; Liberti and Locasale, 2016; Bose and le, 2018). This requires an increased uptake of glucose substantiated by an increased number and expression of glucose transporters (GLUT) (Kozal *et al*., 2021).

The glycolytic pathway involves the catabolism of glucose to pyruvate in nine- ten biochemical steps, in which key transcriptional activations involving GLUT1 and lactate exit transporter monocarboxylate transporter 4 (MCT4), and key glycolytic enzymes such as hexokinase 2, phosphofructokinase 1, enolase 1, the low-activity pyruvate kinase M2 (PKM2) and the over-expressed lactate dehydrogenase A (LDHA) are involved (Vaupel *et al*. 2021).

This metabolism also permits the accumulation and diversion of glycolytic intermediates, allowing biosynthesis of molecules necessary for tumor cells proliferation, including nucleotides, non-essential amino acids, lipids, hexoamines, and key proteins in oncogenesis (Boroughs and Deberardinis, 2015; Liberti and Locasale, 2016; Deberardinis *et al*., 2008; Vaupel *et al*., 2021). As a consequence, a high glycolytic flux is settled. This metabolic reprogramming of tumor cells creates conditions in the microenvironment that permits malignant proliferation through various mechanisms (Liberti and Locasale, 2016; Chen *et al.*, 2021; San-Millán & Brooks, 2017).

Lactate, the final product of the aerobic glycolysis, is a key oncometabolite for malignant progression, allowing angiogenesis, promoting cell motility, local invasion and distant metastasis, resistance to apoptosis and promotion of a stem cell phenotype (Vaupel *et al*., 2019). It permits the suppression of anti-tumor mechanisms through, for example, immune escape allowed T cells altered function and metabolism (Xu *et al*., 2015). Resistance to conventional therapies is also a consequence of extracellular acidosis, resulting from this metabolism (Vaupel *et al*., 2021). Lactate/ Pyruvate, NAD+/NADH and GSH/ GSSG ratio, necessary for redox homeostasis and adaptive stress responses, are responsible for ROS scavenging, reducing DNA damage and causing a decrease in tumor treatment efficacy (Kozal *et al*., 2021; Sattler & Mueller- Klieser, 2009). Lactate has a role in angiogenesis and tumor growth by activating VEGF in endothelial cells (Kumar *et al*.,2007). Interestingly, the Warburg effect was identified to also play a role in human non-tumor diseases,

including pulmonary hypertension, tuberculosis, idiopathic pulmonary fibrosis, cardiovascular disease, atherosclerosis, Alzheimer's diseases and in COVID-19 infection (Chen *et al*., 2017; Kuspriyanti *et al*., 2021; Tuder *et al*., 2012; Xie *et al*., 2015; Hi *et al*., 2015; Newington *et al*., 2011; Icard *et al*., 2021). Studies in veterinary medicine have demonstrated a Warburg effect in chronic atrial fibrillation in dogs (Liu *et al*., 2020).

In veterinary medicine, several epidemiological studies have been carried out in previous decades to try to characterize the prevalence of canine tumours (MacVean *et al*., 1978; Baioni *et al*., 2017; Pinello *et al*., 2022). It has been reported that approximately half of the tumours presented are malignant. Two anatomical sites stand out in terms of prevalence, namely skin and breast tumours. (Grüntzig *et al*., 2015; Gamlem & Nordstoga & Glattre, 2008; Vascelleri *et al*., 2009). In young dogs, tumours are mostly benign and histiocytomas are over-represented, and among the malignant tumours, the mast cell tumours. Mammary tumors ended to be the most common in the older age categories malignant and benign combined. (Schmidt *et al*, 2010; Gamlem & Nordstoga & Glattre, 2008).

Although in human medicine, many studies exist on the Warburg effect and its occurrence, especially in mammary, lung and hepatocellular carcinoma (Lebelo et *al*.,2019; Feng *et al*., 2020; Wu *et al*., 2020), its importance in veterinary medicine is not well defined.

The aim of this study was to evaluate the Warburg effect in in different canine malignant tumours, through the determination of serum concentrations of glucose, fructose, lactate and LDH in animals with oncologic disease and in healthy control animals..

MATERIAL AND METHODS

Animals and samples

This study included 36 dogs with oncologic diseases received at the Onevetgroup University Veterinary Hospital of Coimbra (HVUC), Portugal, and Onegroup Baixo Vouga Veterinary Hospital (HVBV), Portugal, between January 2021 and June 2022. The diseased group was composed of animals with different malignant neoplasms, and in different clinical stages, and is characterized in table 1. A control group with 23 dogs considered clinically healthy based on clinical history, physical examination, and results of complementary exams, including blood analysis (hematology and serum biochemistry), performed as part of the pre-anesthetic protocol for elective surgical procedures or healths check-ups was also evaluated, and is also characterized in table 1. In all cases, surplus serum samples were used for determination of concentrations of glucose, fructosamine, lactate and lactate dehydrogenase. No blood samples were collected exclusively for this study. All tutors gave an informed consent for inclusion of their dogs in this study.

The whole blood sample from each animal included in this study was obtained at diagnosis, before institution of any treatment, and divided into an EDTA tube for hematology and a tube without anticoagulant for serum biochemistry. After settling for 15 minutes at room temperature, the samples obtained in tubes without anticoagulant were centrifuged (10 min, 2000 x g) to separate serum from clot and blood cells. The resulting serum supernatant was used for analyses, and the remaining serum samples were stored at -20 °C until determination of serum glucose, fructsamine, lactate, and LDH concentrations.

Glucose, Fructosamine, Lactate, and Lactate dehydrogenase (LDH) analysis

Serum glucose and lactate were determined using commercially available kits (glucose reagent OSR6121 and lactate reagent OSR6193, respectively, Beckman Coulter, California, USA) following instructions of the manufacturer. Serum LDH and fructosamine were measured using commercially available kits (lactate deshidrogenase ref 11581 and fructosamine ref 11046, respectively, BioSystems SA, Barcelona, Spain) following instructions of the manufacturer.

All analyses were performed at the Interdisciplinary Laboratory of Clinical Analysis, University of Murcia (Interlab-UMU), Spain, using an automated biochemistry analyzer (Olympus AU600 Automatic Chemistry Analyzer, Olympus Europe GmbH, Hamburg, Germany).

Statistical Analysis

The Graphpad Prism version 9 software (La Jolla, California, USA) was used for all data analysis. Comparison of variables between groups was assessed by the Unpaired non-parametric Mann-Whitney test. Values of $P < 0.05$ were considered significant. Serum concentrations of glucose, fructosamine, lactate and LDH were compared between diseased (n=36) and healthy (n=23) dogs. Secondly, dogs with malignant tumors were grouped according with the histopathology diagnosis in four groups, namely carcinoma (n=13), sarcoma (n=10), lymphoma (n=8) and mammary adenocarcinoma (n=8); and serum concentrations of glucose, fructosamine, lactate and LDH were compared between groups, and between groups and controls.

Table 1 – Characterization of the study population

	Malignant tumor group (n=36)	Control group (n=23)
Breed		
Pure breed	26	16
Cross-breed	10	7
Age (years)		
Mean	8,5	4,6
Min-Max	5-12	0,5-14
Gender		
Male	16	10
Female	20	13
Histopathology		
Anaplastic carcinoma	1	-
AGASACA	1	-
Anaplastic sarcoma	1	-
Hemangiosarcoma	3	-
Histiocytic sarcoma	1	-
Leydig cell tumor	1	-
Lymphoma	8	-
Mammary carcinoma	8	-
Mastocytoma	4	-
Nasal squamous cell carcinoma	1	-
Osteosarcoma	4	-
Soft tissue sarcoma	1	-
Transitional cell carcinoma	2	-

AGASACA - apocrine gland anal sac adenocarcinoma; Max – maximim; Min - minimum

RESULTS

Serum concentrations of glucose, fructosamine, lactate and LDH were compared between dogs with malignant tumors (n=36) and healthy controls (n=23). Significant differences in serum concentrations of fructosamine, lactate and LDH were found between diseased and healthy dogs. Animals of the diseased group presented serum concentrations of fructosamine significantly lower than animals of the control group (P=0.007); while serum concentrations of lactate and LDH were significantly higher in diseased dogs when compared with control animals (P=0.009 and P=0.023, respectively). Serum concentrations of glucose were lower in oncologic patients than in controls, but the differences were not significant (P=0.174). Results presented as medians and interquartile range (IQR) for the different analytes between groups are presented in table 2, and boxplots of the different analytes in the two groups of animals analyzed are presented in figure 1.

Table 2 - Serum concentrations of glucose, fructosamine, lactate and Lactate dehydrogenase (LDH) in dogs with oncologic diseases and in healthy control dogs

	Glucose (mg/dL) Median (IQR)	**Fructosamine** (µmol/L) Median (IQR)	**Lactate** (mmol/L) Median (IQR)	**LDH** (U/L) Median (IQR)
Malignant tumor group (n=36)	99.7 (89.1-106.2)	376.5 (302.8-416.0)	2.94 (1.62-4.40)	243.4 (96.3-373.3)
Control group (n=23)	101.7 (98.8-110.6)	405.0 (392.0-430.0)	1.85 (1.55-2.44)	109.4 (71.2- 186.6)

IQR – Interquartile range; LDH – lactate dehydrogenase

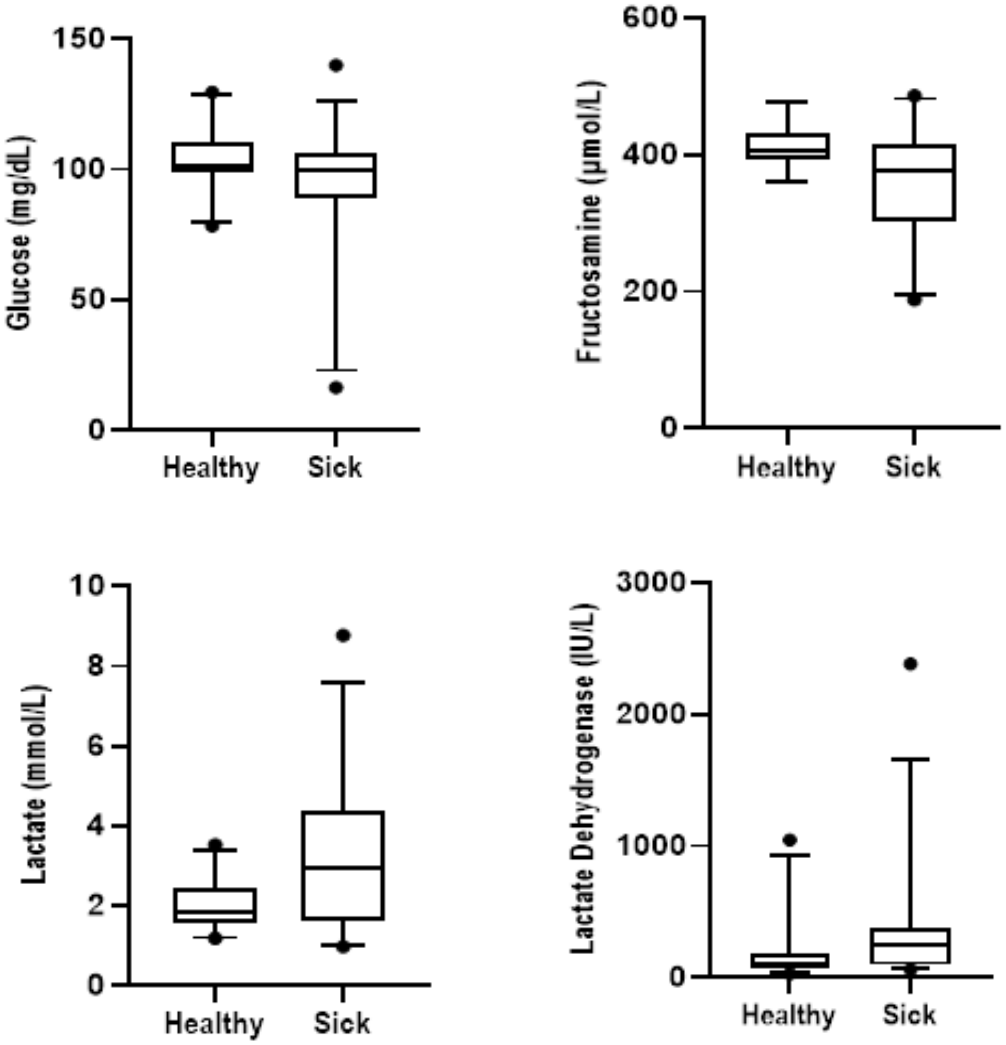

Figure 1 – Glucose, fructosamine, lactate and LDH serum levels observed in diseased and healthy dogs. The plots show median (line within box), 25 th and 75 th percentiles (box), 5 th and 95 th percentiles (whiskers). Outliers are marked with a circle.

Dogs with malignant tumors were grouped according with the histopathology diagnosis in four groups, namely carcinoma (n=13), sarcoma (n=10), lymphoma (n=8) and mammary carcinoma (n=8). Serum concentrations of glucose, fructosamine, lactate and LDH were compared between groups, and also between these groups and control animals (n=23). No significant differences in serum concentrations of the different analytes were found between groups, nor between groups and controls ($P>0.05$ in all cases). Serum concentrations of glucose, fructosamine, lactate and lactate dehydrogenase, presented as medians and IQR, in dogs with carcinomas, sarcomas, lymphoma and mammary carcinoma are presented in table 3.

Table 3 - Serum concentrations of glucose, fructosamine, lactate and lactate dehydrogenase (LDH) in dogs with carcinomas, sarcomas, lymphoma and mammary adenocarcinomas.

	Glucose (mg/dL) Median (IQR)	**Fructosamine** (µmol/L) Median (IQR)	**Lactate** (mmol/L) Median (IQR)	**LDH** (IU/L) Median (IQR)
Carcinoma group (n=13)	104.8 (96.15-107.8)	378 (348.5-428)	3.25 (1.96-5.765)	268.9 (88.3- 536.9)
Sarcoma group (n=11)	98.95 (78.55-107.3)	348.5 (301-419,5)	3.02 (1.418-3.475)	139 (96.18-283.8)
Lymphoma group (n=8)	96.8 (71.8-101.8)	302 (248-400)	1.65 (1.58-4.68)	268.2 (78.6-1116)
Ma. adenocarcinoma (n=8)	105.2 (99.83-107)	405 (392-430)	1.85 (1.55-2.44)	289.8 (84.2-795.3)

LDH – lactate dehydrogenase; Ma - mammary

DISCUSSION

The results of this study showed metabolic alterations in a heterogeneous group of animals with oncological diseases, which can potentially be attributed to the Warburg effect, showing a signifant decrease in serum fructosamine, as well as significant increases in serum lactate and LDH. Serum glucose, although decreased in oncologic patient, did not show significant alteration when compared to the control group.

It is interesting to note that in animals, serum glucose can be impacted by different variables, so other markers such as fructosamine are of interest. Because the serum fructosamine is used for long-term monitoring of glycemia, reflecting blood glucose concentration during the previous one to two weeks, it can help detect chronic hyperglycemia and hypoglycemia and overcome a transitory change in plasma glucose (Loste *et al*., 2001). This difference in serum glucose evaluation might justify the reason why in our study diseased dogs presented significant decreases in serum fructosamine and not in serum glucose. In other perspective, this measurement is interesting because fructose is an alternative energy source to glucose in cancer cells. Indeed, this idea was corroborated by the expression of the main transporter of fructosamine GLUT5 on the cell surface of several types of tumors (Nakagawa *et al*., 2020). In human medicine, clinical studies have demonstrated that GLUT5 protein and GLUT5 gene are expressed in lung adenocarcinoma, colorectal adenocarcinoma and breast cancer (Zamora-León *et al*.,1996; Mahraoui *et al*.,1992; Weng *et al*., 2018).

Increased glucose uptake is considered one of the hallmarks of cancer metabolism (Hanahan and Weinberg, 2011). Glucose is a consistent fuel for tumor cells. Glucose entry into tumor cells is promoted by different GLUT isoforms, between which overexpression of GLUT1 is noticeable in several tumors such as lung carcinoma, mesothelioma, seminoma, or ovary carcinoma (Godoy *et al*., 2006).

The LDH is the enzyme responsible for converting pyruvate to lactate (Lukacova *et al.,* 2008). Because of its distribution in many tissues, increased LDH levels can be encountered in several cardiac, hepatic, skeletal muscle, renal, hematological and neoplastic disorders (Klein *et al*., 2020). Clinical significance can be increased when separated into isoenzyme fractions (Zanatta *et al.,* 2003). However, studies in human medicine have demonstrated that LDH could be a biomarker for prognosis prediction and treatment selection in some types of cancer, like in patients with a diagnosis of hepatocellular carcinoma, in which an increase could be a biomarker for early recurrence of this cancer (Wang *et al*., 2015; Zhu *et al*., 2020). In veterinary medicine, most studies do not show a significant increase in LDH in the different tumor groups (Marconato *et al*., 2009)

As previously mentioned, lactate is a key oncometabolite of the Warburg effect (Vaupel *et al*. 2019). Lactic acidosis results from an imbalance between lactate production and its elimination. It can be distinguished into type A or B depending on its pathophysiology (Touret *et al*., 2010). One of the limitations of our study was that other possible causes of hyperlactatemia in these patients were not evaluated. Moreover, taking into account that the liver is responsible for 70% of lactate metabolism (Touret *et al*., 2012), it would be interesting to evaluate the hepatic function of these patients.

Type A hyperlactatemia results from anaerobic conditions, the most common causes are ischemic bowel syndrome, sepsis, cardiogenic shock, and hypovolemia. Type B hyperlactatemia is not associated with hypoperfusion or other causes of decreased oxygen delivery. Prednisone is one of the drugs whose hyperlactatemic effect has been described (Boysen *et al*., 2009). In human medicine, hyperlactatemia has been described in many tumor types, especially in hematopoietic tumors (Sillos *et al*., 2001; Friedenberg & Brandoff & Schiffman, 2007; Mizock & Glass,1994). Lymphomas, especially in the diffuse large B-cell

lymphoma type (DLBCL) were overrepresented (Masood *et al*., 2017; He & Ong, 2019; Hamada *et al*., 2020). Solid tumors such as small cell lung cancer, cholangiocarcinoma, breast cancer, gynecological cancers, and metastasis from unknown primary carcinoma (de Groot *et al*., 2011) were also described.

When observing the metabolic alterations intrinsic to each group of tumors (carcinoma, sarcoma, lymphoma and mammary carcinoma), no significant variation was observed suggesting a Warburg effect. This lack of results may be due to the small number of patients recruited in each group, as well as to a heterogeneity of the profiles with animals at different clinical stages. In human medicine, hyperlactatemia secondary to tumor is mainly a complication noted at a late stage of the disease. Prognosis associated with the Warburg effect and lactic acidosis is therefore considered as poor (Ubaldo *et al*., 2013). One study has shown that only 2 out 28 lymphoma patients who had lactic acidosis achieved complete remission, and that more than 75% of these patients died within a month (He *et al*., 2007). In veterinary medicine, other studies have demonstrated a potential Warburg effect in feline mammary adenocarcinoma (Almeida *et al.,* 2019), as well as in feline lymphoma (Vitorino *et al*., 2021).

CONCLUSION

In this study, we assessed metabolic changes associated with the Warburg effect in dogs with different malignant tumors and in different clinical states, through evaluation of a biochemical panel that included the determination of serum concentrations of glucose, fructosamine, lactate and LDH in oncologic canine patients. The significant increase in serum concentrations of lactate and LDH, and the significant decrease in fructosamine concentrations detected in the group of sick animals suggests that aerobic glycolysis might be implicated in the carcinogenesis of different canine malignancies. Moreover, and although this study included a group of dogs with different tumors and in different clinical stages, these results also suggest that fructosamine, lactate and LDH might eventually be clinically useful biomarkers in the diagnosis, monitoring of disease evolution and response to treatment, and prognosis in selected canine malignancies; and that alterations in glucose metabolism may be therapeutic targets for some types of neoplasms.

Future studies, with a greater number of animals and with a more homogeneous population, are therefore essential to better understand the importance of the Warburg effect in veterinary oncology, namely in specific tumor types, allowing a better screening of these diseases and the establishment of therapeutic targets, using intervention in metabolism.

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REFERENCES

Almeida A, Almeida M, Barros L, Dias-Pereira P, Canadas-Sousa A, Figueira AC, Craveiro H, Montezinho L, Silvestre-Ferreira AC, Tvarijonaviciute A, Vilhena H, Escribano D. Warburg Effect In Queens With Spontaneous Mammary Tumors. Poster Presented At The 53th Annual Scientific Meeting Of The European Society For Clinical Investigation, 2019.

Baioni, E., Scanziani, E., Vincenti, M. C., Leschiera, M., Bozzetta, E., Pezzolato, M., Desiato, R., Bertolini, S., Maurella, C., & Ru, G. (2017). Estimating canine cancer incidence: findings from a population-based tumour registry in northwestern Italy. *BMC veterinary research*, *13*(1), 203. https://doi.org/10.1186/s12917-017-1126-0

Boysen SR, Bozzetti M, Rose L, Dunn M, Pang DS. Effects of prednisone on blood lactate concentrations in healthy dogs. J Vet Intern Med. 2009 Sep-Oct; 23(5):1123-5. doi: 10.1111/j.1939-1676.2009.0345.x. Epub 2009 Jul 1. PMID:19572915.

Boroughs LK, Deberardinis RJ. Metabolic Pathways Promoting Cancer Cell Survival And Growth. Nature Publishing Group. 2015;17,351–359.

Bose, S., Le, A. Glucose Metabolism In Cancer. Advances In Experimental Medicine And Biology. 2018;1063,3–12.

Chen, J., Cao, X., Li, B., Zhao, Z., Chen, S., Lai, S., Muend, S. A., Nossa, G. K., Wang, L., Guo, W., Ye, J., Lee, P. P., & Feng, M. (2021). Warburg Effect Is a Cancer Immune Evasion Mechanism Against Macrophage Immunosurveillance. Frontiers in immunology, 11, 621757. https://doi.org/10.3389/fimmu.2020.621757

Chen Z, Liu M, Li L, Chen L. Involvement of the Warburg effect in non- tumor diseases processes. J Cell Physiol. 2018; 233: 2839– 2849. https://doi.org/ 10.1002/jcp.25998

Deberardinis, R. J., Lum, J. J., Hatzivassiliou, G., Thompson, C. B. The Biology Of Cancer : Metabolic Reprogramming Fuels Cell Growth And Proliferation. Cell Metabolism. 2008;7,11–20.

Feng, J., Li, J., Wu, L., Yu, Q., Ji, J., Wu, J., Dai, W., & Guo, C. (2020). Emerging roles and the regulation of aerobic glycolysis in hepatocellular carcinoma. Journal of experimental & clinical cancer research : CR, 39(1), 126. https:// doi.org/10.1186/s13046-020-01629-4

Friedenberg, A. S., Brandoff, D. E., & Schiffman, F. J. (2007). Type B lactic acidosis as a severe metabolic complication in lymphoma and leukemia: a case series from a single institution and literature review. Medicine, 86(4), 225 – 232. https://doi.org/10.1097/MD. 0b013e318125759a

Gallo, M., Sapio, L., Spina, A., Naviglio, D., Calogero, A., Naviglio, S. Lactic Dehydrogenase And Cancer : An Overview LDH And Cancer. Frontiers In Biocience. 2015;20,1234–1249.

Gamlem, H., Nordstoga, K., & Glattre, E. (2008). Canine neoplasia-- introductory paper. APMIS. Supplementum, (125), 5–18. https://doi.org/ 10.1111/j. 1600-0463.2008.125m2.x

Godoy, A., Ulloa, V., Rodríguez, F., Reinicke, K., Yañez, A. J., García, M., Medina, R. A., Carrasco, M., Barberis, S., Castro, T., Martínez, F., Koch, X., Vera, J. C., Poblete, M. T., Figueroa, C. D., Peruzzo, B., Pérez, F., & Nualart, F. (2006). Differential subcellular distribution of glucose transporters GLUT1-6 and GLUT9 in human cancer: ultrastructural localization of GLUT1 and GLUT5 in breast tumor tissues. Journal of cellular physiology, 207(3), 614–627. https://doi.org/10.1002/ jcp.20606

de Groot, R., Sprenger, R. A., Imholz, A. L., & Gerding, M. N. (2011). Type B lactic acidosis in solid malignancies. The Netherlands journal of medicine, 69(3), 120–123

Grüntzig, K., Graf, R., Hässig, M., Welle, M., Meier, D., Lott, G., Erni, D., Schenker, N. S., Guscetti, F.,

Boo, G., Axhausen, K., Fabrikant, S., Folkers, G., &Pospischil, A. (2015). The Swiss Canine Cancer Registry: a retrospective study on the occurrence of tumours in dogs in Switzerland from 1955 to 2008. Journal of comparative pathology, 152(2-3), 161–171. https://doi.org/10.1016/j.jcpa.2015.02.005

Hamada, T., Kaku, T., Mitsu, S., Morita, Y., Ohno, N., & Yamaguchi, H. (2020). Lactic Acidosis and Hypoglycemia in a Patient with Gastric Diffuse Large B-Cell Lymphoma due to the Warburg Effect. Case reports in oncology, 13(2), 1047– 1052. https://doi.org/10.1159/000509510

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, *144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

He YF, Wei W, Sun ZM, Ji CS, Wang G, Chen MP, Hu CL, Hu B. Fatal lactic acidosis and hypoglycemia in a patient with relapsed natural killer/ T-cell lymphoma. Adv Ther. 2007

He, Y., Ong, J., & Ong, S. (2019). Refractory Lactic Acidosis and an Approach toits Management - A Case Report. Journal of critical care medicine (Universitatea de Medicina si Farmacie din Targu-Mures), 5(2), 60–65. https://doi.org/10.2478/ jccm-2019-0010

Icard, P., Lincet, H., Wu, Z., Coquerel, A., Forgez, P., Alifano, M., & Fournel, L. (2021). The key role of Warburg effect in SARS-CoV-2replication and associated inflammatory response. Biochimie, 180, 169– 177. https://doi.org/10.1016/ j.biochi.2020.11.010

Klein, R., Nagy, O., Tóthová, C., & Chovanová, F. (2020). Clinical and Diagnostic Significance of Lactate Dehydrogenase and Its Isoenzymes in Animals. Veterinary medicine international, 2020, 5346483. https:// doi.org/ 10.1155/2020/5346483

Kozal, K., Jóźwiak, P. and Krześlak, A. (2021) 'Contemporary Perspectives on the Warburg Effect Inhibition in Cancer Therapy', Cancer Control. doi:10.1177/10732748211041243.

Kumar, V. B., Viji, R. I., Kiran, M. S., & Sudhakaran, P. R. (2007). Endothelial cellresponse to lactate: implication of PAR modification of VEGF. Journal of cellular physiology, 211(2), 477–485. https://doi.org/ 10.1002/jcp.2095

Kuspriyanti, N. P., Ariyanto, E. F., Syamsunaro M. R. (2021). Role of Warburg Effect in Cardiovascular Disease: A Potential Treatment Option

Le, A. (Ed.). (2021). The Heterogeneity of Cancer Metabolism. Advances in experimental medicine and biology doi: 10.1007/978-3-030-65768-0

Levine, A. J., Puzio-Kuter, A. M. The Control Of The Metabolic Switch. Science. 2010;330,1340–1344.

Lebelo, M. T., Joubert, A. M., & Visagie, M. H. (2019). Warburg effect and its role intumourigenesis. Archives of pharmacal research, 42(10), 833–847. https://doi.org/ 10.1007/s12272-019-01185-2

Li, Jiaqi; Eu, Jie Qing; Kong, Li Ren; Wang, Lingzhi; Lim, Yaw Chyn; Goh, Boon Cher; Wong, Andrea L. A. (2020). Targeting Metabolism in Cancer Cells and the Tumour Microenvironment for Cancer Therapy. Molecules, 25(20), 4831–. doi: 10.3390/molecules25204831

Li Z, Yang L, Zhang S, Song J, Sun H, Shan C, Wang D, Liu S. ValproicAcid Suppresses Breast Cancer Cell Growth Through Triggering Pyruvate Kinase M2 Isoform Mediated Warburg Effect. Cell Transplant. 2021;30:9636897211027524.

Li, C., Zhang, G., Zhao, L., Ma, Z., Chen, H. Metabolic Reprogramming InCancer Cells: Glycolysis , Glutaminolysis , And Bcl-2 Proteins As Novel Therapeutic Targets For Cancer. World Journal Of Surgical Oncology.2016;14,1–7.

Liberti, M. V, Locasale, J. W. The Warburg Effect : How Does It Benefit CancerCells ? Trends In Biochemical Sciences. 2016;41,211–218.

Loste, A., Marca, M. C., Pérez, M., & Unzueta, A. (2001). Clinical value of fructosamine measurements in non-healthy dogs. Veterinary research communications, 25(2), 109–115. https:// doi.org/10.1023/a: 1006452818143

Liu, Y., Bai, F., Liu, N., Zhang, B., Qin, F., Tu, T., Li, B., Li, J., Ma, Y., Ouyang, F., & Liu, Q. (2020). Metformin improves lipid metabolism and reverses the Warburg effect in a canine model of chronic atrial fibrillation. BMC cardiovascular disorders, 20(1), 50. https://doi.org/10.1186/s12872-020-01359-7

Lu, J., Tan, M., Cai, Q. The Warburg Effect In Tumor Progression : MitochondrialOxidative Metabolism As An Anti-Metastasis Mechanism. Cancer Letters. 2015;356,156–164.

Luc, R., Tortorella, S. M., Ververis, K., Karagiannis, T. C. Lactate As An InsidiousMetabolite Due To The Warburg Effect. Molecular BiologyReports. 2015;42,835–840.

Lukacova, S., Sørensen, B. S., Alsner, J., Overgaard, J., & Horsman, M. R. (2008). The impact of hypoxia on the activity of lactate dehydrogenase in two different pre-clinical tumour models. Acta oncologica (Stockholm, Sweden), 47(5), 941–947. https://doi.org/10.1080/02841860701644086

Lunt, S. Y., Heiden, M. G. Vander. Aerobic Glycolysis : Meeting The Metabolic Requirements Of Cell Proliferation. Annual Review Of Cell And Developmental Biology. 2011;27,441–464.Mahraoui, L., Rousset, M., Dussaulx, E., Darmoul, D., Zweibaum, A., & Brot-Laroche, E. (1992). Expression and localization of GLUT-5 in Caco-2 cells, human small intestine, and colon. The American journal of physiology, 263(3 Pt 1), G312–G318. https://doi.org/10.1152/ajpgi.1992.263.3.G312

MacVean, D. W., Monlux, A. W., Anderson, P. S., Jr, Silberg, S. L., & Roszel, J. F. (1978). Frequency of canine and feline tumors in a defined population. *Veterinary pathology*, *15*(6), 700–715. https://doi.org/10.1177/030098587801500602

Marconato, L., Crispino, G., Finotello, R., Mazzotti, S., Salerni, F., & Zini, E. (2009). Serum lactate dehydrogenase activity in canine malignancies. Veterinary and comparative oncology, 7(4), 236–243. https://doi.org/10.1111/j. 1476-5829.2009.00196.x

Martinez-Outschoorn, U. E., Whitaker-Menezes, D., Valsecchi, M., Martinez-Cantarin, M. P., Dulau-Florea, A., Gong, J., Howell, A., Flomenberg, N., Pestell, R. G., Wagner, J., Arana-Yi, C., Sharma, M., Sotgia, F., & Lisanti, M. P. (2013). Reverse Warburg effect in a patient with aggressive B-cell lymphoma: is lactic acidosis a paraneoplastic syndrome?. *Seminars in oncology*, *40*(4), 403–418. https://doi.org/10.1053/j.seminoncol.2013.04.016

Masood, U., Sharma, A., Nijjar, S., & Sitaraman, K. (2017). B-cell lymphoma, thiamine deficiency, and lactic acidosis. Proceedings (Baylor University.MedicalCenter), 30(1), 69–70. https://doi.org/10.1080/08998280.2017.11929534

Mizock, B. A., & Glass, J. N. (1994). Lactic acidosis in a patient with multiple myeloma. The Western journal of medicine, 161(4), 417–418.

Mookerjee, S. A., Gerencser, A. A., Nicholls, D. G., & Brand, M. D. (2017). Quantifying intracellular rates of glycolytic and oxidative ATP production and consumption using extracellular flux measurements. The Journal of biological chemistry, 292(17), 7189–7207. https://doi.org/ 10.1074/jbc.M116.774471 Nakagawa, T., Lanaspa, M. A., Millan, I. S., Fini, M., Rivard, C. J., Sanchez-Lozada, L. G., Andres-Hernando, A., Tolan, D. R., & Johnson, R. J. (2020). Fructose contributes to the Warburg effect for cancer growth.Cancer & metabolism, 8, 16. https://doi.org/10.1186/s40170-020-00222-9

Newington, J. T., Pitts, A., Chien, A., Arseneault, R., Schubert, D., & Cumming, R. C. (2011). Amyloid beta resistance in nerve cell lines is mediated by the Warburg effect. PloS one, 6(4), e19191. https://doi.org/10.1371/journal.pone. 0019191

Pinello, K., Pires, I., Castro, A. F., Carvalho, P. T., Santos, A., de Matos, A., Queiroga, F., Canadas-Sousa, A., Dias-Pereira, P., Catarino, J., Faísca, P., Branco, S., Lopes, C., Marcos, F., Peleteiro, M. C., Sattler, U. G., & Mueller-Klieser, W. (2009). The anti-oxidant capacity of tumour glycolysis. International journal of radiation biology, 85(11), 963– 971. https://doi.org/10.3109/09553000903258889

San-Millán, I., & Brooks, G. A. (2017). Reexamining cancer metabolism: lactate production for carcinogenesis could be the purpose and explanation of the Warburg Effect. Carcinogenesis, 38(2), 119–133. https://doi.org/10.1093/carcin/bgw127

Schmidt, J. M., North, S. M., Freeman, K. P., & Ramiro-Ibañez, F. (2010). Caninepaediatric oncology: retrospective assessment of 9522 tumours in dogs up to 12 months (1993-2008). Veterinary and comparative oncology, 8(4), 283–292. https://doi.org/10.1111/j.1476-5829.2010.00226.x

Sillos, E. M., Shenep, J. L., Burghen, G. A., Pui, C. H., Behm, F. G., & Sandlund, J. T. (2001). Lactic acidosis: a metabolic complication of hematologic malignancies: case report and review of the literature. C a n c er92(9)2237–2246 h t t p s : / / d o i . o r g / 10.1002/1097-0142(20011101)92:9<2237::aid-cncr1569>3.0.co;2-9

Shi, L., Salamon, H., Eugenin, E. A., Pine, R., Cooper, A., & Gennaro, M. L. (2015). Infection with Mycobacterium tuberculosis induces the Warburg effect in mouse lungs. Scientific reports, 5, 18176. https://doi.org/10.1038/srep18176

Sun, L., Suo, C., Li, S., Zhang, H., Gao, P. BBA - Reviews On Cancer Metabolic reprogramming for cancer cells and their Microenvironment :Beyond The Warburg Effect. Reviews On Cancer. 2018;1870,51–66.

Touret M, Boysen SR, Nadeau ME. Prospective Evaluation Of ClinicallyRelevant Type B Hyperlactatemia In Dogs With Cancer. J Vet Intern Med. 2010;24:1458-61.

Touret, M., Boysen, S. R., Nadeau, M. Retrospective Evaluation Of Potential Causes Associated With Clinically Relevant Hyperlactatemia In Dogs With Lymphoma. The Canadian Veterinary Journal. 2012;53,511–517.

Tuder, R. M., Davis, L. A., & Graham, B. B. (2012). Targeting energetic metabolism: a new frontier in the pathogenesis and treatment of pulmonary hypertension. American journal of respiratory and critical care medicine, 185(3), 260–266. https://doi.org/10.1164/rccm.201108-1536PP

Vascellari, M., Baioni, E., Ru, G., Carminato, A., & Mutinelli, F. (2009). Animal tumour registry of two provinces in northern Italy: incidence of spontaneous tumours in dogs and cats. BMC veterinary research, 5, 39. https://doi.org/10.1186/1746-6148-5-39

Vascellari M, Capello K, Carminato A, Zanardello C, Baioni E, Mutinelli F. Incidence Of Mammary Tumors In The Canine Population Living In The Veneto Region (Northeastern Italy): Risk Factors And Similarities To Human Breast Cancer. Prev Vet Med. 2016;126:183-9.

Vaupel P, Multhoff G. Revisiting The Warburg Effect: Historical Dogma Versus Current Understanding. J Physiol. 2021;599:1745-1757.

Vaupel, P., Schmidberger, H., & Mayer, A. (2019). The Warburg effect: essential part of metabolic reprogramming and central contributor to cancer progression. International journal of radiation biology, 95(7), 912– 919. https://doi.org/ 10.1080/09553002.2019.1589653

Vitorino C, Figueira AC, Tvarijonaviciute A, Almeida A, Montezinho L, Silvestre- Ferreira AC, Almeida M, Montenegro L, Escribano D, Vilhena H. Warburg Effect In Feline Lymphoma. Poster Presented At The European Society Of Veterinary Oncology Congress, 2021.

Wang, Z. X., Jiang, C. P., Cao, Y., Zhang, G., Chen, W. B., & Ding, Y. T. (2015). Preoperative serum liver enzyme markers for predicting early recurrence after curative resection of hepatocellular carcinoma. Hepatobiliary & pancreatic diseases international : HBPD INT, 14(2), 178–185. https://doi.org/10.1016/s1499-3872(15)60353-8

Warburg O. (1956). On the origin of cancer cells. Science (New York, N.Y.), 123(3191), 309–314. https://doi.org/10.1126/science.123.3191.309

Weng, Y., Zhu, J., Chen, Z., Fu, J., & Zhang, F. (2018). Fructose fuels lung adenocarcinoma through

GLUT5. Cell death & disease, 9(5), 557. https://doi.org/10.1038/s41419-018-0630-x

Xie, N., Tan, Z., Banerjee, S., Cui, H., Ge, J., Liu, R. M., Bernard, K., Thannickal, V. J., & Liu, G. (2015). Glycolytic Reprogramming in Myofibroblast Differentiation and Lung Fibrosis. American journal of respiratory and critical care medicine, 192(12), 1462–1474. https://doi.org/10.1164/rccm.201504-0780OC

Yu L, Chen X, Wang, L. Chen, S. The Sweet Trap In Tumors : Aerobic Glycolysis And Potential Targets For Therapy. Oncotarget. 2016;7,38908–38926.

Zamora-León, S. P., Golde, D. W., Concha, I. I., Rivas, C. I., Delgado- López, F.,Baselga, J., Nualart, F., & Vera, J. C. (1996). Expression of the fructose transporter GLUT5 in human breast cancer. Proceedings of the National Academy of Sciences of the United States of America, 93(5), 1847–1852. https:// doi.org/ 10.1073/pnas.93.5.1847

Zanatta, R., Abate, O., D'Angelo, A., Miniscalco, B., & Mannelli, A. (2003). Diagnostic and prognostic value of serum lactate dehydrogenase (LDH) and LDH isoenzymes in canine lymphoma. *Veterinary research communications*, *27 Suppl 1*, 449–452. https://doi.org/10.1023/b:verc.0000014201.82393.67

Zhu, W. W., Lu, M., Wang, X. Y., Zhou, X., Gao, C., & Qin, L. X. (2020).The fuel andengine: The roles of reprogrammed metabolism in metastasis of primary liver cancer. Genes & diseases, 7(3), 299–307. https:// doi.org/10.1016/j.gendis. 2020.01.016