UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CÂMPUS DE JABOTICABAL

DETERMINING LACTATE AND GLUCOSE THRESHOLDS AND HEART RATE DEFLECTION POINT IN DOGS UNDERGOING INCREMENTAL EXERCISE TESTING

Wilmer Alejandro Zamora Restan

Medico Veterinário

2017

UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CÂMPUS DE JABOTICABAL

DETERMINING LACTATE AND GLUCOSE THRESHOLDS AND HEART RATE DEFLECTION POINT IN DOGS UNDERGOING INCREMENTAL EXERCISE TESTING

Wilmer Alejandro Zamora Restan

Orientador: Prof. Dr. Guilherme de Camargo Ferraz

Dissertação apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Mestre em Medicina Veterinária (Clínica Medica Veterinária)

2017

Zamora Restan, Wilmer Alejandro
Z25d Determining lactate and glucose thresholds and heart rate deflection point in dogs undergoing incremental exercise testing / Wilmer Alejandro Zamora Restan. – – Jaboticabal, 2017
xv, 41 p. : il. ; 29 cm

Dissertação (mestrado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2017
Orientador: Guilherme de Camargo Ferraz
Banca examinadora: Herbert Gustavo Simões, Aparecido Antonio Camacho
Bibliografia

1. Limiares anaeróbicos. 2. Cão. 3. Teste de Conconi. 4. Estado estacionário. 5. Fisiologia do exercício. I. Título. II. Jaboticabal-Faculdade de Ciências Agrárias e Veterinárias.

CDU 619:612.766.1:636.7

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação – Serviço Técnico de Biblioteca e Documentação - UNESP, Câmpus de Jaboticabal.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: DETERMINING LACTATE AND GLUCOSE THRESHOLDS AND HEART RATE DEFLECTION POINT IN DOGS UNDERGOING INCREMENTAL EXERCISE TESTING

AUTOR: WILMER ALEJANDRO ZAMORA RESTAN
ORIENTADOR: GUILHERME DE CAMARGO FERRAZ

Aprovado como parte das exigências para obtenção do Título de Mestre em MEDICINA VETERINÁRIA, área: CLÍNICA MÉDICA VETERINÁRIA pela Comissão Examinadora:

Prof. Dr. GUILHERME DE CAMARGO FERRAZ
Departamento de Morfologia e Fisiologia Animal / FCAV / UNESP - Jaboticabal

Prof. Dr. HERBERT GUSTAVO SIMOES
Departamento de Educação Física / Universidade Católica de Brasília,DF

Prof. Dr. APARECIDO ANTONIO CAMACHO
Depto. de Clínica e Cirurgia Veterinária / FCAV / UNESP - Jaboticabal

Jaboticabal, 22 de fevereiro de 2017

DADOS CURRICULARES DO AUTOR

Wilmer Alejandro Zamora Restan – nascido em Cúcuta, NS, Colômbia em 12 de agosto de 1990. Em agosto de 2007 ingressou no curso de Medicina Veterinária e Zootecnia da Faculdade de Medicina Veterinária, da Universidade Cooperativa de Colômbia, campus Ibague. Graduou-se em setembro de 2014 e em março de 2015 ingressou no curso de Mestrando do Programa de Medicina Veterinária, área de Clínica Médica da Universidade Estadual Paulista Júlio de Mesquita Filho - Campus Jaboticabal. É mestrando do Laboratório de Fisiologia do Exercício Equino e Farmacologia (LAFEQ) e colaborador do Setor de Cardiologia Veterinária de Pequenos Animais do Hospital Veterinário “Governador Laudo Natel”, da Faculdade de Ciências Agrárias e Veterinárias (FCAV) - UNESP Jaboticabal.

“A crença na vida após a morte é um conto de fadas para quem tem medo de morrer.”

Stephen Hawking

À minha mãe, por acreditar e sempre me apoiar, por seu amor e incentivo

AGRADECIMENTOS

Minha gratidão eterna a minha mãe, Omaira Judith Restan Hincapie, pelo apoio e incentivo ao longo desses quatro anos em Jaboticabal, mesmo estando longe, você foi meu o motivo para terminar meu trabalho, meu apoio e meu maior orgulho.

À Bruna, minha namorada, pelo amor e apoio, por me acompanhar diariamente nas etapas finais do meu trabalho e por brindar-me a oportunidade de viver uma grão história do seu lado, e especialmente a meu peludinho, motivo de acordar cada dia.

Ao Prof. Guilherme, por sua orientação, paciência e ajuda na elaboração desse trabalho, por ter compartilhado seu conhecimento, seu valioso tempo e sua amizade e, especialmente, por ter acreditado em mim, num momento difícil. Obrigado por ter dado a oportunidade de aprender sobre uma das minhas paixões, a Fisiologia do Exercício.

Ao Prof. Camacho, que me aceitou no estágio, confiou em mim e permitiu fazer parte do Serviço de Cardiologia, minha área preferida, onde aprendo muito e conheci profissionais excelentes e grandes amigos. Muito obrigado por toda ajuda e auxílio para execução desse projeto.

Ao Evandro, pela amizade, pela paciência e ajuda na elaboração desse trabalho, exemplo de pessoa e profissional que tenho como modelo para seguir.

Um agradecimento, muito especial a Prof$_a$ Dunia Trujillo, pela amizade, confiança e ajuda para poder realizar meus sonhos de realizar meus estudos, obrigado por ter acreditado em mim.

Um agradecimento, a Edna pela ajuda desde o momento que cheguei em Jaboticabal, pelos conselhos e amizade.

Um agradecimento, muito especial, a Samara Beretta pela amizade, pela ajuda nos momentos onde estava começando nosso trabalho, você foi parte fundamental deste. Agradeço imensamente a você pela parceria, companheirismo e paciência e pelo presente que você deu na minha vida junto com o Yuri.

Um agradecimento, muito especial, a Juliana Cerqueira pela ajuda para finalizar este trabalho, pela amizade e paciência, obrigado pela parceria, nosso equipe de trabalho foi ótima.

Um agradecimento, muito especial, a Jessica pela ajuda, pela amizade, pelos momentos de apoio nos momentos difíceis em meu experimento, pela ajuda nos testes de esforço e no manejo dos cães.

Ao Jorge Hurtua, pela ajuda incondicional nos testes de esforço e demais momentos.

Aos meus colegas e amigos de serviço, Evandro, Jorge, Raphael, Fernando, Jais, Michelle, Bruno, Beth e Roberto só tenho a agradecer por todo ensinamento, paciência, ajuda e carinho. Em especial ao Evandro e o Jorge pela colaboração, paciência e ensino durante as avaliações.

Aos graduandos, pós-graduandos e funcionários do LAFEQ pela confiança e colaboração na realização da parte experimental desse projeto.

Ao Professor Aulus, a Thayla, a Raquel, a Bruna a Elaine e Diego pela paciência e confiança acreditada e pelos Beagles cedidos para realização do Trabalho.

Aos funcionários do hospital veterinário. Ao seu Isaís que foi o motorista durante o início do meu trabalho; a Lidiane, pelo auxílio casual no transporte dos animias, assim como o Marcelo; a Flavia e Maria Luiza.

Aos meus amigos Colombianos Ricardo, Yuri, Oscar, Jessica, Jorge, Didimo, Edna, Diana, Elwi, Yani, Donicer e outros por fazer a minha estadia no Brasil mais fácil.

Aos Beagles por toda sua ajuda e permitir realizar meu experimento.

Um agradecimento, CAPES pela concessão da bolsa de estudos.

LISTA DE TABELAS

LISTA DE FIGURAS

CAPÍTULO 2 - **DETERMINING LACTATE AND GLUCOSE THRESHOLDS AND HEART RATE DEFLECTION POINT IN BEAGLE DOGS UNDERGOING INCREMENTAL EXERCISE TESTING**

SUMÁRIO

Página

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo nº 3.624/15 do trabalho de pesquisa intitulado "Determinação do limiar glicêmico de cães submetidos a um programa de condicionamento aeróbio", sob a responsabilidade do Prof. Dr. Guilherme de Camargo Ferraz está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 03 de março de 2015.

Jaboticabal, 03 de março de 2015.

Prof.ª Dr.ª Paola Castro Moraes
Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n CEP 14884-900 - Jaboticabal - SP - Brasil
Tel. 16 3209 2600 - fax 16 3202 4275 www.fcav.unesp.br

RESUMO

O propósito deste estudo foi comparar métodos para determinação dos limiares relacionados com curvas de lactato plasmático e suas intensidades. Determinaram-se os limiares (1) de lactato (LTv) e (2) glicêmico (GTv) visualmente, (3) o limiar de lactato por meio do modelo polinomial (LTp), (4) limiar de glicose pelo modelo polinomial (GTp) e (5) o ponto de deflexão da frequência cardíaca (FCdp). Dezoito Beagles foram submetidos a teste de esforço incremental (IET). O IET foi realizado numa esteira, sendo concluído quando os cães mostraram sinais de fadiga. Os LTv GTv e FCdp foram determinados visualmente; LTp e GTp foram obtidos matematicamente por meio de função polinomial. Análise de variância (ANOVA), correlação de Pearson, regressão ordinária dos produtos mínimos e Bland-Altman foram utilizadas para avaliar a concordância entre as variáveis. Não houve diferença entre as velocidades relacionadas aos limiares ($P > 0.05$). Houve correlação entre LTv e GTv ($r = 0,91$), LTv e LTp ($r = 0,96$), LT e GTp ($r = 0,94$) e a velocidade de corrida no ponto de deflexão da freqüência cardíaca (Vdp) e LT ($r = 0,95$) ($P < 0,05$). Foi observado viés constante entre LTv e LTp. Foi possível a determinação dos limiares de lactato e glicêmico em cães, sendo que a concordância entre LTv e FCdp indicou que a FC pode ser utilizada como método indireto para a obtenção do limiar de lactato.

Palavras-chave: Limiar anaeróbio, Cão, Teste de Conconi, Estado estacionário, Fisiologia do exercício.

ABSTRACT

The aim of this study was to compare methods to determining visually (1) lactate threshold (LTv), (2) glycemic threshold (GTv) and (3) lactate threshold by the polynomial model (LTp), (4) glucose threshold by the polynomial model (GTp), and (5) heart rate deflection point (HRdp). Eighteen Beagles underwent an incremental exercise testing (IET). IET was performed on the treadmill. The IET was completed when the dogs showed signs of fatigue. The LTv, GTv and HRdp were determined visually; LTp and GTp were obtained from the polynomial function. One-way analysis of variance, Pearson correlation, ordinary least product regressions and Bland-Altman plot were used to assess the agreement between the variables. There was no difference between the velocities corresponding to the thresholds (P >0,05). There was a correlation between LTv and GTv (r = 0.91), LTv and LTp (r = 0.96), LT and GTp (r = 0.94) and the running speed at the heart rate deflection point (Vdp) and LT (r = 0.95) (p <0.05). A constant bias was observed between LTv and LTp. It was possible to determine the lactate and glycemic thresholds in dogs, and the agreement between LTv and FCdp indicated that the HR can be used as an indirect method to obtain the lactate threshold.

Keywords: Anaerobic threshold, Dog, Conconi test, Steady state, Exercise physiology.

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS.

1. INTRODUÇÃO

De maneira geral a fisiologia do exercício é ramo da ciência que estuda alterações momentâneas na homeostasia durante o exercício, e como estas modificam a estrutura e funcionamento do corpo. Isso inclui benefícios para a saúde e as respostas crônicas provocadas pelo condicionamento em atletas, indivíduos enfermos, bem como, nas modalidades de alto rendimento. Isto acontece no organismo como um todo. O interesse na fisiologia do exercício é demostrado por muitas áreas, tais como, fortalecimento, condicionamento e fisiologia do exercício clínico, tudo isto englobado na medicina esportiva (HERSHEL, 2000).

A utilização de esteira para cães permite o estudo, sob condições laboratoriais controladas das respostas metabólicas e clínicas, frente à prática de esforço físico. Neste contexto, existe claro interesse no desenvolvimento de métodos para avaliação da aptidão física, com destaque para a área que estuda a relação entre a concentração de lactato sanguíneo e a intensidade de esforço, por meio de testes físicos realizados em esteiras (FERASIN et al., 2009).

Segundo Simões et al. (1999), a lactatemia e a glicemia quando relacionadas à intensidade (velocidade) de esforço podem ser utilizadas tanto para prescrever a intensidade das sessões de exercício, como para detectar adaptações decorrentes da prática de exercício crônico (condicionamento). As determinações dos limiares de lactato e glicêmico são utilizadas intensivamente para o diagnóstico da capacidade aeróbia, que está correlacionada com a resistência (“endurance”). Nesta idéia, existem testes com incrementos gradativos da intensidade (teste incremental) que utilizam a resposta da lactatemia e da glicemia para o diagnóstico aeróbio em equinos (FERRAZ et al., 2008) e seres humanos (SIMÕES, 2003). Outra metodologia alternativa para determinação indireta da capacidade aeróbia foi sugerida originalmente por Conconi et al. (1982), que utilizaram um método não invasivo determinado a partir da FC durante um teste de esforço incremental (TEI) em esteira. Este método foi definindo pela redução da inclinação da relação entre a HR vs

velocidade, conhecida como o ponto da deflexão da FC (FCdp) (CONCONI et al.,1996; GRAZZI et al., 2005). Em outras palavras, a FCdp é caracterizada pela tendência curvilínea e perda do aumento linear da FC durante um TEI, sendo que alguns estudos na espécie humana revelaram correlação positiva com o LL (CONCONI et al., 1982; SENTIJA et al., 2007; IGNJATOVIC et al., 2008).

2. REVISÃO DE LITERATURA

2.1. Bioenergética

Para a realização de exercício faz-se necessário que haja conversão de energia química em trabalho físico, energia cinética. O músculo esquelético utiliza várias fontes de energia (MURPHY et al., 1997). A molécula de trifosfato de adenosina (ATP) é a principal fonte de energia para os músculos (BURGER, 1993; MURPHY et al., 1997). Essa concentração é baixa de ATP no músculo, em comparação com as necessidades do exercício. Por esta razão, a ATP tem de ser formada a partir de outros substratos musculares (GRANDJEAN; PARAGON, 1992).

O fosfato de creatina é um combustível armazenado endogenamente no sarcoplasma, sendo utilizado para liberar rapidamente o fosfato para formação da ATP (TOLL; REYNOLDS, 2000). Esta fonte de energia repõe a ATP numa taxa rápida. Entretanto, é suficiente apenas para alguns segundos (5-15), já que os armazenamentos de fosfato de creatina nos músculos são limitados (BURGER, 1993; TOLL; REYNOLDS, 2000).

Uma fonte de ATP anaeróbia é feita por meio do metabolismo da glicose (GRANDJEAN; PARAGON, 1992). Esse processo quebra a glicose ou glicogênio a piruvato que pode ser convertido em lactato, com a liberação de ATP. A glicogenólise é a quebra do glicogênio armazenado nos músculos em glicose, que pode então produzir ATP e com liberação de íons H^+ e lactato (GRANDJEAN; PARAGON 1992; MURPHY et al., 1997). Glicogenólise e glicólise podem produzir ATP em taxas muito rápidas (até 6 vezes mais rápido do que o metabolismo aeróbio). Entretanto, ambos mecanismos apenas podem produzir um baixo rendimento de ATP, já que a produção

só pode ser mantida por um curto período de tempo (GRANDJEAN; PARAGON, 1992; WORTINGER, 2007).

A glicose têm três destinos possíveis: pode ser armazenada na forma de uma macromolécula; pode ser oxidada e depois utilizada na via das pentoses fosfato; ou pode ser oxidada até formar piruvato por meio da glicólise e gerar ATP (NELSON & COX, 2004). A glicólise é o primeiro passo do metabolismo da glicose, sendo um processo anaeróbio que ocorre no citoplasma das células (ALLEN; HOLM, 2008). Durante este processo, uma molécula de glicose é degradada por meio de uma série de reações em cadeia, dando origem ao piruvato. Parte da energia contida na molécula de glicose é perdida sobre a forma de calor, enquanto outra parte é conservada na forma de ATP (NELSON; COX, 2004).

O piruvato que se forma ao final desta sequência tem dois destinos possíveis.Pode ser oxidado em acetilcoenzima A e entrar numa nova fase de geração de ATP, difundindo-se para a mitocôndria e entrando no ciclo de Krebs e na fosforilação oxidativa (ALLEN; HOLM, 2008). Neste momento ocorre transferência de elétrons e oxidações dos diferentes co-factores levando à produção de ATP, reduzindo o piruvato a lactato, aceitando elétrons do NADH, formando-se o NAD+ (NELSO; COX, 2004). Este processo é muito mais eficiente que a glicólise e ocorre particularmente em células com elevada capacidade oxidativa, como os músculos esquelético e cardíaco (STEVENSON et al., 2007). Por outro lado, o lactato formado durante a glicólise, por meio da redução do piruvato pela ação da enzima lactato desidrogenase (LDH), é transportado pelo sangue até o fígado e convertido em glicose, processo conhecido como neoglicogênese ou Ciclo de Cori. (STEVENSON et al., 2007; ALLEN; HOLM, 2008). Importante ressaltar que é nesse momento que pode haver coincidência entre as curvas lactato versus velocidade e glicose versus velocidade (SIMÕES et al., 1999; FERRAZ et al., 2008).

Outra fonte de ATP para o cão é o metabolismo das gorduras, aminoácidos e glicose. Trata-se da via aeróbia (com participação essencial da molécula oxigênio), este processo ocorre na mitocôndria e também é conhecido como aerobiose

(GRANDJEAN; PARAGON, 1992; WORTINGER, 2007). O metabolismo aeróbio de gorduras, aminoácidos e glicose, produz ATP num ritmo mais lento quando comparado ao metabolismo anaeróbio (GRANDJEAN; PARAGON, 1992).

2.2 Limiar de lactato e exercício

O lactato forma-se durante a glicólise anaeróbia por meio da redução do piruvato pela atividade enzimática da lactato desidrogenase (LDH). O lactato começa a ser produzido quando observamos a necessidade de aceleração da glicólise, pelas fibras musculares, com o objetivo de produzir a energia que é necessária para sustentar determinada intensidade de exercício. O aumento da lactatemia durante o exercício é a consequência da produção exponencial de lactato pelo músculo e pelo transporte deste lactato para a corrente sanguínea (FERASIN; NGUYENBA, 2008; FERASIN; MARCORA, 2009). A deflexão (limiar de lactato) observada na curva lactato-velocidade ocorre na espécie equina nas concentrações de lactato que podem variar entre 1,5 a 4 mmol/L sendo observado a utilização frequente da uma unidade arbitrária de 4 mmol/L tanto para a prescrição e avaliação como para comparação de programas de condicionamento físico (CAMPBELL, 2011).

Segundo estudo na espécie canina, diferentemente daquilo que é observado nas espécies humana e equina, o exercício parece não induzir alterações abruptas na concentração do lactato sanguíneo (FERASIN; MARCORA, 2009). Não obstante, apesar de variações significativas na lactatemia observadas entre as diferentes intensidades de esforço, na espécie canina, até o presente momento, não foi possível encontrar nenhum estudo científico que determinou o limiar de lactato tal como está descrito em equinos ou humanos. Portanto, a determinação do limiar de lactato em cães é uma lacuna que se observa na literatura. Esta dissertação de mestrado pretendeu propor uma metodologia para determinação do LL em cães da raça Beagle.

2.3 Glicemia e exercício

A glicemia após o exercício manteve-se inalterada em relação às concentrações antes do esforço, em vários estudos utilizando Beagles, cães sem raça definida e cães de trenó (NAZAR et al., 1992; HINCHCLIFF et al., 1993; BURR et al., 1997; CHANOIT et al., 2002). Entretanto, em outros estudos foi demosntrado que a glicose aumenta durante os exercícios de alta velocidade em cães galgos (SNOW; HARRIS; STULTARD, 1988; ILKIW et al., 1989; ROSE et al.,1989). O mesmo comportamento foi observado em atletas da espécie humana e equina (SIMÕES et al., 1999; FERRAZ et al., 2008).

A liberação de hormônios que, possuem ação antagônica, como o cortisol e a insulina tem um papel primordial na regulação do metabolismo da glicose, cuja concentração pode sofrer alterações significativas durante o exercício, de modo a controlar o aporte energético que as células necessitam (ROVIRA et al., 2008). Ademais, durante o exercício, há um aumento na concentração das catecolaminas, hormônio do crescimento e glucagon (SCHNABEL et al., 1982; HARGREAVES; RICHTER, 1988; URHAUSEN et al., 1994.). Rovira et al. (2008) verificaram em cães que a glicemia permaneceu constante ao longo de um exercício de agilidade, imputando este fato a um balanço entre a mobilização hepática pelo aumento das concentrações de catecolaminas e o aporte de glicose pelo músculo para o seu metabolismo. Adicionalmente, as catecolaminas tem uma influência sobre a produção de lactato, já que levam ao aumento da concentração de glicose sanguínea devido ao estímulo da glicólise/glicogenólise (ROVIRA et al., 2008). O exercício causa uma ação adrenérgica, sendo que a liberação de adrenalina estimula a glicogenólise e a produção de lactato durante o exercício (EXTON, 1979; STAINSBY et al., 1991).

2.4 Frequência cardíaca

As alterações cardiovasculares durante o exercício, podem ser monitoradas e, portanto, usada como um indicador da intensidade do exercício. A resposta da FC

aumentam linearmente conforme a intensidade do exercício (MCGOWAN; HAMPSON, 2007). Contudo, Brooke, Hamley e Thomason (1968), e novamente Brooke e Hamley (1972) observaram que a resposta da FC aos testes incrementais nem sempre indica um aumento linear.

Baseando-se nas observações feitas em pesquisas anteriores, Conconi et al. (1982) propuseram um teste indireto não invasivo para determinar o limiar anaeróbio, por meio da análise da FC. Conconi et al. (1982) relataram que durante o exercício incremental progressivo ocorre uma deflexão na relação linear da FC com a velocidade, onde o aumento da FC e linear com a velocidade até velocidades submáximas, após disto a relação torna-se curvilínea. O ponto de transição de linear para curvilíneo foi denominado como ponto de deflexão da frequência cardíaca (FCdp) e a sua velocidade de deflexão correspondente (Vdp). Esta metodologia é denominada teste de Conconi. O teste foi posteriormente aplicado em outras modalidades de exercícios em ambientes laboratoriais e de campo. FCdp é relatado na faixa de 88 a 94% de HR máxima (BODNER; RHODES, 2000) para atletas em vários esportes e sob diferentes protocolos.

Em cães a FCdp foi determinada por Radin et al. (2015) em *Border collies* submetidos a teste de esforço incremental em esteira. A média de FCdp nos Border collies nesta pesquisa correspondeu a 80% de sua FCmáx, com intervalos de entre 162-229 bpm. Contudo, Radin et al. (2015) ressaltaram a necessidade de estudos futuros, para verificar se os valores de limiar anaeróbio obtidos usando a técnica não-invasiva correlacionam-se com o limiar de lactato.

2.5 Testes para avaliação da aptidão física e prescrição de um programa de condicionamento

Durante e após um teste de esforço, a capacidade funcional ou aptidão física do indivíduo é determinada indiretamente pela capacidade aeróbia por meio da frequência cardíaca (FC) e/ou por meio do limiar de lactato.

Na espécie humana e nos equinos, a determinação tanto da lactatemia e glicemia, como a FC durante os testes de esforço são bem conhecidos, sendo que a prescrição

de programas de condicionamento pode ser guiada pela percentagem da FC máxima ou pelo limiar de lactato (CAMPBELL, 2011). Ademais, as adaptações metabólicas e musculares provocadas por um programa de condicionamento físico podem ser determinadas pela lactatemia (FLETCHER et al., 1990; FERRAZ et al., 2008). Segundo Simões et al. (1999), Trilk et al. (2002) e Erck et al. (2007) a concentração de lactato sanguíneo é utilizada tanto para avaliação do condicionamento físico como para prescrever a intensidade de treinamento e detectar adaptações decorrentes da prática de exercício crônico (condicionamento ou treinamento). A determinação do LL é empregada intensivamente para o diagnóstico da capacidade aeróbia que está correlacionada com a resistência (“endurance”). Existem vários testes com incrementos gradativos da intensidade de esforço (teste incremental) que utilizaram a resposta da lactatemia para o diagnóstico aeróbio (CAMPBELL, 2011).

Mudanças na concentração plasmática do lactato foram principalmente estudadas no homem e no cavalo (EVANS et al., 1993; SIMÕES et al., 1999; FERRAZ et al., 2008). A lactatemia elevada depende do ponto onde o estado estacionário dinâmico (maxima fase estável de lactato) entre a produção, utilização e remoção de lactato é perdido devido à produção excessiva, e a concentração de lactato no sangue começa a subir exponencialmente (FERRAZ et al., 2008). Ademais, na espécie humana, como nos equinos, a determinação do limiar glicêmico pode ser utilizada como indicadora da capacidade aeróbia (SIMÕES et al., 1999; FERRAZ et al., 2008). Entretanto, na espécie canina pouco se sabe a respeito destas importantes variáveis fisiológicas, uma vez que os testes de esforço são empregados na cardiologia veterinária somente com finalidade de diagnóstico e prognóstico (KITTLESON; JOHNSON; PION, 1996; BODDY et al., 2004; FERASIN; MARCORA, 2007). Será que o limiar de lactato e o limiar glicêmico possuem concordância entre si e podem ser utilizados para avaliação da aptidão física em cães? Esta é a pergunta que os resultados da presente dissertação pretendem responder.

A correspondência entre a velocidade obtida na esteira e o limiar de lactato e glicêmico costuma ser utilizada na avaliação do potencial atlético e na quantificação dos possíveis efeitos de programas de condicionamento físico na espécie humana e em equinos (SIMÕES et al., 1999, FERRAZ et al., 2008).

Atualmente, os testes ergométricos numa esteira rolante são os mais utilizados na espécie humana (WISLOFF et al., 2005), camundongos (ALMEIDA et al., 2011) e equinos (FERRAZ et al., 2008). Estes permitem a prescrição individualizada para o tratamento de doenças e auxilia na compreensão dos mecanismos envolvidos na melhoria do condicionamento físico e da capacidade aeróbia. Com este propósito, não existem testes específicos padronizados para cães.

Com base no conhecimento reduzido sobre o LL, LG e a FCdp, o segundo capítulo da presente dissertação abordará alguns métodos para determinação do LL e LG por metodologia visual, assim como modelo polinominal e a FCdp, e a sua correlações. Este artigo será enviado para o periódico **BMC Veterinary Research.**

REFERÊNCIAS

ALMEIDA, W. S.; LIMA, L. C. J.; CUNHA, V. N. C.; CUNHA, R.; ARAÚJO, R. C.; BARROS, C. C.; SIMÕES, H. G.; CAMPBELL, C. S. G. Assessment of aerobic capacity during swimming exercise in ob/ob mice. **Cell Biochemistry and Function**, Oxford, v. 29, n.8, p.666-672, 2011.

ALLEN, S.; HOLM, J. Lactate: physiology and clinical utility. **Journal of Veterinary Emergency and Critical Care,** San Antonio**,** v. 18, n. 2, p. 123-132, 2008.

BODDY, K. N.; ROCHE, B. M.; SCHWARTZ, D. S.; NAKAYAMA, T.; HAMLIN, R. L. Evaluation of six-minute walk test in dogs. **American Journal of Veterinary Research**, Chicago, v. 65, n. 3, p. 311-313, 2004.

BODNER, M.; RHODES, E. A review of the concept of the heart rate deflection point. **Sports Medicine**, Auckland, v. 30, p. 31–46, 2000.

BROOKE, J. D.; HAMLEY, E. J.; THOMASON, H. The relationship of heart rate to physical work. **The Journal of physiology**, London, v. 197, p. 61-3, 1968.

BROOKE, J.D.; HAMLEY, E. J. The heart-rate-physical work curve analysis for the prediction of exhausting work ability. **Medicine and science in sports**, Madison, v, 4 p. 23-6, 1972.

BURGER, I. H. The Waltham Book of Companion Animal Nutrition. **Pergamon Press Limited**, Oxford, UK, 1993.

BURR, J.R.; REINHART, G. A.; SWENSON, R. A.; SWAIM, S. F.; VAUGHN, D. M.; BRADLEY, D. M. Serum biochemical values in sled dogs before and after competing in long distance races. **Journal of the American Veterinary Medical Association**, Ithaca, v. 211, n. 2, p. 175, 1997.

CAMPBELL, E. H. Lactate-driven equine conditioning programmes. **The veterinary journal**, London, v. 190, n. 2, p. 199-207, 2011.

CHANOIT, G. P.; CONCORDET, D.; LEFEBURE, H. P.; ORCEL, K.; BRAUN, J. P. Exercise does not inducemajor changes in plasma muscle enzymes, creatinine, glucose and total proteins concentrations in untrained beagle dogs. **Journal of Veterinary Medicine Series A**, v. 49, p. 222-224, 2002.

CONCONI, F.; FERRARI, M.; ZIGLIO, P. G.; DROGHETTI, P.; CODECA, L. Determination of the anaerobic threshold by a non-invasive field test in runners. **Journal of applied physiology**, Bethesda, v. 52 p. 869–87, 1982.

EXTON, J. H. Hormonal control of gluconeogenesis. **Advances in Experimental Medicine and Biology**, v. 111, p.125-167, 1979.

EVANS, D. L.; HARRIS, R. C.; SNOW, D. H. Correlation of racing performance with blood lactate and heart rate after exercise in thoroughbred horses. **Equine veterinary journal**, Newmarke, v. 25, p. 441-445, 1993.

FERASIN, L.; MARCORA, S. A pilot study to assess the feasibility of a submaximal exercise test to measure individual response to cardiac medication in dogs with acquired heart failure. **Veterinary Research Communications,** Amsterdam, v. 31, n. 6, p. 725-737, 2007.

FERASIN, L.; MARCORA, S. Reliability of an incremental exercise test to evaluate acute blood lactate, heart rate and body temperature responses in Labrador retrievers. **Journal of comparative physiology.** B, v. 179, n. 7, p. 839-845, 2009

FERRAZ, G. C.; D'ANGELIS, F. H. F.; TEIXEIRA-NETO, A. R.; FREITAS, E. V. V.; LACERDA-NETO, J. C.; QUEIROZ-NETO, A. Blood lactate threshold reflects glucose responses in horses submitted to incremental exercise test. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v. 60, n. 1, p. 256-259, 2008.

FLETCHER, G. F.; FROELICHER, V.F.; HARTLEY, L. H.; HASKELL, W. L.; POLLOCK, M. L. Exercise standards: A statement for health professionals from the American Heart Association. **Circulation,** Dallas, v. 82, n. 6, p. 2286-2322, 1990.

GRANDJEAN, D.; PARAGON, B. M. Nutrition of racing and working dogs part I: Energy Metabolism of Dogs. **Compendium on Continuing Education for the Practicing Veterinarian**, v. 14 n. 12, p. 1608, 1992.

HARGREAVES, M.; RICHTER, E. A. Regulation of skeletal muscle glycogenolysis during exercise. Canadian journal of sport sciences, Downsview, v. 13, n. 4, p. 197-203, 1988.

HERSHEL, R. **Secretos de la Fisiología: Fisiología del ejercicio y bioenergética de la contracción muscular**. México: Mc Graw Hill, 2000. p. 265, 269

HINCHCLIFF, K. W.; OLSON, J.; CRUSBERG, C.; KENYON, J.; LONG, R.; ROYLE, W.; WEBER, W.; BURR, J. Serum biochemical changes in dogs competing in a long distance sled race. **Journal of American Veterinary Medical Association,** Ithaca, v. 202, n. 3, p. 401-405, 1993.

ILKIW, J. E.; DAVIS, P. E.; CHURCH, D. B. Hematologic, biochemical, blood gas and acid base values in greyhounds before and after exercise. **American Journal of Veterinary Research,** Chicago, v. 50, n. 4, p. 583- 586, 1989.

KITTLESON, M. D.; JOHNSON, L.E.; PION, P.D. Submaximal exercise testing using lactate threshold and venous oxygen tension as endpoints in normal dogs and dogs with heart failure. **Journal of Veterinary Internal Medicine**, Philadelphia, v. 10, n. 1, p. 21-27, 1996.

MCGOWAN, C. M.; HAMPSON, B. Comparative exercise physiology. In: MCGOWAN, C.; STUBBS, N. G. **Animal physiotherapy, assessment, treatment and rehabilitation of animals.** Blackwell Pub, Oxford, 2007. p 56–71

MURPHY, M. G.; CONROY, S.; LEWE, J. A. Aspects of exercise physiology in the dog. **Irish Veterinary Journal** v. 50, n. 1, p. 65, 1997.

NAZAR, K.; GREENLEAF, J. E.; POHOSKA, E.; TURLEJSKA, E.; KACIUBA-USCILKO, H.; KOZLOWSKI, S. Exercise performance, core temperature and metabolism after prolonged restricted activity and retraining in dogs. **Aviation, Space and Environmental Medicine**, United States, v 63, n. 8, p. 684-688, 1992.

NELSON, N.; COX, M. **Lehninger principles of biochemestry** (4ª edição). W. H. Freeman & Co. Ltd, 2004.

ROSE, R. J.; BLOOMBERG, M. S. Responses to sprint exercise in the greyhound: effects on haematology, serum biochemistry and muscle metabolites. **Research in Veterinary Science**, London, v. 47, n. 2, p. 212-218, 1989.

ROVIRA, S.; MUÑOZ, A.;BENITO, M. Effect of exercise on physiological, blood and endocrine parameters in search and rescue-trained dogs. **Veterinarni Medicina,** Prague, v. 53, n. 6, p. 333-346, 2008.

SCHNABEL, A.; KINDERMANN, W.; SCHMITT, W. M.; BIRO, G.; STEGMANN, H. Hormonal and metabolic consequences of prolonged running at the individual anaerobic threshold. **International journal of sports medicine**, Stuttgart, v. 3, n. 3, p. 163-168, 1982.

SIMÕES, H. G.; CAMPBELL, C. S. G.; KOKUBUM, E.; DENADAI, B. S.; BALDISSERA, V. Blood glucose responses in humans mirror lactate responses for individual anaerobic threshold and for lactate minimum in track tests. **European journal of applied physiology and occupational physiology,** Berlin, v.80, n.1, p.34-40, 1999.

SIMÕES, H. G.; CAMPBELL, C. S.; KUSHNICK, M. R.; NAKAMURA, A.; KATSANOS C. S.; BALDISSERA, V.; MOFFATT, R. J. Blood glucose threshold and the metabolic responses to incremental exercise tests with and without prior lactic acidosis induction. **European Journal of Applied Physiology**, Udine, v. 89, p. 603-611, 2003.

SNOW, D.H.; HARRIS, R. C.; STULTARD, E. Changes in haematology and plasma biochemistry during maximal exercise in greyhounds. **Veterinary Record,** London, v. 123, n. 19, p. 487-489, 1988.

STAINSBY , W. N.; BRECHUE, W. F.; O'DROBINAK, D. M. Regulation of muscle lactate production. **Medicine and science in sports and exercise**, Madison, v. 23, n. 8, p. 907-911, 1991.

STEVENSON, C.; KIDNEY, B.; DUKE, T.; SNEAD, E.; JAIME, R.; JACKSON, M. Serial blood lactate concentrations in systemically ill dogs. **Veterinary Clinical Pathology**, Baton Rouge, v. 36, n.3, p. 234-239. 2007.

URHAUSEN, A.; WEILER, B.; COEN, B.; KINDERMANN, W. Plasma catecholamines during endurance exercise of different intensities as related to the individual anaerobic threshold. **European journal of applied physiology and occupational physiology**, Berlin, v. 69, n. 1, p. 16-20, 1994.

WISLØFF, U.; NAJJAR, S. M.; ELLINGSEN, O.; HARAM, P. M.; SWOAP, S.; AL-SHARE, Q.; FERNSTRÖM, M.; REZAEI, K.; LEE, S. J.; KOCH, L. G.; BRITTON, S. L. Cardiovascular Risk Factors Emerge After Artificial Selection for Low Aerobic Capacity. **Science**, New York, v. 307, n. 5708, p. 418-420, 2005

CAPÍTULO 2 - **DETERMINING LACTATE AND GLUCOSE THRESHOLDS AND HEART RATE DEFLECTION POINT IN BEAGLE DOGS UNDERGOING INCREMENTAL EXERCISE TESTING**

ABSTRACT

Background: The aim of this study was to compare methods to determining the lactate, glucose and the heart rate deflection threshold (LTv, GTv, and HRdp) in dogs. Eighteen Beagles underwent an incremental exercise testing (IET). IET was performed on the treadmill. The IET was completed when the dogs showed signs of fatigue. The LT_v, GT_v and the HR_{dp} were determined visually; LT_p and GT_p were obtained from the polynomial function. One-way analysis of variance, Pearson correlation, ordinary least product regressions and Bland-Altman plot were used to assess the agreement between the variables.

Results: No differences were observed (P>0.05), between the velocities on the LTv (3.87±0.82), GTv (3.71±0.85), LTp (3.66±0.84), GTp (3.63±0.78) and Vdp (3.82±0.82). High agreement between the variables and high correlations were obtained between the velocities at LTv and GTv (r = 0.91), LTv and LTp (r = 0.96), LT and GTp (r = 0.94) and the running speed at the heart rate deflection point (Vdp) and LT (r = 0.95) (p <0.05).

Conclusions: Our study found no statistical difference between the intensities (velocities) related to the studied variables, A constant bias was observed between LT_v and LT_p only. The agreement between LT_v and HR_{dp} indicated that the physiological variable HR could be used to both determine the intensity

Keywords: anaerobic threshold, dog, conconi test, steady state, exercise physiology,

Background

The visual determination of lactate threshold (LT_v) or anaerobic threshold is used to prescribe training intensity and/or evaluating physical fitness in humans, horses, and rats [1,2,3]. Furthermore, it is characterized by the highest exercise intensity that does not cause an exponential increase in plasma lactate concentrations [lactate]. It is the point at which lactate concentration increases above baseline during an incremental exercise test (IET). Essentially, it indicates the steady state between lactate production and removal. In the literature, there is an intense and ongoing debate about the physiological mechanisms and the methodology for determining the LT [4,5].

Most methodological studies on the plasma lactate curve and lactate thresholds (LTs), obtained from an IET, were performed in humans [4] and horses [6]. In recent years, few research groups [7,8,9], have shown relative interest in determining some useful variables related to [lactate] for studying physical fitness in dogs using the IET on a treadmill. The performance tests are used in healthy dogs, undertaken to work as police dogs, tracking/detection and athletics dogs for the determination of functional capacity, as well as the measure the exercise tolerance of untrained canine cardiac patients [7,8,9]. To our knowledge, determining the LT_v in dogs by visual inspection of the exponential growth of lactate-velocity curve has not yet been performed, and the studies conducted with canine species used submaximal intensity tests [7,8,9].

Besides LT_v effectiveness, other studies have investigated the behavior of blood glucose during an IET in runners and athlete horses. The visual Glucose threshold (GT_v) was identified and used as an alternative method to assess aerobic capacity [2,3,10]. Another method developed for human athletes [10,11], and used for horses as well [5], is to estimate the lactate-minimum-speed (LMS). This method consists of carrying out a conventional IET, after inducing hyperlactatemia by a high-intensity effort (sprint). However, this test protocol is not recommended for elderly or untrained individuals. As an alternative to the LMS protocol, the LT is determined by a polynomial mathematical model, by dividing the plasma lactate concentration and intensity ([lactate]/velocity). The Polynomial lactate threshold (LT_p), methodology produces a U-shaped curve, which has been previously established for humans and rodents [1,10]. It is noteworthy that it was not found in the literature on LT_s, studies applying the polynomial function to glucose vs. effort intensity curves.

Another non-invasive method for indirect determination of LT using an IET was suggested by Conconi et al [6]. This variable is obtained from the relationship between heart rate (HR) and exercise intensity. The

relationship is linear and non-linear, and this method consists of reducing the linearity (breakpoint) between the HR vs. velocity, known as heart rate deflection point (HR_{dp}) [6,12,13]. In other words, HR_{dp} is characterized by the curvilinear trend and loss of the linear increase in HR during an IET, and the corresponding velocity deflection point (V_{dp}) is positively correlated with LT in humans [14]. From mechanical point of view when has used the HR_{dp}, it is not necessary to use types of equipment or invasive techniques to determine the aerobic threshold [15]. In dogs, HR_{dp} and V_{dp} were determined visually, and the authors reported that it is necessary to verify whether the values obtained by this deflection correlate with those obtained by invasive techniques, such as using blood samples for LT [16]. Thus, this study is an attempt to fill in this gap.

Over the past 50 years, approaches that correlated plasma concentrations of lactate, glucose and HR to exercise intensity and methodologies to determine lactate or glucose threshold have become an important tool to diagnose endurance performance. Indeed, the few studies found were not able to determine the lactate threshold. Therefore, this study hypothesized that dogs submitted to an EIT with progressive loads, reach to maximum capacity by forming lactate-velocity curve that allows determining the lactate threshold. Thus, the objectives this study were 1) to verify the possibility to determine the LT in dogs by using visual inspection; and 2) to compare above-mentioned methods for LT, GT and Vdp determination. The possible constant and/or proportional biases were also determined using ordinary least product regression.

Methods

Animals

Eighteen clinically healthy Beagles (10 males and 8 females), weighing on average 12.6 ± 0.9 kg and aged on average 1.4 ± 0.1 years old, were used. The dogs belonged to the kennel of the Laboratory of Nutrition and Nutritional Diseases of dogs and cats of the UNESP – Univ Estadual Paulista, in Jaboticabal, SP. Before being included in the study, all animals underwent a complete clinical examination, seeking to discard concomitant diseases that could influence the results. Moreover, dogs undergoing drug treatment were not admitted in the study. The dogs were fed the commercial ration Sabor & Vida® (Guabi, Campinas, SP Brazil), enough to meet the energy needs according to NCR.[17] The study followed the Ethical Principles in Animal Experimentation adopted by The Ethics Committee on the Use of Animals (CEUA - 3.624/15).

Incremental exercise test and blood samples

The experiment was conducted at the Equine Exercise Physiology and Pharmacology Laboratory (LAFEQ) of the UNESP – Univ Estadual Paulista, in Jaboticabal, Brazil. The dogs underwent an adaptation period of ten consecutive days to get used to the exercise room. On the first day, they were encouraged to climb on the turned-off treadmill (Galloper® 5500, Sahinco, Brazil), receiving positive reinforcement as reward. On the second day, the dog climbed on the treadmill, which was then turned on at the initial speed of 1.5 m/s, causing the dog to move while the staff members encouraged the animal to move so that later, they could perform the exercise test comfortably and safely. The dogs were rewarded biscuits after the exercise sessions as positive reinforcement. During the IET, the room temperature varied between 19 and 22°C, relative humidity between 50 and 60%. The altitude was 597 meters. The IET and blood collection were previously determined in pilot tests, which demonstrated the feasibility of collecting the blood at this time (90 seconds) since the release of lactate from the muscle to the blood is slower in dogs [8], compared with the humans and horses. At the beginning, the dogs were placed on the mat, with no inclination, at a speed of 1.5 m/s for 5 minutes, as a warm up. Subsequently, the belt was set at 5 and 7.5 % slope while velocity was increased gradually in increments of 0.5 m/s with duration of 5 min each. The treadmill remained still for 120 seconds between incremental steps. During this period, at precise 90 seconds, blood samples were collected through a venous catheter placed in the left jugular vein. Subsequently, the belt was restarted, to continue the test. The IET was interrupted when the dogs showed signs of exhaustion, characterized by sudden stop or loss of motor coordination. The whole blood samples (2 ml) of each incremental step were collected in tubes containing EDTA and sodium fluoride, centrifuged for plasma separation and determination of [lactate] and [glucose] using an electro-enzymatic Bioanalyzer (YSI 2300, Yellow Springs Instrument, USA) previously validated in dogs [18].

Velocity related to lactate and glucose thresholds

The objective of the IET is to increase the concentrations of [lactate]. Thus, the LT_v was identified through visual analysis from the observation of three evaluators (LT_v). The evaluators have been blinded to the dog's identity and the other variables associated with that dog's exercise test. Thus, the evaluators, working independently, visually set the lactate threshold. The velocity at lactate threshold consisted of the mean values informed by the three evaluators. The LT is determined at the point where the linear increase of the plasma lactate vs. velocity shows an abrupt and exponential increase of [lactate], considering the workload corresponding to the inflection point of [lac]

within a range of 1.30 – 5 mmol/l of plasma lactate concentration. The lactate peak (Lac Peak) produced in each test was also determined. The GT was identified visually (GT_v) as the minimum point in relation to the x-axis (abscissa) in the glucose-velocity curve [23]. The LT_p and GT_p thresholds were determined mathematically using a second-order polynomial function. To determinate the LT_p, the polynomial function has been applied to the [lac]/velocity ratio versus velocity in the IET, producing the U-shape plot curve similarly to lactate minimum test [1]. The point has been used to determine LT_p and GT_p mathematically, considering the visually identified minimum in relation to the x-axis, and two points immediately before and after. This function was applied to each dog individually.

Heart Rate

The HR was determined using Cardioflash® equipment (digital - Cardio Systems, São Paulo, Brazil), belonged to the Laboratory of Cardiology of the Veterinary Hospital "Governor Laudo Natel" of the UNESP - Univ Estadual Paulista, in Jaboticabal, Brazil. The signals were captured using 2223BRQ® adhesive electrodes (3M São Paulo, Brazil) adhered to the previously shaved skin surface. Placement of electrodes in the animal for continuous electrocardiographic recording three channels: red (xiphoid), white (notch), black (left hemithorax) and green (right hemithorax). Leads were arranged to approximate the frontal leads I, II, and III. A bandage was applied to the dogs' chest, which worn a vest for storing the digital 3-channel recording device. The electrocardiographic tracings were processed by specific software (Cardiosmart©, Cardios Systems, Brazil) and always reviewed by the same veterinarian.

HR_{dp} and V_{dp} determination

The mean HR values obtained for each incremental step were used to determine the heart deflection point (HR_{dp}) and the velocity deflection point (V_{dp}) by visual inspection using as a guideline the methodology published by Radin et al [16]. The HRmax values were determined for each animal in each exercise testing.

Statistical analysis and mathematical procedures

The Shapiro-Wilk normality test was applied. The average velocities corresponding to the LT_v, GT_v, LT_p, GT_p and HR_{dp} (V_{dp}), were obtained individually from each dog. Analysis of variance (ANOVA), used to compare the mean intensity (velocity) corresponding to LT_v, GT_v, LT_p, GT_p and V_{dp}. Tukey test was performed to compare the different methods. The relationships between the LT_v, GT_v, LT_p, GT_p and V_{dp} variables were determined using the

Pearson correlations coefficient, r. The coefficients of variation (CV) of the studied variables were calculated following the procedures described by Widjaja et al [19].

The thresholds were compared by two methods. First, the Bland-Altman plots at 95% confidence level were built to analyze the limits of agreement between LT_v, GT_v, LT_p, GT_p and V_{dp} intensities. However, the least products regression analysis was employed because the constant and proportional biases could not be determined independently using the Bland-Altman approach. Thus, the validity and accuracy of the obtained values were determined from the Pearson correlation coefficient combined with bias degree. The degree of constant bias was determined using the 95% confidence interval (CI) of the y-interceptor. There was no constant bias if the value 0 was included in the CI of the interceptor. The proportional bias was determined from the 95% CI for the slope of the straight line. In this case, there was no proportional bias if the CI included 1.0. Importantly, an ordinary least products regression analysis was performed instead of a least-squares regression analysis because the errors of all intensities were identified by different methods [20,21]. The level of significance was set at P-value < 0.05. Statistical analyses were performed using the SigmaPlot v.12.0 software.

Results

This study evaluated two methods to determine variables that assess the physical fitness of dogs submitted to an incremental exercise test to evaluate aerobic endurance performance capacity. Table 1 shows the mean values of a few variables related to plasma lactate, HR and velocity obtained from the IET. The exponential rise in lactate concentration observed during IET, allows determining the LT_v (Fig. 1). The same figure shows the behavior of blood glucose, allowing determining the GT_v. The LT_v was determined for all dogs (n = 18). However, GT_v was determined in 16 dogs only (89%). Thus, 16 dogs were compared regarding the visual LT_v and GT_v thresholds. The same number of comparisons was carried out for the polynomial thresholds, LT_p and GT_p. Figure 2 shows the mean curves obtained from the polynomial mathematical model.

The HR deflection point used as an alternative method to identify the LT, allowed determining the HR_{DP} and V_{DP} in 17 dogs only (94.4%). Thus, it was possible to compare the intensities of LT_v and LT_p with V_{dp} in 17 dogs. Figure 3 shows one of the results. The intensities of GT_v and GT_p with V_{dp} were possible to compare in 15 dogs only. Table 2 shows no difference between the velocities (P = 0.834).

The Bland-Altman method (Figure 4) was used to assess the agreement between the velocities corresponding to the studied methods. The [bias (± 95% of confidence interval)] between LT_v vs. GT_v (Figure 4A) was [-0.15 (0.53) m/s], LT_v vs. LT_p (Figure 4B) [-0.21 (0.23) m/s], LT_v vs. GT_p (Figure 4C) [-0.23 (0.30) m/s], LT_p vs. V_{dp} (Figure 4D) [- 12:17 (0.52) m/s], GT_p vs. V_{dp} (Figure 4E) [0.20 (0.66) m/s], GT_v vs. V_{dp} (Figure 4F) [-0.44 (0.64) m/s] and the LT_v vs. V_{dp} [0.00 (0.25) m/s] (Figure 5). All correlations were significantly positive and strong ($0.90 \leq r < 1.0$) and the results of the least product regression analysis revealed a fixed bias only between LT_v vs. LT_p. There was no proportional bias between the studied methods (Table 3).

Discussion

Beagle dogs were subjected to a single IET to determine some variables considered hallmarks of aerobics fitness. In addition, the results indicated a correlation between the variables, which should be a basis for future studies in dogs. A new contribution was determining the LT_V in dogs, and its strong correlation with GT_v, LT_p, GT_p and HR_{dp}. There was agreement, without bias, between lactate and glycemic responses, and this finding supports the possibility of using plasma glucose concentrations to identify the aerobic capacity in dogs. Also, the good agreement and the absence of fixed and proportional biases between LT_v and HR_{dp} indicated the possibility of using HR, a non-invasive physiological variable, which can be obtained easily with a frequency meter for prescribing intensities and evaluating training programs of athlete dogs. It is known in the literature that specific IET_s and/or modified protocols may alter test results. Therefore, to minimize these effects we used the same IET to determine all variables. The protocol of this test was adapted from a previous study [8], which showed test-retest reliability for the correlations between lactate concentrations (r = 0.89) and HR (r = 0.96). Another issue that should be mentioned is the possible limitation of the visual determinations related to the possible subjectivity in the kinetic analysis of lactate/glucose in response to exercise, and the mitigation of this event depends on the experience of researchers.

After careful literature review, it is safe to state that most of the results produced herein have not been published before. Although other studies with dogs have tried to demonstrate the responses of lactate concentrations and plasma glucose, as well as HR and IETs, no study was able to get the abrupt increase in lactate concentration (lactate threshold), or its correlation with GT_v, LT_p, GT_p and HR_{dp} [7,8,9,16]. The correlations, mean differences, and the absence of biases between the variables detected by regression analysis show the importance of the results obtained in this study.

Another novelty of this study was the choice of ordinary least products regression analysis to characterize the relationship between the measurements obtained using the LT_v with GT_v, LT_p, GT_p and V_{dp}. This type of regression allows both the y- and x-values to be attended by the random error [20,21]. Clinical studies of dogs use the combination of Bland-Altman plots and Pearson correlation to determine the degree of agreement among the methods [16]. When using the Bland-Altman plots, the mean differences between the values obtained from the analyzers resulted from the interaction of the constant and proportional biases. Therefore, the mean differences between the values from the different methods do not reflect the validity of using the method or the constant bias associated with its use. The occurrence of biases or the proportional reduction of correlation may be determined in some situations. Therefore, the use of ordinary least products regression analysis to compare the two methods prevents these two limitations, allowing a more accurate determination of any constant or proportional bias [20,21].

Our study found no statistical difference between the intensities (velocities) related to the studied variables, and these results are similar to those obtained in either healthy or unhealthy humans [10,22,23]. These findings were similar to those obtained in mice previously [1]. However, the comparison of the methods revealed a constant bias (0.30 m/s ~ 1.08 km/h) between LT_v and LT_p. A constant bias means that two different methods (LT_p vs. LT_v) produce different results, and this difference is constant. From a practical viewpoint, the bias was small and should not affect the ability to estimate the lactate threshold from the LT_P without the prior need to induce hyperlactatemia from a maximum effort (sprint). LT_P was identified in human patients with type 2 diabetes [22,24], as an alternative to the LMS method and, in rats, it was the best variable to predict the maximal lactate steady state (MLSS) intensity [1], the "gold standard" to determine endurance capacity. The MLSS is the highest metabolic rate capable of maintaining a dynamic steady state of production and elimination of lactate. This study was the first to determine the LT_p in beagle dogs using the [lac]/velocity ratio with subsequent application of a second order polynomial. Another noteworthy advantage is the possibility of using this protocol to estimate the aerobic capacity in a single IET. Therefore, this method has the potential to be applied for prescribing and evaluating conditioning programs for high performance or health reasons, especially in obese, cardiac or elderly dogs. Moreover, LT_v and GT_p showed a strong correlation ($r = 0.94$), with no bias, demonstrating that blood glucose levels also has the potential to be used to evaluate aerobic fitness in dogs.

The lactate threshold is used to determine the endurance capacity [1,2,25,26,27], and its determination is always protocol-dependent [5,26,28]. Our findings differed from previous studies in dogs [7,8,29], in which the IET did not induce abrupt and obvious increases in plasma lactate concentrations. In this study, LT_v was

determined by the exponential behavior of the lactate-velocity curve. This discrepancy between results may be related to the IET protocol used and possible physiological differences between dog breeds, to the adrenergic activity induced by stressful stimuli that promote glycogenolysis [26]. Additionally, must also be mentioned the possible low density of monocarboxylate transporters in dogs, proteins responsible for transporting the lactate produced by the muscle to the blood [8].

It is also noteworthy that the dogs underwent an adjustment period and this learning step before the exercise testing may have improved the biomechanical efficiency [1], and positively influenced the IET results. Another fact that may have helped was the pause time between the test steps, which were longer compared to previous studies in dogs [8]. This measure can minimize the possible "delay" in muscle lactate transport in dogs. Another aspect was the relatively high speeds of the incremental steps [mean: 4.79 m/s (~ 17.2 km/h); range 3.5 m/s (~ 12.6 km/h) to 6 m/s (~ 21.6 km/h)]. The speed component is relevant to raise the adrenergic activity and mobilize the glycogenolysis metabolism of type II fibers. Nevertheless, it was observed that the exercise-induced rise of lactate does not occur similarly between dogs, especially breeds used in submaximal activity. This can be seen when we observe the lacpeak values (mean: 2.72; range 1.35 to 5.02 mmol/L). Future studies should focus on adrenergic activity, characterization, and quantification of monocarboxylate transporters in the muscle.

In any case, the used effort protocol allowed determining the GT_v, from glucose during exercise. Initially, glucose concentrations decreased gradually until an intensity that coincided with the LT_v, and increased again as the IET intensity also increased. Similar behavior has been previously described by other authors during IETs in humans. The minimum glucose concentration was highly correlated ($r = 0.90$, $P < 0.05$) with the LT_v [10,19,26], and in the evaluation and prescription of exercises for patients with type 2 diabetes [22,24].

There was also a correlation between LT_v and GT_v in horses subjected to an IET [2]. Other authors have suggested that the decreasing blood glucose response at the start of the physical activity to its lowest point during exercise may be related to increased muscle protein phosphorylation that captures glucose, such as GLUT-4 [30,31]. As the effort intensified, the control mechanism of blood glucose was regulated by hormones such as glucagon and cortisol, and the catecholamines [19]. It has been demonstrated that the latter increased the glycogenolysis rate and lactate production during progressive exercise [10]. Few studies in humans have found that the concentrations of lactate, glucose, and catecholamines are higher during the physical activity performed above the lactate threshold compared to exercise performed below the threshold. However, the GT_v cannot always

be determined in human athletes subjected to an IET [19]. Two dogs had no GT_v in our study. The first dog did not adapt to high speeds while the other always had high glucose concentrations, from the start of the test. We speculate that in the case of the latter, the response may be related to behavioral factors, such as anxiety, which causes release of catecholamines, raising blood glucose.

An alternative to evaluate aerobic capacity are fixed methods (i.e. LT_4, speed at which lactate concentration reached 4 mmol/L), which are used in human and equine sports medicine [32]. Both methodologies (visual and fixed) can be used to predict MLSS. In our study, it was not possible to determine the fixed methods because the dogs did not show sufficiently high lactate concentrations, demonstrating the importance of the visual assessment of each dog. On the other hand, MLSS has not been evaluated in dogs, which is a gap in the literature. In this sense, our study becomes a basis for further studies to determine the MLSS in dogs.

HR increased linearly with increasing IET speed. Indeed, these results corroborate other studies [7,16,33]. Amazingly, these results differ from previous findings [8]. The latter authors found no linear increase of HR in incremental exercise test. However, HR linear increase was observed in human and equine athletes, including a plateau phase when the IET became more intense [6,34]. This behavior allowed identifying the aerobic capacity through indirect and non-invasive methods based on HR and HR_{dp} [15]. Our results allowed determining the lactate threshold indirectly by determining the HR_{dp}.

Thus, the visual determination of HR_{dp} and its corresponding speed (V_{dp}) was possible in 94.4% of dogs, similar to results obtained in humans [6,35]. However, these findings were different from those reported by a seminal study in dogs,[16] where the visual and computerized determination of HR_{dp} was possible in 11 of 14 (78.5%) dogs. Possibly one of the reasons for the discrepancy observed with the results of Radin et al. [16] was the different protocols. According to these authors, the non-occurrence of HR_{dp} may be influenced by inadequate warm up, differences in intensity (speed) increments, and degree of fitness. Another aspect to consider is that perhaps some dogs used in the study of Radin et al. [16] did not reach the aerobic-anaerobic transition during the exercise testing. Therefore, the exercise intensity was not sufficient to raise the HR and determine the HR_{dp} and V_{dp}. This fact shows that our protocol was intense enough to raise both lactatemia and HR. One dog did not present the HR deflection point, even with the LT_V, and this finding has already been reported in humans [14,15].

There are several protocols for identifying HR breakpoint and determine the HR_{dp} [15,36,37], especially visual and mathematical methods. Some authors have suggested using mathematics to determine HR_{dp} [13]. Visual

inspection can be used to determine HR_{dp} in humans and dogs [16,38], the methodology used in this study. This latest study evaluated the agreement and correlation between the mathematical and visual methods for identifying HR_{dp} and reported a mean difference of -1 bpm, agreement limits from -17 bpm to 15 bpm, and a positive correlation of r = 0.92. However, these authors concluded that its correlation with LT is a gap in dog literature, and this research contributed to fill this gap.

A study with Border Collies [16] reported that HR_{dp} was on average 80% HRmax. This discrepancy whit our study occurred, possibly due to the protocol with maximum fixed speed (20 km/h) used in the study. In this case, if few dogs were stimulated with more intense speeds, the HRmax could reach higher values leading to a more significant reduction in relation to HRmax. In our study, the HRmax in one dog reached 291 bpm with an average 246. Studies with dogs detected a medium range between 230-300 bpm [16,38,39], showing that the dogs' effort was near maximum capacity. The average HR_{dp} was 235 bpm the equivalent to 95% HRmax. The HR_{dp} in humans is often between 88-95% HRmax [4,13]. The proximity of the HRdp with HRmax is possibly related to the major parasympathetic component in the autonomic modulation in HR in dogs, when compared to humans and other species [40,41]. These latter studies have speculated that the physiological difference in HR modulation and the higher vagal tone in dogs could explain the HRdp and the lactate threshold in our work were close to HRmax, since the withdrawal of the vagal tone would be slower compared with other species during an EIT. Several studies in exercise testing in humans have shown that during exercise from low to moderate intensity, increase in HR is controlled by withdrawal of vagal tone [42,43]. On the contrary, during intensities above LT, there is an increase in the activity of the sympathetic system [43], being modulated by the action and the increase of circulating catecholamine concentrations in the blood, which occurs simultaneously with the glycogenolysis and increase of blood lactate concentrations, in response to high intensity exercise [44]. The transition between increased sympathetic nervous system activity and withdrawal from the parasympathetic system has been shown to be strongly correlated with LT and the ventilatory threshold in humans [45,46]. Certainly, these mechanisms could be subjects for future studies in dogs.Exercise protocols for identifying the anaerobic threshold may be determined from a single exercise testing [16] using different assessments to determine the physical capacity, which can become a practical tool in the evaluation, prescription and control of aerobic training, especially for dogs, just as proposed in human studies [27]. Moreover, the protocol for determining the LT in dogs proposed in this study suggests further studies to assess the effects of chronic exercise training in athletes dogs, with the intensities prescribed based on LT_V, GT_V, LT_P, GT_P or HR_{dp}. Just as in the development of therapeutic protocols,

nonpharmacological of diseases such as hypertension, heart disease, respiratory, diabetes, obesity, issues to be explored by the academic community. The modern lifestyle also causes metabolic changes in dogs associated with obesity. In this sense, the results obtained should work as a basis for other studies focusing on the relationship between physical activity and health of the species. Also, the effect of exercise on dogs can be used as an experimental model to detect the effects of exercise on health maintenance and treatment of diseases of interest in medicine.

Conclusion

This study provides a basis for further studies to determine LTv, GT, LT_p, GT_p and HR_{dp} in dogs. The protocol of the incremental exercise test used in this study provided information on the plasma responses of lactate, glucose, and HR in dogs. Our study found no statistical difference between the intensities (velocities) related to the studied variables, being the first study that compared and correlated the LTv, GTv, LT_P, GT_P and HR_{dp} in dogs.

Abbreviations

ANOVA: Analysis of variance; CI: confidence interval; CV: Coefficients of variation; GT: Glucose threshold; GT_p: Polynomial glucose threshold; HR: Heart rate; HR_{dp}: Heart rate deflection point; IET: Incremental exercise test; LT_v: Visual lactate threshold; LT_p: Polynomial lactate threshold; [lactate]: Lactate concentrations; LMS: Lactate-minimum-speed; V_{dp}: Velocity deflection point.

Ethics approval and consent to participate

The study followed the Ethical Principles in Animal Experimentation adopted by The Ethics Committee on the Use of Animals (CEUA - 3.624/15).

Consent for publication:

Not applicable.

Availability of data and material:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Funding:

The study was partly funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Authors' contributions:

During the last nearly 5 decades, the plasma lactate-velocity curve and lactate thresholds (LTs) is a rapidly developing field in athletic human and horses. Many studies on the plasma and blood lactate derived thresholds have been published, few of them in dogs. This paper evaluates methods for evaluating changes in blood lactate during incremental exercise in Beagle dogs, being the first to demonstrate the lactate threshold in dogs. A new contribution was determining visually the LT in Beagles dogs.

Acknowledgements:

This manuscript was reviewed by a professional science editor and by a native English-speaking copy editor to improve readability. The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support and the Pharmacology and Exercise Physiology Laboratory (LAFEQ) for the logistics and assistance to the study.

References

1. Cunha RR, Carvalho CVN, Segundo PR, Moreira SR, Kokubun E, Campbell CSG, Simões HG. Determination of the lactate threshold and maximal blood lactate steady state intensity in aged rats. Cell Biochem Funct. 2009;27:351-357.

2. Ferraz GC, Dangelis FHF, Teixeira-Neto AR, Freitas EVV, Lacerda-Neto JC, Queiroz-Neto A. Blood lactate threshold reflects glucose responses in horses submitted to incremental execise test. Arq Bras Med Vet Zootec. 2008;60:256-259.

3. Webster T, Harber, Bell R, Bell G. Hormonal responses associated with the nadir in blood glucose during graded cycling exercise. J Exerc Sci Fit. 2013;11:6-11.

4. Faude O, Kindermann, W, Meyer T. Lactate threshold concepts. Sports Medicine. 2009;39:469-490.

5. Miranda MCPC, Queiroz-Neto A, Silva-Júnior JR, Pereira MC, Soares OAB, Borghi RT, Ferraz GC. Comparison of the lactate minimum speed and the maximal lactate steady state to determine aerobic capacity in purebred Arabian horses. N Z Vet J. 2013;62:15-20.

6. Conconi F, Ferrari M, Ziglio PG, Droghetti P, Codeca L. Determination of the anaerobic threshold by a non-invasive field test in runners. J Appl Physiol. 1982;52:869–87.

7. Alves J, Santos A, Brites P, Ferreira-Dias G. Evaluation of physical fitness in Police dogs using an incremental test. Comp exerc physiol. 2012;8:219-226.

8. Ferasin L, Marcora S. Reliability of an incremental exercise test to evaluate acute blood lactate, heart rate and body temperature responses in Labrador retrievers. J Comp Physiol B. 2009;179:839 – 845.

9. Kittleson MD, Johnson LE, Pion PD. Submaximal exercise testing using lactate threshold and venous oxygen tension as endpoints in normal dogs and dogs with heart failure. Journal of Veterinary Internal Medicine. 1996;10:21-27.

10. Simões HG, Campbell CS, Kushnick MR, Nakamura A, Katsanos CS, Baldissera V, Moffatt RJ. Blood glucose threshold and the metabolic responses to incremental exercise tests with and without prior lactic acidosis induction. Eur J Appl Physiol. 2003;89:603-611.

11. Zagatto AM, Padulo J, Silva ARSD, Müller PDTG, Miyagi WE, Gobatto CA. Physiological responses at the lactate-minimum-intensity with and without prior high-intensity exercise. Journal of sports sciences. 2016;1-8.

12. Delevatti, RS, Kanitz, AC, Alberton, CL, Pantoja, PD, Marson, EC, Pinho, CDF, Lisboa, SC, Bregagnol LP, Kruel, LFM. Heart rate deflection point as an alternative method to identify the anaerobic threshold in patients with type 2 diabetes. Apunts. Medicina de l'Esport. 2015;50:123-128.

13. Grazzi G, Casoni I, Mazzoni G, Uliari S, Conconi F Protocol for the Conconi test and determination of the heart rate deflection point. Physiol Res. 2005; 54:473–475.

14. IgnjatovićA, Hofmann P, RadovanovićD. Non-invasive determination of the anaerobic threshold based on the heart rate deflection point. Facta Univ Ser Phys Educ Sport. 2008;6:1–10.

15. Bodner M, Rhodes E. A review of the concept of the heart rate deflection point. Sports Med. 2002;30:31–46.

16. Radin L, Belić M, Bottegaro NB, Hrastić H, Torti M, Vučetić V, Stanin D, Vrbanac Z. Heart rate deflection point during incremental test in competitive agility border collies. Vet Res Commun. 2015;39:137-142.

17. NRC. Nutrient requirements of dogs and cats. Washington, D.C.: National Academies Press, 2006.

18. Berkman C, Pereira MC, Nardi KB, Pereira GT, Soares OAB, Restan WAZ, Queiroz-Neto A, Ferraz GC . Agreement between i-STAT and YSI 2300 devices to determine lactate concentrations in dogs undergoing exercise Comp Exer Physiol. 2016;1-8.

19. Widjaja A, Morris RJ, Levy JC, Frayn KN, Manley SE, Turner RC. Within - and between-subject variation in commonly measured anthropometric and biochemical variables. Clinical Chemist. 1999;45:561–566.

20. Hart S, Drevets K, Alford M, Salacinski A, Hunt BE. A method-comparison study regarding the validity and reliability of the Lactate Plus analyzer. BMJ open. 2013;3:e001899.

21. Ludbrook J. Statistical techniques for comparing measurers and methods of measurement: a critical review. Clin. Exp. Pharmacol. Physiol. 2002;29**:**527-536.

22. Simões HG, Hiyane WC, Benford RE, Madrid B, Prada FA, Moreira SR, Campbell CSG. Lactate threshold prediction by blood glucose and rating of perceived exertion in people with type 2 diabetes 1, 2. Perceptual and motor skills. 2010;111:365-378.

23. Sotero RC, Pardono E, Landwehr R, Campbell CSG, Simoes HG. Blood Glucose Minimum Predicts Maximal Lactate Steady State on Running. Int J Sports Med. 2009;30:643 – 646.

24. Moreira SR, Arsa G, Oliveira HB, Lima LC, Campbell CS, Simões HG. Methods to identify the lactate and glucose thresholds during resistance exercise for individuals with type 2 diabetes. J Strength Cond Res. 2008;22:1108-1115.

25. Albouaini K, Egred M, Alahmar A Cardiopulmonary exercise testing and its application. Postgrad Med J. 2007;83:675– 682.

26. Simões HG, Campbell CSG, Kokubum E, Denadai BS, Baldissera V. Blood glucose responses in humans mirror lactate responses for individual anaerobic threshold and for lactate minimum in track tests. Eur J Appl Physiol Occup Physiol. 1999;80:34-40.

27. Svedahl K, MacIntosh BR. Anaerobic threshold: the concept and methods of measurement. Can J Appl Physiol. 2003;28:299–323.

28. Lindner AE. Maximal lactate steady state during exercise in blood of horses. Journal of Animal Scienc. 2010;88:2038-2044.

29. Ferasin L, Marcora SA. pilot study to assess the feasibility of a submaximal exercise test to measure individual response to cardiac medication in dogs with acquired heart failure. Vet Res Commun. 2007;31:725-737.

30. Rocha C, Canellas A, Monteiro D, Antoniazzi M, Azevedo PHS. Changes in individual glucose threshold during military training. Int J Sports Med. 2010;31:482-485.

31. Rose AJ, Richter EA. Skeletal muscle glucose uptake during exercise: how is it regulated?. Physiology. 2005;20:260-270.

32. Soares OAB, Ferraz GC, Martins CB, Dias DPM, Lacerda-Neto JC, Queiroz-Neto A Comparison of maximal lactate steady state with V2, V4, individual anaerobic threshold and lactate minimum speed in horses. Arq Bras Med Vet Zootec. 2014;66:39-46.

33. Piccione G, Casella S, Panzera M, Giannetto C, Fazio F. Effect of Moderate Treadmill Exercise on Some Physiological Parameters in Untrained Beagle Dogs. Experimental Animal. 2012;61:511-515.

34. Gramkow HL, Evans, DL. Correlation of race earnings with velocity at maximal heart rate during a field exercise test in thoroughbred racehorses. Equine Vet J 2006;36:118-122.

35. Sentija D, Vucetic V, Markovic G. Validity of the modified Conconi running test. Int J Sports Med. 2007;28:1006–1011.

36. Mikulic P, Vucetic V, Sentija D. Strong relationship between heart rate deflection point and ventilatory threshold. Journal of Strength and Conditioning Research. 2011;25:360-366.

37. Hofmann P, Pokan R. Value of the application of the heart rate performance curve in sports. Int J Sports Physiol Perform. 2010;5:437–447.

38. Hampson B and McGowan C. Physiological responses of the Australian cattle dog to mustering exercise. Equine Comp Exerc Physiol. 2007;4:37-41.

39. Rovira S, Muñoz A, Riber C, Benito M. Heart rate, electrocardiographic parameters and arrythmias during agility exercises in trained dogs. Revue de Medicine Veterinaire. 2010;161:307-313.

40. Manzo A, Ootaki Y, Ootaki C, Kamohara K, Fukamachi K. Comparative study of heart rate variability between healthy human subjects and healthy dogs, rabbits and calves. Lab Anim. 2009;43:1:41-45.

41. Matsunaga T, Harada T, Mitsui T, Inokuma M, Hashimoto M, Miyauchi M, Murano H, Shibutani Y. Spectral analysis of circadian rhythms in heart rate variability of dogs. Am J Vet Res. 2001;62:1:37-42.

42. Sales MM, Campbell CSG, Morais PK, Ernesto C, Soares-Caldeira LF, Russo P, Motta DF, Moreira SR, Nakamura FY, Simões HG. Noninvasive method to estimate anaerobic threshold in individuals with type 2 diabetes. Diabetol Metab Syndr. 2011;3:1:1.

43. Warren JH, Jaffe RS, Wraa CE, Stebbins CL. Effect of autonomic blockade on power spectrum of heart rate variability during exercise. Am J Physiol. 1997;273:2:495-502.

44. Mazzeo RS, Marshall, PETER. Influence of plasma catecholamines on the lactate threshold during graded exercise. J Appl Physiol. 1989;67:4:1319-1322.

45. Cottin F, Medigue C, Lopes P, Leprêtre PM, Heubert R, Billat V. Ventilatory thresholds assessment from heart rate variability during an incremental exhaustive running test. Int J Sports Med. 2007;28:04:287-294.

46. Karapetian GK, Engels HJ, Gretebeck RJ. Use of heart rate variability to estimate LT and VT. Int J Sports Med. 2008;29:08:652-657.

Figure Captions

Fig. 1 Parameters of the incremental exercise test for a single dog. Plasma lactate and glucose responses for determination of LT and GT by visual identification.

Fig. 2 Determination of the lactate threshold and glucose threshold by a second order polynomial function and SD applied to: A. [Glu] (GT_P), and B. [Lac]/intensity (LT_P) during an incremental exercise testing in 16 dogs.

Fig. 3 Changes in blood lactate and heart rate in a single dog submitted to an incremental exercise test on a treadmill. The arrow indicates physiological coincidence between lactate threshold determined by visual inspection (LT_v) and heart rate deflection point (HR_{dp}).

Fig. 4 Limits of agreement between; **a,** visual lactate threshold (LT_v) and polynomial lactate threshold (LT_p); **b,** LT_v and polynomial glucose threshold (GT_p); **c,** LT_v and visual glucose threshold (GT_v); **d,** GT_p and heart ratdeflection point and the corresponding velocity (V_{dp}); **e,** GT_v and V_{dp}, and **f**, LT_p and V_{dp} by the technique of Bland and Altman.

Fig. 5 Bland Altman plot showing the limits of agreement for the visual lactate threshold (LTv) and heart rate deflection point and the corresponding velocity (Vdp) in 17 dogs (mean difference = 0.00 [0.49 to -0.49]).

Table 1. Descriptive variables for Beagles submitted to an incremental exercise test on a treadmill.

	Mean	**±SD**	**Max**	**Min**
Lac_{peak} (mmol/l)	2.72	0.9	5.02	1.35
HRdp (bpm)	235	18,12	277	207
HR_{max} (bpm)	246	14.3	291	229
V_{max} (m/s)	4,79	0,75	6	3.5

Plasma lactate peak (LAC_{peak}), heart rate deflection point (HRdp), maximum heart rate during the test (HR_{max}), maximum running speed achieved (V_{max}).

Table 2. Values are means ± SD of the parameters identified by different methods.

	Measured Thresholds				Polynomial Models Identified Threshold		HR_{dp}
	LT (m/s)	**Lactate (mmol/l)**	**GT (m/s)**	**Glucose (mmol/l)**	**LT_p (m/s)**	**GT_p (m/s)**	**V_{dp} (m/s)**
Mean	3.87	1.32	3.71	5.48	3.66	3.63	3.82
SD	0.82	0.36	0.85	0.38	0.84	0.78	0.82
CV	20.93	27.11	22.91	6.97	22.95	21.48	21.46

SD: Standard deviation; CV: coefficient of variation (%); intensity (velocities); lactate threshold (LT), glucose threshold (GT); lactate threshold (LT_p) and glucose threshold (GT_p) by the polynomial model, plasma lactate, plasma glucose, heart rate deflection point (HR_{dp}) and the corresponding velocity (V_{dp}).

Table 3. Analyses by the ordinary least products regression

Proportional	*n*	*r*	*a*	95% CI	*b*	95% CI	PB	CB
LT_v - GT_v	16	0.91*	0.29	-0.61 - 0.02	1.03	0.71 - 1.35	No	No
LT_v - LT_p	16	0.96*	0.30	0.05 - 0.56	0.97	0.71 - 1.22	No	Yes
LT_v - GT_p	16	0.94*	0.06	-0.21 - 0.35	1.04	0.76 - 1.33	No	No
LT_p - V_{pd}	15	0.90*	-0.15	-0.49 - 0.18	0.99	0.65 - 1.33	No	No
GT_p - V_{pd}	15	0.92*	0.01	-0.25 - 0.27	0.94	0.67 - 1,21	No	No
GT_v - V_{pd}	15	0.92*	0.04	-0.36 - 0.27	0.98	0.66 - 1.30	No	No
LT_v - V_{dp}	17	0.92*	0.08	-0.17 - 0.34	0.98	0.71 - 1.23	No	No

r - product–moment correlation coefficient, *a* e *b* - coefficients in the ordinary least products regression model E(A) =a+b(B); a, LT (*y* axis) intercept; b, slope. PB, proportional bias; CB constant bias *P<0.0001. Visual lactate threshold (LT_v), glucose threshold (GT_v), lactate threshold (LT_p) and glucose threshold (GT_p) by the polynomial model, plasma lactate and running speed at the heart rate deflection point (V_{dp}).

Fig 1

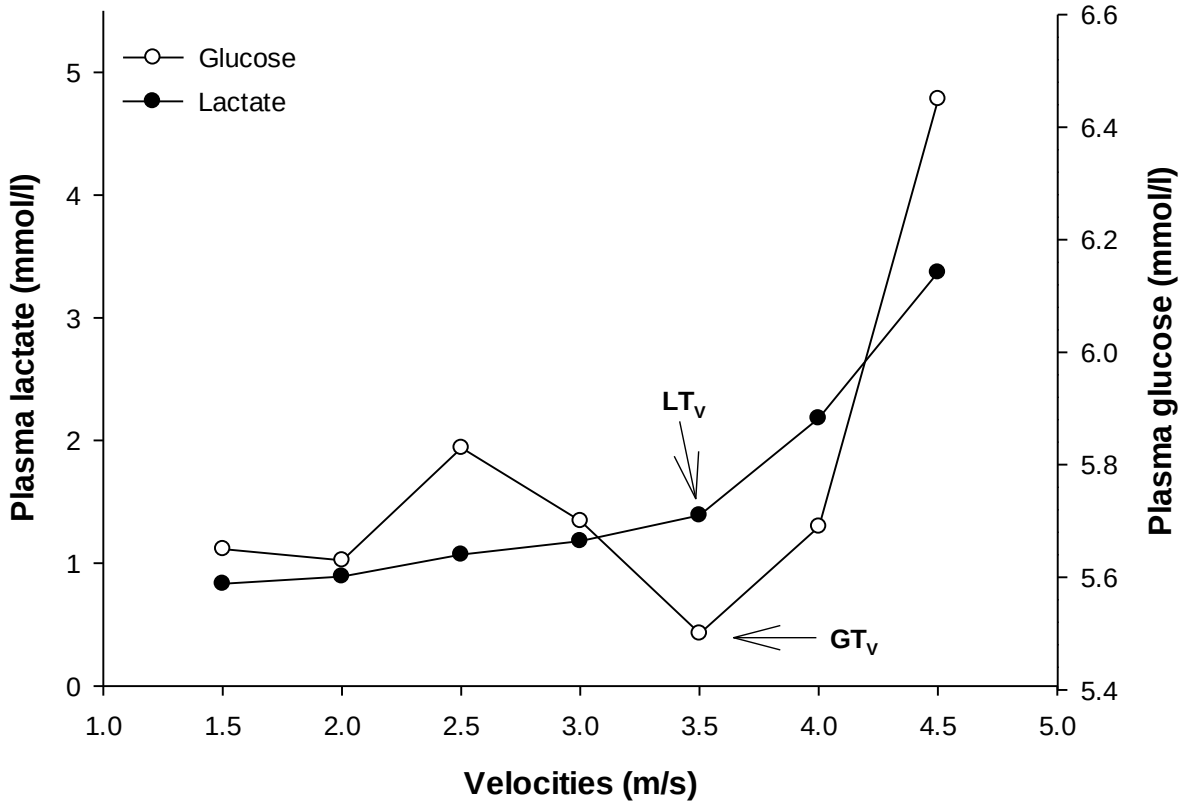
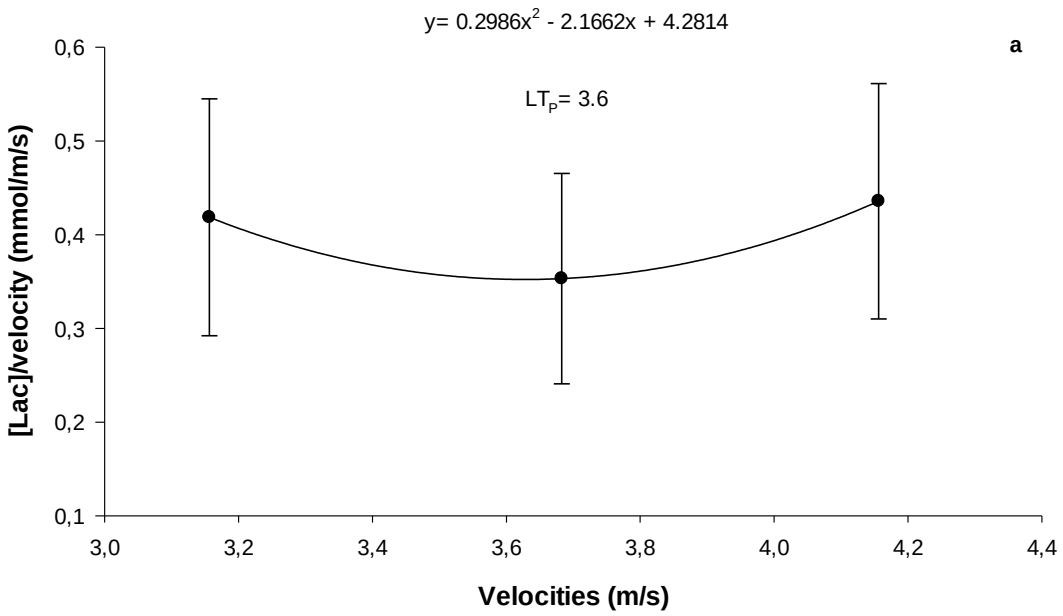

Glucose
Lactate
Plasma lactate (mmol/l)
Plasma glucose (mmol/l)
Velocities (m/s)
LT$_V$
GT$_V$
0
1
2
3
4
5
5.4
5.6
5.8
6.0
6.2
6.4
6.6
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0

Fig 2


y= 0.2986x2 - 2.1662x + 4.2814
a
LTP= 3.6
[Lac]/velocity (mmol/m/s)
0,6
0,5
0,4
0,3
0,2
0,1
3,0
3,2
3,4
3,6
3,8
4,0
4,2
4,4
Velocities (m/s)

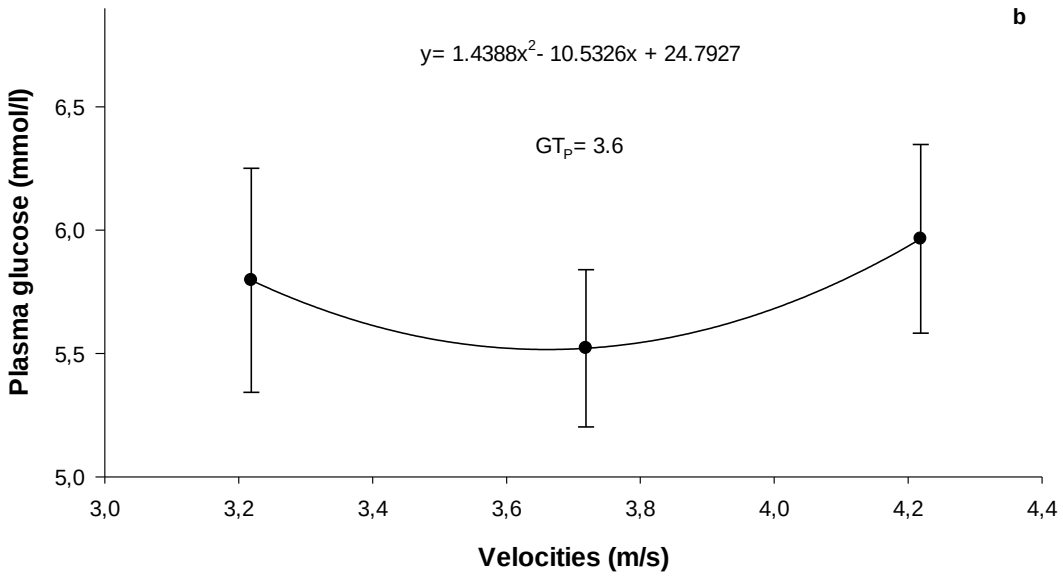

b
y= 1.4388x2- 10.5326x + 24.7927
GTP= 3.6
Plasma glucose (mmol/l)
6,5
6,0
5,5
5,0
3,0
3,2
3,4
3,6
3,8
4,0
4,2
4,4
Velocities (m/s)

Fig 3

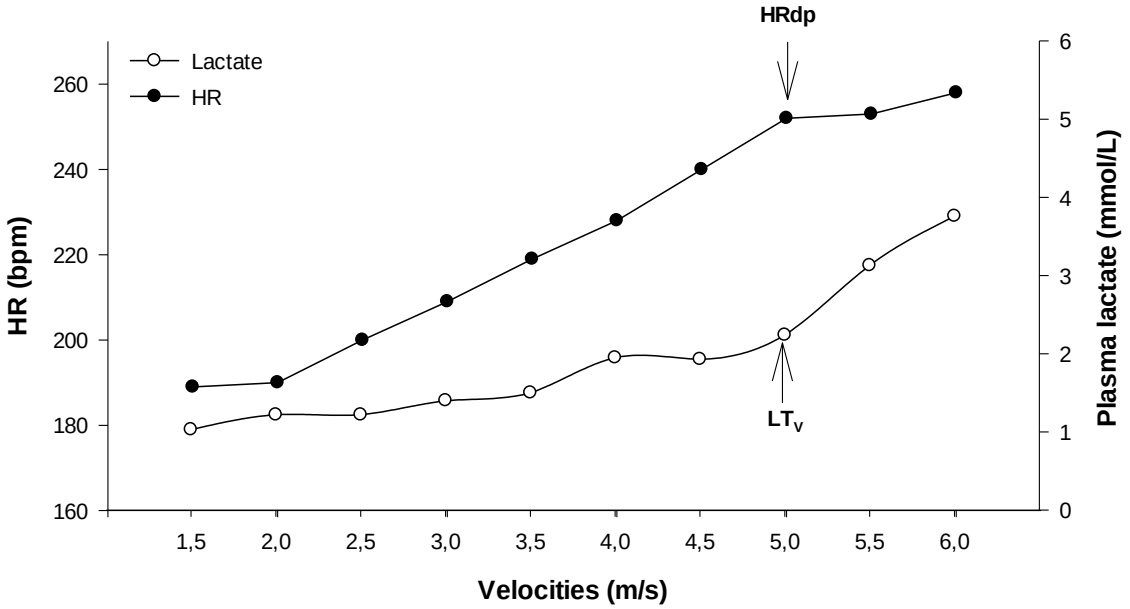

HRdp
Lactate
HR
260
240
220
200
180
160
HR (bpm)
6
5
4
3
2
1
0
Plasma lactate (mmol/L)
LTv
1,5
2,0
2,5
3,0
3,5
4,0
4,5
5,0
5,5
6,0
Velocities (m/s)

Fig 4

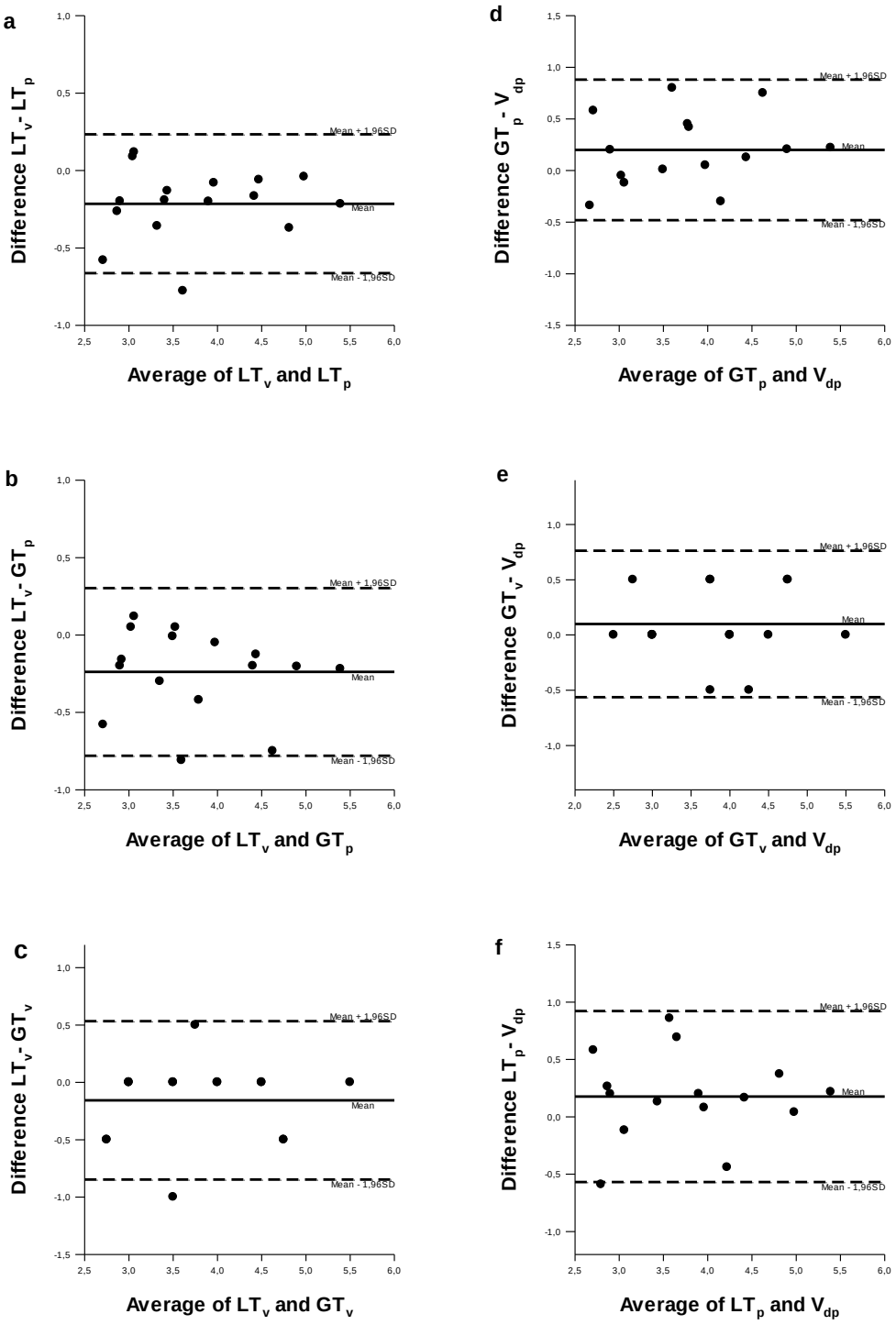
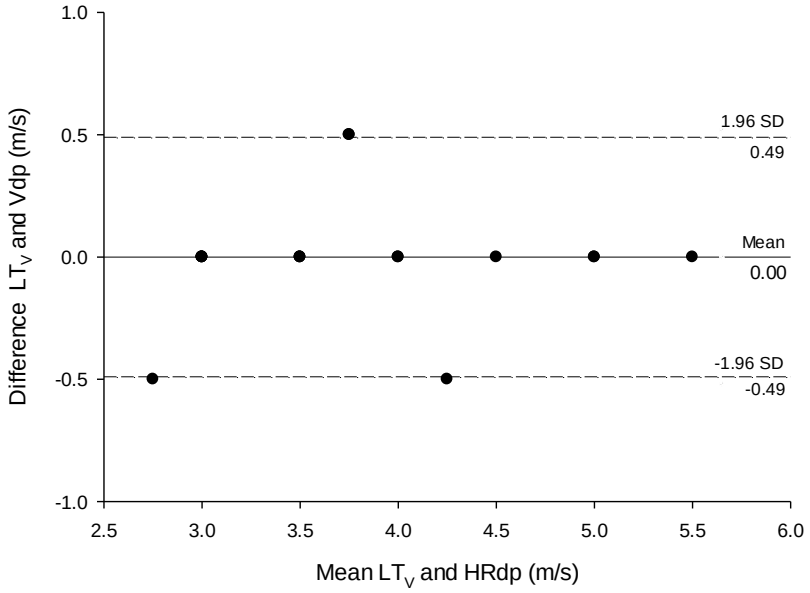

a
Difference LTv- LTp
Mean + 1,96SD
Mean
Mean - 1,96SD
Average of LTv and LTp
b
Difference LTv- GTp
Mean + 1,96SD
Mean
Mean - 1,96SD
Average of LTv and GTp
c
Difference LTv- GTv
Mean + 1,96SD
Mean
Mean - 1,96SD
Average of LTv and GTv
d
Difference GTp - Vdp
Mean + 1,96SD
Mean
Mean - 1,96SD
Average of GTp and Vdp
e
Difference GTv- Vdp
Mean + 1,96SD
Mean
Mean - 1,96SD
Average of GTv and Vdp
f
Difference LTp- Vdp
Mean + 1,96SD
Mean
Mean - 1,96SD
Average of LTp and Vdp

Fig 5



Morrer morer