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Chetoacidosi diabetica nel cane e nel gatto: nuove prospettive terapeutiche e strumenti di monitoraggio

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Sommario

ABSTRACT

La presente tesi di dottorato affronta il tema della chetoacidosi diabetica (DKA), una emergenza endocrina che, quando inappropriatamente gestita, può associarsi ad un elevato rischio di mortalità per l'intervenire di complicazioni in genere conseguenti ad una terapia troppo aggressiva, ad un monitoraggio clinico inadeguato, oppure all'impossibilità di rivalutare sistematicamente alcuni parametri laboratoristici. La tesi si articola in 6 studi incentrati sulle nuove prospettive terapeutiche e sugli strumenti impiegati per il monitoraggio dei pazienti in corso di trattamento.

Il **Capitolo 2** costituisce un'introduzione all'argomento e riassume l'attuale stato dell'arte sulla DKA. Successivamente è stato riportato uno studio il cui scopo era quello di indagare l'efficacia e la sicurezza dell'infusione endovenosa di insulina Lispro, un analogo insulinico a rapida azione, nella specie felina, dimostrando che il suo impiego è associato a minori effetti collaterali e alla stessa efficacia rispetto all'insulina cristallina regolare **(Capitolo 3)**.

A seguire, sono esposti due studi condotti in parallelo finalizzati a indagare l'accuratezza e la precisione di un glucometro (Gluco Calea, WellionVet) e di un glucometro/chetometro (Belua, WellionVet) nella specie canina **(Capitolo 4)** e in quella felina **(Capitolo 5)**. Nessuno dei due dispositivi è risultato essere sufficientemente accurato da consentirne un utilizzo sicuro nel cane; nel gatto, invece, il Belua ha mostrato delle performance superiori che ne supportano l'impiego clinico.

Negli ultimi anni, la ricerca ha rivolto un grande interesse nei confronti dei dispositivi che misurano il glucosio interstiziale in maniera continuativa. Nel **Capitolo 6** è riportato lo studio che indaga le performance del FreeStyle Libre in 14 cani con DKA, determinando anche l'effetto esercitato dal body condition score, dalla lattatemia, dalla gravità della chetosi e dell'acidosi sulla sua accuratezza. Dai risultati è emerso che, sebbene il FreeStyle non rispetti pienamente i criteri ISO 15197:2913, la sua accuratezza clinica, non compromessa dalle variabili metaboliche, ne supporta l'impiego nei cani con DKA, anche se l'effetto esercitato dal BCS sulle performance merita ulteriori indagini.

Infine, la tesi si conclude con uno studio il cui obiettivo era quello di indagare quale parametro tra AcAc urinario e 3-HB ematico fosse più idoneo per definire l'endpoint della terapia insulinica in corso di trattamento della DKA nel cane. Lo studio dimostra che l'impiego del 3-HB ematico, comparato con l'AcAc urinario, non riduce la durata dell'infusione e dell'ospedalizzazione. Tuttavia, trattandosi di un parametro più veloce e semplice da monitorare, se ne consiglia comunque l'impiego come strumento di monitoraggio della terapia della DKA in sostituzione all'AcAc urinario **(Capitolo 7)**.

Capitolo 1

OBIETTIVI E SCOPI DELLA TESI

1. Obiettivi e scopi della tesi

La chetoacidosi diabetica (DKA, *diabetic ketoacidosis*) è una complicanza acuta e potenzialmente fatale del diabete mellito, tipicamente caratterizzata dalla triade iperglicemia, chetosi e acidosi metabolica. Il meccanismo patogenetico che ne sta alla base è la carenza assoluta o relativa di insulina, responsabile della ridotta capacità dei tessuti periferici di utilizzare il glucosio.
La conoscenza dei meccanismi fisiopatologici che si innescano in corso di DKA risulta di fondamentale importanza per la comprensione degli aspetti clinici e clinicopatologici che caratterizzano tale patologia, per la scelta delle indagini diagnostiche da effettuare e delle strategie terapeutiche da attuare **(Capitolo 2)**.

L'obiettivo del trattamento del paziente in DKA consiste nel correggere l'acidosi metabolica ripristinando le perdite di acqua ed elettroliti, ed interrompendo i processi di lipolisi, chetogenesi e gluconeogenesi a livello epatico tramite la somministrazione di insulina e glucosio. La risposta del paziente al trattamento insulinico è estremamente individuale e difficile da prevedere, rendendo necessaria la scelta di una preparazione insulinica caratterizzata da un rapido inizio dell'effetto e una breve durata d'azione. In letteratura sono stati descritti diversi protocolli insulinici, la maggior parte dei quali prevede la somministrazione di insulina cristallina regolare per via intramuscolare o endovenosa. Tuttavia, la disidratazione e lo stato di shock che spesso caratterizzano i pazienti chetoacidotici possono causare un assorbimento incostante di insulina quando somministrata per via intramuscolare. Per questo motivo, l'infusione endovenosa, ad oggi, è considerata la strategia più efficace per il trattamento della DKA, in quanto consente una maggiore prevedibilità dell'andamento glicemico e permette di modificare tempestivamente la terapia in funzione della risposta del paziente.
Negli ultimi anni, gli analoghi insulinici a rapida azione (Lispro, Aspart) hanno preso piede in medicina umana e il loro successo ha gradualmente ridotto l'impiego dell'insulina cristallina regolare, al punto da metterne in discussione la futura produzione. Gli analoghi insulinici sono molecole geneticamente "ingegnerizzate" in cui minime modificazioni della sequenza aminoacidica assicurano un assorbimento ed una eliminazione più rapidi dal sito di iniezione sottocutaneo, consentendo un inizio dell'azione ipoglicemizzante più rapido e, allo stesso tempo, di durata più breve.
Due studi in letteratura veterinaria hanno valutato l'impiego endovenoso di tali molecole nei cani in DKA, ottenendo risultati promettenti. In quest'ottica, si è deciso di effettuare uno studio per indagare l'efficacia e la sicurezza dell'insulina Lispro nella specie felina. Sono stati inclusi 18

gatti con diagnosi di DKA, tutti sottoposti allo stesso protocollo insulinico standard con infusione endovenosa lenta di insulina; in 9 soggetti è stata somministrata l'insulina cristallina regolare, e negli altri 9 è stata utilizzata l'insulina Lispro. L'efficacia è stata valutata confrontando i tempi mediani di risoluzione dell'iperglicemia, della chetosi e dell'acidosi metabolica tra i due gruppi; la sicurezza è stata giudicata sulla base della frequenza con cui i gatti sviluppavano effetti avversi secondari alla terapia insulinica (ipoglicemia, ipokaliemia e ipofosfatemia) **(Capitolo 3)**.

Nonostante l'applicazione di nuove strategie di trattamento, la DKA rimane una malattia difficile da trattare. Ciò è dovuto, in parte, all'effetto deleterio che la DKA esercita su numerosi sistemi e apparati, oltre che alla frequente associazione a patologie concomitanti, spesso gravi, che sono responsabili dell'alta percentuale di mortalità. Tuttavia, un monitoraggio frequente delle variabili cliniche e clinicopatologiche aumenta la probabilità di successo terapeutico. In questo contesto, il monitoraggio della glicemia e dello stato di chetosi sono di cruciale importanza per valutare la risposta clinica del paziente ed effettuare opportuni adeguamenti nella terapia insulinica.

Negli ultimi decenni, sono stati messi in commercio diversi glucometri e chetometri portatili ad uso umano. Dal momento che l'accuratezza di questi dispositivi potrebbe essere soggetta ad una variabilità inter-specifica, il loro impiego in ambito veterinario richiede l'obbligo della validazione. A tal proposito, si è deciso di realizzare due studi in parallelo al fine di stabilire l'accuratezza e la precisione di un glucometro (Gluco Calea, WellionVet) e di un glucometro/chetometro (Belua, WellionVet) nella specie canina **(Capitolo 4)** e in quella felina **(Capitolo 5)**, basandoci sui requisiti stabiliti dalla norma ISO 15197:2013, e valutando l'interferenza esercitata dal packed cell volume (PCV) sull'accuratezza dei due dispositivi.

L'impiego dei glucometri portatili nel monitoraggio dei pazienti chetoacidotici ha, però, diversi limiti, tra cui quello di richiedere frequenti prelievi ematici o il posizionamento di un catetere venoso centrale (potenziale causa di complicazioni, quali infezioni e flebiti), di consentire un monitoraggio esclusivamente intermittente della glicemia a dei costi comunque onerosi per il proprietario e, ancora, quello di costituire una fonte di stress per il paziente.

Negli ultimi due decenni, in medicina umana, la ricerca ha rivolto un grande interesse nei confronti dei dispositivi che misurano il glucosio interstiziale in maniera continuativa. In ambito veterinario, questi strumenti potrebbero essere di grande ausilio in quei pazienti che

necessitano di un monitoraggio più stretto della glicemia, come i soggetti in DKA o quelli in fase di stabilizzazione del diabete. Le prime generazioni di questi strumenti non erano prive di difetti; tuttavia, il FreeStyle Libre (Abbott, UK), che ha recentemente ottenuto la licenza per l'impiego in ambito umano, sembrerebbe superare molti di questi limiti. Le performance del FreeStyle Libre sono state valutate in cani con diabete mellito non complicato, ma non in cani in DKA, i quali presentano importanti alterazioni metaboliche che potrebbero interferire con l'accuratezza del dispositivo. A questo proposito, si è deciso di effettuare uno studio con l'obiettivo di indagare le performance del FreeStyle Libre in 14 cani con DKA e determinare l'effetto esercitato dal body condition score (BCS), dalla lattatemia, dalla gravità della chetosi e dell'acidosi sull'accuratezza dello strumento **(Capitolo 6)**.

Per quanto concerne lo stato di chetosi, fino a qualche anno fa, in medicina veterinaria, il parametro più comunemente usato per la diagnosi e il monitoraggio della terapia della DKA era l'acetoacetato (AcAc) urinario, valutato mediante strisce reattive urinarie. Queste ultime hanno il limite di fornire una stima esclusivamente semiquantitativa dell'AcAc e, inoltre, non sono in grado di rilevare la presenza del 3-β-idrossibutirrato (3-HB). Questo corpo chetonico viene prodotto a partire dall'AcAc in presenza di idrogenioni; quindi più grave è lo stato di acidosi maggiore sarà la quantità di 3-HB circolante a scapito dell'AcAc. Ciò comporta che la chetonuria valutata mediante strisce reattive non rifletta il reale stato acido-base del paziente e, inoltre, potrebbe dare esiti diagnostici falsamente negativi in stadi precoci della malattia. Inoltre, una volta iniziato il trattamento insulinico, si assiste ad una complessiva riduzione dei livelli di corpi chetonici circolanti e ad una contemporanea conversione del 3-HB in AcAc. Per questi motivi, la concentrazione di AcAc urinario è considerata un parametro tardivo per valutare la risoluzione della chetosi. Diversi studi, in medicina umana, hanno dimostrato che la concentrazione del 3-HB ematico risulta essere meglio correlata con la gravità dell'acidosi ed è pertanto impiegata come endpoint per stabilire quando interrompere l'infusione endovenosa di insulina.
In quest'ottica, si è deciso di effettuare uno studio allo scopo di indagare quale parametro tra AcAc urinario e 3-HB ematico fosse più idoneo per definire l'endpoint della terapia insulinica in corso di trattamento della DKA nel cane. Sono stati inclusi 20 cani con diagnosi di DKA, tutti sottoposti allo stesso protocollo insulinico standard con infusione endovenosa lenta di insulina cristallina regolare. In 10 soggetti l'infusione è stata interrotta nel momento in cui l'acidosi fosse risolta e il 3-HB ematico fosse risultato inferiore a 1 mmol/L per due volte consecutive a

distanza di un'ora; diversamente, negli altri 10, l'interruzione aveva luogo quando l'acidosi fosse risolta e la valutazione dell'AcAc urinario avesse dato esito negativo **(Capitolo 7)**.

Nel **Capitolo 8** sono riassunte la discussione e le conclusioni della presente tesi.

Capitolo 2

LA CHETOACIDOSI DIABETICA NEL CANE E NEL GATTO

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RIASSUNTO

La chetoacidosi diabetica (DKA) è una grave complicazione del diabete mellito. Nonostante le conoscenze inerenti la fisiopatologia della DKA siano in continua espansione e nonostante l'applicazione di nuove strategie di trattamento per le complicazioni ad essa connesse, la chetoacidosi diabetica rimane una malattia difficile da trattare. Ciò è dovuto, in parte, all'effetto deleterio che la DKA esercita su numerosi sistemi e apparati, oltre che alla frequente associazione a patologie concomitanti, spesso gravi, che sono responsabili dell'alta percentuale di mortalità. Tuttavia, attuando una strategia terapeutica adattata al singolo individuo e monitoraggi frequenti delle variabili cliniche e clinicopatologiche la probabilità di successo terapeutico è elevata.

ABSTRACT

Diabetic ketoacidosis (DKA) is a serious complication of diabetes mellitus. Despite the expanding knowledge regarding the pathophysiology of DKA and the application of new treatment techniques for the complications, it remains a challenging disorder to treat. It is, in part, due to the deleterious impact that DKA has on multiple organ systems and the frequent occurrence of concurrent often serious disorders that are responsible for the high mortality rate. Nevertheless, with logical therapy adapted to the individual and careful monitoring of clinical and clinicopathological parameters, the rate of therapeutic success is high.

INTRODUZIONE

La chetoacidosi diabetica (DKA) è una complicanza acuta e potenzialmente fatale del diabete mellito (DM), tipicamente caratterizzata dalla triade iperglicemia, chetosi e acidosi metabolica[1]. Il meccanismo patogenetico che ne sta alla base è la carenza assoluta o relativa di insulina, responsabile della ridotta capacità dei tessuti periferici di utilizzare il glucosio[2]. In condizioni di deficit energetico, infatti, si instaurano dei meccanismi ormonali che portano alla sintesi dei corpi chetonici (CC; acetoacetato, betaidrossibutirrato e acetone), molecole prodotte a livello epatico e derivanti dal metabolismo degli acidi grassi. I CC costituiscono una fonte di energia alternativa al glucosio quando questo non è prontamente disponibile. Il controllo sui meccanismi che regolano la chetogenesi è subordinato all'attività di ormoni quali insulina e glucagone, ma

anche all'influenza dei cosiddetti "ormoni controregolatori del glucosio" (catecolamine, cortisolo e ormone della crescita), i quali, oltre ad esercitare un'azione diretta sulla sintesi dei CC, sono anche responsabili del fenomeno dell'insulinoresistenza. La produzione di questi ormoni aumenta in un'ampia varietà di patologie o in corso di situazioni stressanti per l'organismo e, sebbene il loro ruolo sia inizialmente difensivo, in corso di DKA questi contribuiscono a peggiorare l'iperglicemia e la chetonemia già indotte dalla carenza insulinica[2]. Quando la concentrazione plasmatica di glucosio e CC supera la soglia di riassorbimento da parte dei tubuli renali, queste molecole rimangono nelle urine inducendo una grave diuresi osmotica. Essendo i CC degli anioni, la loro persistenza nelle urine induce l'escrezione di ioni positivi (sodio, potassio, calcio e magnesio) che si accumulano all'interno dei tubuli impedendo il riassorbimento di acqua. Le conseguenze sono un'ipovolemia e ipoperfusione tissutale associate ad un'ipertonicità del fluido del compartimento extracellulare[2].

La produzione di CC nel fegato è associata alla formazione di idrogenioni e quando la loro concentrazione è tale da saturare i sistemi tampone dell'organismo si instaura un'acidosi metabolica. Questa condizione comporta sintomi quali vomito, diarrea, anoressia e conseguente ulteriore perdita di liquidi. L'ipovolemia peggiora la perfusione tissutale, specialmente a livello renale dove riduce il tasso di filtrazione glomerulare e quindi anche la capacità di eliminare glucosio e idrogenioni. L'iperosmolarità secondaria all'iperglicemia si autoalimenta aggravando la diuresi osmotica e causa contestualmente una disidratazione cellulare, conseguente al passaggio di acqua dall'interno all'esterno delle cellule, responsabile dell'ottundimento del sensorio dei pazienti chetoacidotici[2].

Queste gravi conseguenze metaboliche della DKA, quali grave acidosi, diuresi osmotica, iperosmolarità, disidratazione e alterazioni elettrolitiche, possono risultare fatali per il paziente.

ASPETTI CLINICI E DIAGNOSTICI DELLA CHETOACIDOSI DIABETICA

Segnalamento, anamnesi e reperti clinici

La DKA insorge più frequentemente in cani e gatti con DM non ancora diagnosticato, meno comunemente anche in soggetti già trattati con terapia insulinica per problematiche connesse al regime di trattamento (dosaggio inadeguato, errori nella procedura di somministrazione o di conservazione dell'insulina) oppure qualora sopraggiungano delle patologie concomitanti. In

quest'ultimo caso, riconoscere e trattare tempestivamente tali patologie è di fondamentale importanza affinché il trattamento della DKA si concluda con successo. Le patologie più spesso associate alla DKA nella specie felina sono pancreatite acuta, lipidosi epatica, infezione delle vie urinarie, malattia renale cronica, altre infezioni batteriche o virali e neoplasie[3,4]; nel cane sono pancreatite acuta, infezioni batteriche e neoplasie[1]. Oltre a queste, vanno considerate altre condizioni in grado di determinare insulinoresistenza tra cui le più comuni sono la Sindrome di Cushing (cane) o la somministrazione di corticosteroidi (cane e gatto), l'ipertiroidismo (gatto) e il diestro (nelle cagne intere). L'iperprogesteronemia che caratterizza la fase diestrale determina una potente condizione di insulinoresistenza tramite la produzione di ormone della crescita (GH, *Growth Hormone*) da parte del tessuto mammario.

Il segnalamento dei pazienti chetoacidotici ricalca quello dei diabetici non chetoacidotici. In genere la DKA insorge in soggetti di media età o anziani, non presenta predisposizione di razza, mentre relativamente al sesso sembrerebbe essere più frequente nelle femmine per la specie canina e nei maschi per la specie felina[2].

I rilievi anamnestici possono spaziare dai comuni poliuria/polidipsia, polifagia e perdita di peso (spesso sottovalutati dai proprietari) ai più evidenti anoressia, vomito e letargia, la cui gravità è direttamente correlata all'entità dell'acidosi metabolica e al tipo di patologia concomitante.

Reperti clinici comunemente riscontrati includono letargia, disidratazione, tachipnea, tachicardia, debolezza, un forte odore di acetone del respiro, il quale spesso è lento e profondo (respiro di Kussmaul) in funzione della gravità dell'acidosi. Altre alterazioni possono variare in funzione della patologia concomitante. Una scrupolosa indagine anamnestica e un attento esame fisico sono necessari per svelare sintomi trascurabili per il proprietario e per riconoscere eventuali patologie concomitanti.

Alterazioni clinico-patologiche

La diagnosi di DM prevede la presenza di determinati segni clinici (poliuria/polidipsia, polifagia, perdita di peso) e la documentazione di uno stato di iperglicemia persistente associato a glicosuria. Il riscontro di chetonemia/chetonuria riflette uno stato di chetosi e la valutazione dello stato acido-base consente la differenziazione tra chetosi diabetica (DK) e DKA.

Le strisce reattive urinarie forniscono una stima semiquantitativa dell'acetoacetato (AcAc) e acetone urinari, ma non sono in grado di rilevare la presenza del betaidrossibutirrato (BHB). Quest'ultimo viene prodotto a partire dall'AcAc in presenza di idrogenioni, quindi più grave è lo

stato di acidosi maggiore sarà la quantità di BHB circolante a scapito dell'AcAc. Ciò comporta che la chetonuria valutata mediante strisce reattive non riflette il reale stato acido-base del paziente e, inoltre, potrebbe dare esiti negativi in stadi precoci di DK o DKA[2]. Inoltre, una volta iniziato il trattamento insulinico, si assiste ad una complessiva riduzione dei livelli di corpi chetonici circolanti e ad una contemporanea conversione del BHB in AcAc[6]. Per questi motivi le concentrazioni di AcAc urinario vengono considerate un parametro tardivo per valutare la risoluzione della chetosi; viceversa la concentrazione del BHB ematico risulta essere meglio correlata con la gravità dell'acidosi[7].

In commercio sono disponibili dei glucometri/chetometri portatili (Precision Xtra, Abbott; Optium Xceed, Abbott; Belua, Wellion Vet) che misurano la concentrazione ematica di BHB. Un valore di 3,8 mmol/L nel cane (sensibilità 70%, specificità 92%) e di 2,55 mmol/L nel gatto (sensibilità 94%, specificità 68%) sono stati definiti come cutoff per diagnosticare uno stato di chetoacidemia[8,9].

La tipologia e la gravità degli squilibri metabolici che si instaurano in corso di DKA possono essere molto variabili da un caso all'altro in funzione del tempo intercorso da quando la patologia si è stabilita, dalla risposta soggettiva dell'organismo e dalla presenza di patologie concomitanti. Per confermare il sospetto diagnostico di DKA e per impostare un protocollo terapeutico mirato al singolo individuo, sono assolutamente indispensabili la misurazione della glicemia, la valutazione della chetonemia/chetonuria, l'esame emogasanalitico comprendente anche le concentrazioni elettrolitiche (sodio e potassio) e l'Anion Gap, l'esame chimico-fisico delle urine, l'azotemia (urea e creatinina), la fosfatemia e l'osmolarità sierica. L'Anion Gap rappresenta la concentrazione degli anioni plasmatici non misurati, quali lattati, acidi uremici (fosfati, solfati) e chetoacidi; pertanto la misurazione di questo parametro in corso di DKA riflette, anche se in maniera poco precisa, la concentrazione circolante di corpi chetonici.

A completamento dell'iter diagnostico e al fine di identificare la presenza di eventuali patologie concomitanti, è sempre consigliato effettuare un esame emocromocitometrico, un profilo biochimico, un esame batteriologico delle urine prelevate per cistocentesi, un esame ecografico dell'addome e la lipasi pancreatica-specifica o la DGGR-lipasi quando vi sia il sospetto di pancreatite. Nella Tabella 1 sono elencate le più comuni alterazioni clinicopatologiche di cani e gatti con DKA.

Tabella 1: Alterazioni clinicopatologiche generalmente riscontrate in cani e gatti con chetoacidosi diabetica.

Alterazioni clinicopatologiche	Cane (Hume *et al*, 2006)	Gatto (Cooper *et al*, 2015)
Iperglicemia	98%	91%
Glicosuria	100%	-
Chetonemia/chetonuria	100%	100%
Acidosi metabolic	100%	100%
Riduzione dei bicarbonate	93%	96%
Riduzione della CO_2	84%	84%
Aumento dell'Anion Gap	77%	99%
Aumento dell'osmolarità plasmatica calcolata	53%	51%
Leucocitosi neutrofilica	66%	54%
Left shift (neutrofilia immatura, in corso di setticemia)	62%	22%
Monocitosi	54%	38%
Anemia	52%	35%
Emoconcentrazione	1%	3%
Aumento dell'aspartato amino transferasi (AST)	65%	96%
Aumento dell'alanina amino transferasi (ALT)	57%	54%
Aumento della fosfatasi alcalina (SAP)	97%	25%
Aumento della gamma glutamil transferasi (GGT)	41%	7%
Aumento della bilirubina	20%	59%
Ipercolesterolemia	47%	61%
Iponatremia	54%	35%
Ipokaliemia	45%	58%
Ipocloremia	59%	87%
Ipocalcemia	86%	71%
Ipofosfatemia	29%	33%
Aumento della creatinina	18%	30%
Infezione del tratto urinario	20%	14%

TERAPIA

L'approccio terapeutico è molto differente a seconda che il paziente si trovi in una condizione di chetoacidosi oppure di semplice chetosi diabetica.

La *chetosi diabetica* può essere individuata in pazienti con diabete mellito neodiagnosticato ma anche in pazienti già diabetici e sottoposti a terapia insulinica. In quest'ultimo caso, lo sviluppo di una condizione di chetosi è da attribuire ad una inefficacia della terapia insulinica (a causa di problemi connessi al regime di trattamento), allo sviluppo di patologie concomitanti in grado di determinare insulinoresistenza, oppure ad entrambe le condizioni. La presentazione clinica di questi pazienti è caratterizzata dai segni clinici di un diabete mellito non controllato ma in assenza di malattia sistemica. I soggetti che mantengono un discreto appetito e non risultano

estremamente depressi o disidratati possono essere gestiti direttamente con le insuline utilizzate per la gestione diabetica nel lungo periodo (NPH, insulina lenta, PZI, glargina, detemir). L'approccio terapeutico al paziente in chetosi maggiormente instabile (depressione, forte disappetenza/anoressia, marcata disidratazione) prevede la somministrazione per via sottocutanea di insulina cristallina regolare a breve durata d'azione al dosaggio iniziale di 0,1-0,2 U/kg ogni 8 ore. Per minimizzare il rischio di fenomeni ipoglicemici, il paziente deve essere alimentato con gli stessi intervalli di tempo somministrando un terzo della razione giornaliera della dieta[2]. I monitoraggi di glicemia e chetonemia/chetonuria sono fondamentali per valutare la risposta clinica del paziente ed effettuare eventuali adeguamenti nel dosaggio insulinico. Generalmente questa gestione richiede dalle 48 alle 96 ore per la correzione dello stato di idratazione e della chetosi; tempistiche maggiori possono essere suggestive della presenza di una patologia concomitante (es. pancreatite cronica). Una volta risolta la chetosi, si attua il passaggio ad una terapia insulinica di mantenimento (NPH, insulina lenta, PZI, glargina, detemir).

Ben diversa è la presentazione clinica, e quindi l'approccio terapeutico, dei pazienti con *chetoacidosi diabetica*. Questi ultimi presentano segni clinici di malattia sistemica (es. letargia, anoressia e/o vomito), all'esame fisico si evidenziano disidratazione, depressione, astenia ed è presente una grave acidosi metabolica. L'obiettivo del trattamento del paziente in DKA consiste nel correggere l'acidosi metabolica ripristinando le perdite di acqua ed elettroliti, ed interrompendo i processi di lipolisi, chetogenesi e gluconeogenesi a livello epatico tramite la somministrazione di insulina e glucosio. Per garantire il successo terapeutico è fondamentale anche l'identificazione e il trattamento di eventuali fattori predisponenti o patologie concomitanti. Ciò potrebbe implicare delle modificazioni nel protocollo terapeutico o la somministrazione di terapie aggiuntive specifiche (Tabella 2); tuttavia, essendo il trattamento insulinico indispensabile per la risoluzione della DKA, esso non dovrebbe mai essere ritardato o interrotto a causa delle patologie concomitanti. Nei casi in cui non sia possibile ottenere rapidamente una diagnosi e, quindi, impostare l'opportuna terapia (ad esempio in caso di Sindrome di Cushing), il clinico dovrà considerare una minore efficacia del protocollo terapeutico e una risoluzione più lenta della DKA.

Tabella 2: Principali patologie concomitanti/scatenanti riscontrate in pazienti con DKA e terapie associate.

Patologia concomitante/scatenante	Terapie associate
Pancreatite	Fluidoterapia (soluzioni cristalloidi e/o colloidi) Terapia antibiotica Terapia analgesica Trattamento della CID (plasma o sangue intero) Terapia con antiemetici e gastroprotettori Dieta povera di grassi
Infezioni batteriche	Terapia antibiotica specifica
Insufficienza renale	Fluido terapia Monitoraggio dell'output urinario mediante cateterismo Terapia con diuretici (furosemide, mannitolo, dopamina)
Lipidosi epatica, epatite o colangioepatite	Fluidoterapia Terapia antibiotica Epatoprotettori Lattulosio (in caso di encefalopatia epatica) Dieta specifica Applicazione di un sondino esofageo in caso di anoressia in assenza di vomito Nutrizione parenterale in caso di anoressia in presenza di vomito
Iperadrenocorticismo	La diagnosi deve essere posticipata a quando il paziente è stabile, non viene applicata alcuna terapia specifica

A causa delle importanti variazioni biochimiche e osmolari che subisce un organismo in chetoacidosi, una terapia troppo aggressiva può essere controproducente. Conoscere le alterazioni metaboliche (soprattutto pH, elettroliti e glicemia) presenti alla diagnosi e le loro modificazioni durante il corso della terapia permette al clinico di scegliere il tipo di fluido più idoneo a sopperire alle carenze idriche ed elettrolitiche del soggetto, se e quanto supplementare gli elettroliti (potassio e fosforo) tenendo in considerazione che, una volta iniziata la terapia insulinica, le loro concentrazioni subiranno un ulteriore calo conseguentemente allo shift intracellulare, e infine quanto aggressivi essere con la terapia fluida ed insulinica senza correre il rischio di una brusca riduzione dell'osmolarità plasmatica e quindi di edema cerebrale. Una lenta ma progressiva normalizzazione dei parametri alterati in un periodo di 24-48 ore garantisce una maggiore percentuale di successo.

FLUIDOTERAPIA

La terapia fluida, sebbene non sia sufficiente a sopprimere i processi di chetogenesi[10,11], rappresenta comunque un tassello fondamentale della terapia del paziente chetoacidotico. Gli

obiettivi sono: (1) ripristinare e mantenere un normale bilancio idrico necessario per garantire un adeguato output cardiaco, una pressione sanguigna idonea e per assicurare un'opportuna perfusione tissutale, specialmente a livello renale; (2) correggere il deficit di sodio e potassio; (3) prevenire e trattare le alterazioni elettrolitiche indotte dalla terapia insulinica; (4) ridurre la glicemia mediante una maggiore escrezione renale di glucosio e di ormoni diabetogeni[2].

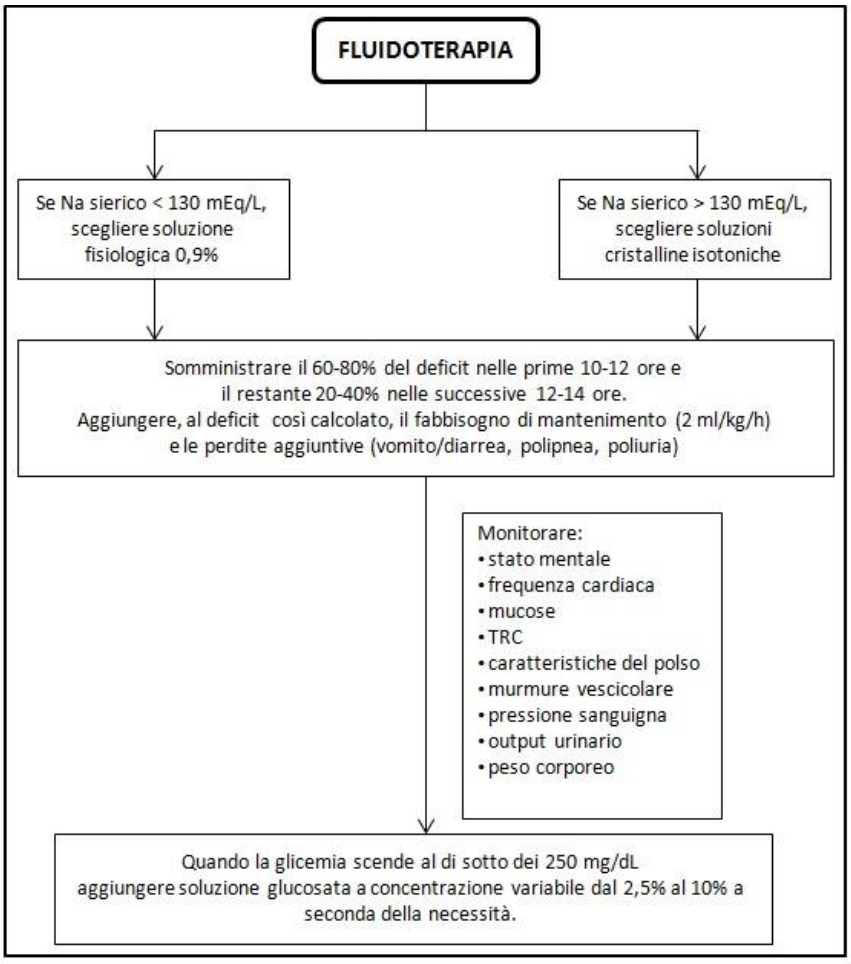
L'inizio della fluidoterapia deve precedere di 2 o più ore l'inizio della terapia insulinica al fine di minimizzare le complicazioni connesse a quest'ultima. Infatti, una riduzione graduale della glicemia insieme all'apporto di sodio evitano il rischio di una riduzione troppo repentina dell'osmolarità del compartimento extracellulare, minimizzando il passaggio di acqua a livello intracellulare e quindi l'insorgenza di edema cerbrale[12].

Tipologia e quantità di fluidi

La scelta della tipologia di fluido dipende dallo stato elettrolitico e osmolare del paziente oltre che dalla concentrazione di glucosio nel sangue (Figura 1). In caso di grave iponatremia (<130 mEq/L) il fluido di prima scelta è la soluzione fisiologica 0,9%[13] cui deve essere apportata un'adeguata integrazione di potassio[2] (Tabella 3). Tuttavia, tale soluzione non possiede proprietà tampone e, anzi, potrebbe causare un'acidosi metabolica ipercloremica[13].

Pertanto, per evitare tale complicazione e nei pazienti con iponatremia lieve (>130 mEq/L) si può ricorrere a soluzioni cristalloidi isotoniche a

Figura 1: Schema esemplificativo per la fluidoterapia endovenosa da seguire in corso di trattamento della DKA nel cane e nel gatto.



debole azione tampone quali Ringer (lattato o acetato), Plasma-Lyte 148 e Normosol-R[12,13]. Una controindicazione all'utilizzo di soluzioni contenenti lattato dipende dal fatto che questa molecola viene metabolizzata a livello epatico con un meccanismo simile a quello dei chetoni. Pertanto, in condizioni di iperchetonemia, il metabolismo del lattato sarà rallentato mentre la sua concentrazione ematica crescerà determinando una maggiore escrezione renale di sodio e potassio[14]. Tuttavia questa controindicazione sembra esclusivamente teorica e nella esperienza degli autori non sono state rilevate complicazioni conseguenti all'utilizzo di tali soluzioni.

Tabella 3: Linee guida per la supplementazione di potassio secondo le linee guida classiche e secondo le linee guida della chetoacidosi diabetica.

Potassiemia sierica (mEq/L)	Supplementazione di potassio secondo le linee guida classiche (mEq/L)	Supplementazione di potassio secondo le linee guida della chetoacidosi diabetica* (mEq/L)
>5.0	Attendere	Attendere
4.0-5.0	10	Da 20 a 30
3.5-4.0	20	Da 30 a 40
3.0-3.5	30	Da 40 a 50
2.5-3.0	40	Da 50 a 60
2.0-2.5	60	Da 60 a 80
<2.0	80	80

**La supplementazione di potassio non deve superare gli 0,5 mEq/kg/h.*

L'utilizzo di fluidi ipotonici (es. soluzione salina 0,45%), non è quasi mai indicato, nemmeno quando presente uno stato di grave iperosmolarità, in quanto, oltre a non apportare un'adeguata quantità di sodio e non essere capaci di ristabilire un corretto bilancio idrico, riducono troppo rapidamente l'osmolarità ematica con maggiore probabilità di sviluppare edema cerebrale[12].

Il calcolo del volume di fluidi da infondere e la velocità di somministrazione devono tenere conto dello stato di disidratazione/ipovolemia, la concentrazione proteica plasmatica e la presenza o meno di malattie cardiache. Poco comuni sono i casi di pazienti in shock al momento della presentazione; questi richiedono una fluidoterapia più aggressiva fino a quando l'equilibrio emodinamico non viene ristabilito.

Ad eccezione di questi rari casi, il criterio generale prevede il ripristino graduale nelle 24 ore del deficit idrico, calcolato secondo la formula:

deficit (ml) = % di disidratazione x peso corporeo (Kg) x 10

Circa un 60-80% di tale deficit deve essere integrato nel giro delle prime 10-12 ore; successivamente una fluidoterapia pari a 1,5-2 volte il fabbisogno di mantenimento dovrebbe

garantire un apporto sufficiente al paziente, da modificare in funzione dello stato di idratazione, output urinario, iperazotemia, persistenza di vomito/diarrea. La fluidoterapia richiede un monitoraggio molto stretto per evitare le complicazioni connesse ad una sovraidratazione (es. edema polmonare, perdite liquide nel "terzo-spazio") tramite parametri soggettivi e oggettivi ogni 4 ore almeno. L'esame emogasanalitico deve essere ripetuto a intervalli di 4-8 ore almeno nelle prime 24 ore in quanto le variazioni delle concentrazioni elettrolitiche e dei gas ematici sono comuni e non prevedibili, richiedendo frequenti adeguamenti della terapia fluida.

La glicemia deve essere valutata ogni ora nelle prime 24 ore di terapia. Quando essa scende al di sotto dei 250 mg/dl oppure quando il suo decremento risulti maggiore a 75 mg/dl/h (evenienze che si verificano, in genere, una volta iniziata la terapia insulinica) e la chetosi sia ancora marcata, è necessario supplementare la fluidoterapia con soluzione glucosata alla percentuale di 2,5-5%, a volte arrivando fino al 10%, in funzione delle esigenze del singolo paziente[12,13,15]. L'importanza di questa strategia terapeutica, spesso sottovalutata, oltre che per evitare fenomeni ipoglicemici e shock osmotici, risulta di fondamentale importanza per garantire all'organismo un substrato glucidico che, insieme alla terapia insulinica, sia sufficiente ad interrompere i processi di chetogenesi.

Nella maggior parte dei casi di DKA la terapia fluida e insulinica sono sufficienti per risolvere l'acidosi metabolica[13], pertanto la somministrazione di soluzioni a base di bicarbonato risulta superflua o addirittura controproducente se si considerano i rischi connessi al loro utilizzo. Questi ultimi sono: l'esacerbazione dell'ipokaliemia secondaria all'ingresso del potassio dentro le cellule, l'ipossia tissutale conseguente alla ridotta dissociazione dell'ossigeno dall'emoglobina quando l'acidosi viene risolta troppo rapidamente, e un peggioramento delle funzioni nervose derivante da una repentina riduzione del pH del liquido cerebrospinale (acidosi paradossa). Per questi motivi il trattamento con bicarbonato è sconsigliato e va considerato solo in quelle condizioni di grave acidosi metabolica (bicarbonati <12 mEq/L). Il deficit di bicarbonato viene calcolato secondo la formula:

deficit di bicarbonato = peso corporeo (kg) x 0,4 x (12 – bicarbonati del paziente)

Per evitare gli effetti avversi della terapia con bicarbonato, solo la metà di questo deficit deve essere somministrata in un periodo di 6 ore. Allo scadere di questo tempo, lo stato acido-base dovrà essere rivalutato e il deficit ricalcolato finché non si raggiunga una concentrazione di bicarbonati maggiore a 12 mEq/L.

TERAPIA INSULINICA

Il ruolo dell'insulina è di importanza cruciale per la risoluzione della DKA. La sue funzioni sono quelle di inibire la lipolisi e, indirettamente, la chetogenesi; agire sul metabolismo epatico favorendo il processo di lipodeposizione e sopprimendo la gluconeogenesi; promuovere l'utilizzo del glucosio e dei CC da parte dei tessuti[2,16,17]. Tutto ciò determina un calo della glicemia e della chetonemia che si riflette a livello renale con una riduzione della diuresi osmotica e delle perdite elettrolitiche, e a livello organico con la correzione dell'acidosi metabolica.

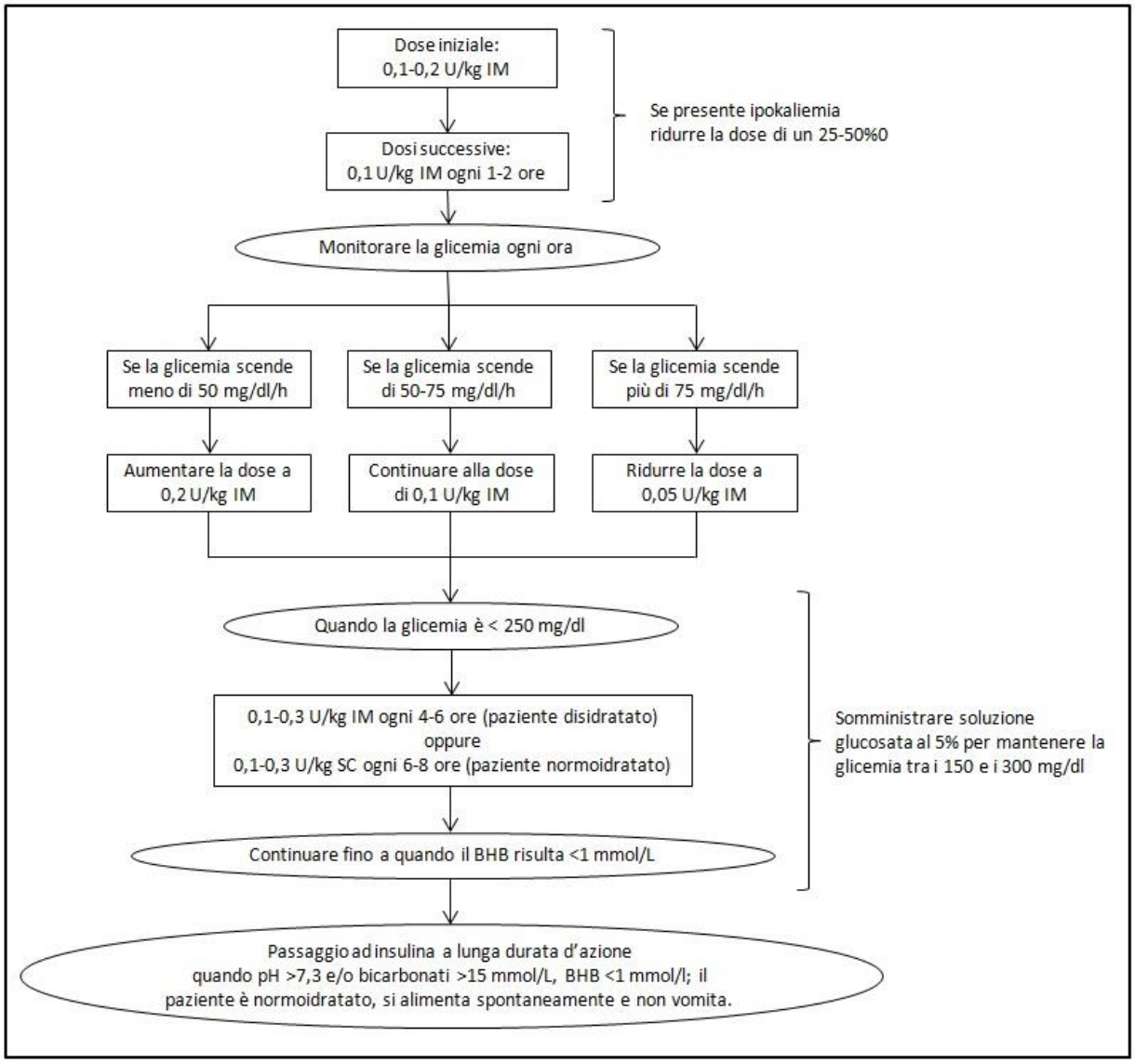
Possibili rischi connessi alla terapia insulinica sono gravi ipokaliemia, ipofosfatemia e ipoglicemia. Tali conseguenze possono essere evitate mediante la scelta di una opportuna fluidoterapia, monitoraggi frequenti delle concentrazioni sieriche di elettroliti e glucosio, e ritardando l'inizio del trattamento insulinico in funzione di questi parametri. Nelson[2] consiglia di somministrare insulina solo dopo aver instaurato la fluidoterapia da un minimo di 2 ad un massimo di 4 ore; diversamente O'Brien[13] suggerisce di aspettare 4-8 ore. DiFazio et al.[18] hanno dimostrato che iniziare precocemente, entro le 6 ore, la terapia insulinica si associa ad una più rapida risoluzione della DK/DKA senza maggiori rischi di complicazioni. Gli autori della presente review solitamente iniziano la terapia insulinica dopo 3-4 ore di fluidoterapia.

La risposta del paziente al trattamento insulinico è estremamente individuale e difficile da prevedere, rendendo necessaria la scelta di una preparazione insulinica caratterizzata da un rapido inizio dell'effetto e una breve durata d'azione, quale l'insulina cristallina regolare[19]. Negli ultimi anni, gli analoghi insulinici a rapida azione (Lispro, Aspart) hanno preso piede in medicina umana, e due studi hanno dimostrato risultati promettenti anche nella specie canina[20,21].

Protocollo intramuscolare

La somministrazione per via intramuscolare piuttosto che sottocutanea trova giustificazione nel fatto che, in pazienti fortemente disidratati, l'assorbimento dell'insulina dal sottocute è fortemente compromesso. Questo protocollo insulinico prevede la somministrazione di una dose iniziale di insulina cristallina regolare a 0,1-0,2 U/kg, seguita da successive somministrazioni a 0,1 U/kg ogni 1-2 ore, monitorando la glicemia ogni ora (Figura 2). Nel caso in cui sia presente ipokaliemia, il dosaggio insulinico deve essere ridotto di un 25-50% nelle prime ore di terapia.

Figura 2: Schema esemplificativo del protocollo per la somministrazione intramuscolare intermittente di insulina cristallina regolare in cani e gatti affetti da DKA.



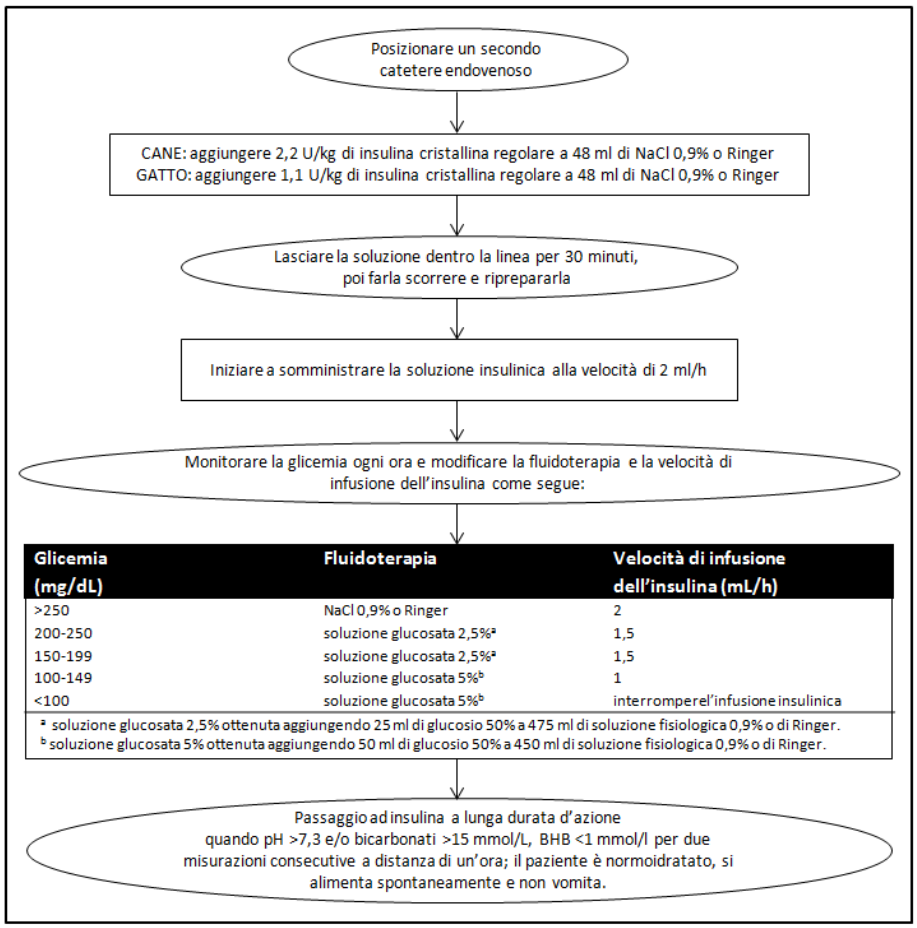
L'obiettivo della terapia insulinica è quello di ridurre gradualmente la glicemia fino a raggiungere valori di 200-250 mg/dl nel giro di circa 6-10 ore. L'entità di riduzione della glicemia dovrebbe essere idealmente di 50-75 mg/dl/h[22]; diversamente risulta necessario modificare il dosaggio insulinico. Quando la glicemia raggiunge valori inferiori a 250 mg/dl, l'insulina deve essere somministrata alla dose di 0,1-0,3 U/kg per via intramuscolare ogni 4-6 ore se lo stato di idratazione del paziente non è ancora stato ripristinato, oppure allo stesso dosaggio ma per via sottocutanea ogni 6-8 ore se il paziente si presenta normoidratato. In questa fase, è opportuno alimentare il paziente e/o supportarlo con soluzione glucosata al 5% al fine di mantenere la glicemia in valori compresi tra 150 e 300 mg/dl.

Marshall et al.[23] hanno dimostrato l'efficacia, nella specie felina, di un protocollo che prevede l'utilizzo di insulina lenta glargine somministrata per via intramuscolare alla dose di 1-2 U/gatto, poi ripetuta ad intervalli di 2-22 ore, associata o meno alla somministrazione per via sottocutanea della stessa insulina al dosaggio di 1-3 U/gatto ogni 12 ore. Gallagher et al.[24], infine, hanno confrontato un protocollo che prevede la somministrazione di insulina glargine SC associata a insulina regolare IM, con la gestione classica in infusione continua endovenosa lenta di insulina regolare, concludendo che tale protocollo offre un'alternativa efficace per il trattamento della DKA nel gatto.

Protocollo in infusione continua endovenosa lenta

Questo protocollo richiede il posizionamento di un secondo catetere endovenoso, utilizzato esclusivamente per l'infusione insulinica, e la disponibilità di una pompa da infusione[25,26]. Per ottenere la soluzione insulinica, 2,2 U/kg per il cane e 1,1 U/kg per il gatto di insulina regolare vengono aggiunte a 48 ml di soluzione fisiologica 0,9%[2,25] o di Ringer[13,15] (Figura 3).

Figura 3: Schema esemplificativo del protocollo per la somministrazione endovenosa lenta e continua di insulina cristallina regolare in cani e gatti affetti da DKA.



Dal momento che l'insulina aderisce al vetro e alla plastica, per saturare la linea, la soluzione insulinica così ottenuta deve essere lasciata al suo interno per 30 minuti e poi fatta scorrere[27]. A questo punto la soluzione va ripreparata e può essere somministrata al paziente ad una velocità iniziale di 2 ml/h; la velocità deve essere inferiore nel caso in cui sia presente ipokaliemia. L'obiettivo, anche in questo caso, è quello di apportare una quota di insulina sufficiente a garantire un decremento lento della glicemia che andrà monitorata ogni ora. Quando questa raggiunge valori inferiori ai 250 mg/dl, la velocità di infusione dell'insulina dovrà essere modificata e aggiunta una integrazione variabile di glucosio alla fluidoterapia in funzione della risposta del paziente (Figura 3). Nell'esperienza degli autori, il momento migliore per interrompere l'infusione continua di insulina è quando la chetoacidosi è risolta (pH >7,3 e/o bicarbonati >15 mmol/L, BHB <1mmol/L per due misurazioni consecutive a distanza di un'ora l'una dall'altra), il paziente è normoidratato, si alimenta spontaneamente e non vomita.

In uno studio condotto su 29 gatti con DKA, Claus et al.[28] hanno confrontato l'efficacia di 3 diversi dosaggi insulinici (1,1 U/kg/giorno, 2,2 U/kg/giorno e dosi crescenti da 1,1 a 2,2 U/kg/giorno) e non hanno ottenuto differenze statisticamente significative relativamente al tempo necessario per ottenere glicemie inferiori a 250 mg/dl, tempo di risoluzione della chetonuria, tempo di ospedalizzazione e complicazioni quali ipopotassiemia e ipofosfatemia. Due studi sulla DKA canina, hanno valutato l'efficacia di due analoghi insulinici a breve durata d'azione, la lispro e l'aspart[20,21]. I risultati ottenuti hanno dimostrato che entrambe queste insuline costituiscono un'alternativa efficace e sicura per il trattamento della DKA, qualora la produzione dell'insulina regolare venisse interrotta.

Passaggio all'insulina a lunga durata d'azione

Il passaggio alla terapia definitiva di mantenimento con insulina a lunga durata d'azione (NPH, insulina lenta, PZI, glargina, detemir) deve avvenire secondo i criteri stabiliti dal relativo protocollo insulinico adottato. La regola generale prevede che la dose iniziale debba essere analoga alla dose di insulina regolare somministrata prima del passaggio, con successivi adeguamenti effettuati sulla base della risposta clinica del paziente.

MONITORAGGIO DEL PAZIENTE

Per la complessità che caratterizza questa patologia, quando non oculatamente gestita, la DKA può associarsi ad un elevato rischio di mortalità per l'intervenire di complicazioni in genere conseguenti ad una terapia troppo aggressiva, ad un monitoraggio clinico inadeguato, oppure all'impossibilità di rivalutare sistematicamente alcuni parametri laboratoristici. Il rischio è maggiore durante le prime 24-48 ore di ricovero poiché in queste fasi i livelli glicemici, le concentrazioni elettrolitiche e l'osmolarità sierica possono subire delle fluttuazioni imponenti. L'obiettivo del clinico deve essere quello di normalizzare i parametri alterati in maniera lenta ma continua. Le complicazioni più frequenti sono l'ipoglicemia, l'ipokaliemia, l'edema cerebrale, l'ipofosfatemia (e anemia emolitica), l'ipernatremia e l'ipercloremia (Tabella 4).

Tabella 4: Complicazioni comuni che possono insorgere a seguito della terapia della chetoacidosi diabetica canina e felina.

Complicazione	Meccanismi responsabili
Ipoglicemia	Eccessivo dosaggio insulinico Inadeguata somministrazione di glucosio Monitoraggi glicemici non sufficientemente ravvicinati
Ipokaliemia	Inadeguata supplementazione di potassio
Ipofosfatemia (e anemia emolitica)	Inadeguata supplementazione di fosforo
Ipernatremia	Somministrazione di volumi eccessivi di soluzione fisiologica 0,9% Insufficiente apporto di fluidi
Oliguria persistente	Inadeguato o insufficiente apporto di fluidi
Ipotensione persistente	Inadeguato o insufficiente apporto di fluidi
Edema cerebrale e sintomi neurologici	Decremento repentino della glicemia e/o dell'osmolarità sierica
Acidosi cerebrale paradossa e sintomi neurologici	Somministrazione di bicarbonati troppo rapida

I pazienti con DKA possono subire rapide escursioni delle concentrazioni di glucosio plasmatico a causa della compromissione dei normali meccanismi omeostatici oltre che per gli interventi terapeutici, quali la somministrazione di insulina e di soluzioni contenenti glucosio. Il monitoraggio glicemico viene effettuato, mediante glucometri portatili, da una goccia di sangue ottenuta generalmente dal padiglione auricolare. Questa tecnica presenta sicuramente dei vantaggi in termini di semplicità di esecuzione e rischi di complicazioni (infezioni e flebiti)

rispetto al classico prelievo di sangue, tuttavia comporta comunque uno stress non indifferente per il paziente. Per questa ragione, negli ultimi anni, è aumentato l'interesse nei confronti di dispositivi per il monitoraggio continuo del glucosio (CGMS, Continuous interstitial Glucose Monitoring System) (Foto 1), già testati su soggetti diabetici non chetoacidotici[29,30].

Foto 1: A. Posizionamento del sensore FreeStyle Libre sulla regione dorsale del collo e fissaggio tramite scotch di rinforzo; B. Scansione tramite il lettore; C. Visualizzazione del risultato sullo schermo; D. Bendaggio protettivo del collo.

Uno studio del 2010 ha dimostrato che questi sistemi di monitoraggio costituiscono uno strumento utile e affidabile anche per cani e gatti in DKA, e che il margine di errore di lettura che può derivare dallo stato di idratazione e di perfusione del paziente o dalla gravità della chetosi impatta in maniera solo trascurabile sull'accuratezza del dispositivo[31].

Elettroliti quali potassio, fosforo e magnesio possono andare incontro a deplezione durante la terapia fluida e insulinica a seguito di diversi meccanismi (es. effetto diluizione, passaggio dal compartimento extracellulare a quello intracellulare, perdite renali e gastroenteriche e correzione dell'acidosi) determinando conseguenze che possono compromettere la vita del paziente. Per questo motivo il loro monitoraggio ogni 4-12 ore ed eventuale supplementazione,

soprattutto nelle prime 24-48 ore di terapia, risulta indispensabile per il successo del trattamento (Tabella 5).

Tabella 5: Carenze elettrolitiche: segni clinici comunemente riscontrati in corso delle principali carenze elettrolitiche, modalità, controindicazioni e potenziali effetti avversi della supplementazione.

Elettrolita	Segni clinici conseguenti alle deplezione elettrolitica	Supplementazione	Controindicazioni della supplementazione	Effetti avversi potenziali conseguenti alla supplementazione
Potassio	Astenia Ventroflessione del collo	Vedi tabella 2	Oliguria	Iperkaliemia
Fosforo	Anemia emolitica e problemi neuromuscolari per valori <1,5 mg/dl	0,01-0,12 mmol/kg/h (fosfato di sodio o potassio), incompatibile con soluzioni contenenti calcio	Ipercalcemia Iperfosfatemia Oliguria Necrosi tissutale	Ipocalcemia iatrogena Ipernatremia Ipotensione Calcificazioni metastatiche
Magnesio	Letargia Anoressia Debolezza Ipokaliemia o ipocalcemia refrattarie per valori di Magnesio sierico totale <1,0 mg/dl o Magnesio ionico <0,4 mg/dl	Rapida: 0,5-1 mEq/kg/giorno Lenta: 0,3-0,5 mEq/kg/giorno Incompatibile con Bicarbonato di sodio o soluzioni contenenti calcio	Terapia con glicosidi digitalici	Ipocalcemia Ipotensione Blocchi cardiaci atrioventricolari o di branca Depressione respiratoria Arresto cardiaco (L'overdose va trattata con gluconato di calcio)

PROGNOSI

La DKA rappresenta ancora oggi una tra le patologie metaboliche di più difficile gestione medica. I punti chiave per il successo terapeutico sono rappresentati dalla fluidoterapia e l'integrazione di glucosio, dalla terapia insulinica e dalla supplementazione di potassio. Affinché il clinico possa attuare delle scelte non controproducenti per il paziente, sono necessari uno stretto monitoraggio clinico e clinicopatologico, oltre che una tempestiva identificazione e trattamento delle patologie concomitanti. Questi accorgimenti hanno reso possibile una riduzione della percentuale di mortalità dal 26-30% di qualche anno fa[1,3,26] fino al 5%[2,28] dei giorni nostri. Nel gatto, infine, va ricordato che è possibile una remissione del DM dopo risoluzione della DKA, soprattutto in soggetti che, al momento della diagnosi, presentano una patologia pancreatica o hanno subito trattamenti con corticosteroidi[23,32].

2. La chetoacidosi diabetica nel cane e nel gatto

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Capitolo 3

USE OF LISPRO INSULIN FOR THE TREATMENT OF DIABETIC KETOACIDOSIS IN CATS

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3. Use of lispro insulin for the treatment of diabetic ketoacidosis

ABSTRACT

Objectives: The aim of this study was to evaluate the efficacy and safety of lispro insulin for the treatment of feline diabetic ketoacidosis (DKA). Times to resolution of hyperglycaemia, ketosis and acidosis were compared between cats treated with continuous rate infusion (CRI) of lispro insulin and cats treated with CRI of regular insulin.

Methods: Client-owned cats with naturally occurring DKA, newly diagnosed with diabetes mellitus (DM) or already receiving treatment for DM, were included. Diagnosis of DKA involved the presence of at least two clinical signs consistent with DKA (eg, polyuria/polydipsia, anorexia, severe lethargy, vomiting and dehydration), blood glucose (BG) concentration >13.9 mmol/l (>250 mg/dl), blood beta hydroxybutyrate (BHB) concentration >2.5 mmol/l and venous pH <7.3 or bicarbonate <15 mEq/l.

Cats were treated with a standard protocol of an intravenous (IV) CRI of regular insulin (group R) or lispro insulin (Group L). The time to resolution of DKA was defined as the time interval from when the IV CRI of insulin began until marked hyperglycaemia (BG >13.9 mmol/l [>250 mg/dl]), ketosis (BHB concentration >1 mmol/l) and acidosis (venous pH <7.3 and/or bicarbonate <15 mEq/l) resolved.

Results: Eighteen DKA cases (nine per group) were enrolled into the study. There were no significant differences in the median time to resolution of three variables (hyperglycaemia, ketosis and acidosis) between the two groups. Two cats in group R developed hypoglycaemia during the CRI of insulin. One cat in group L and three cats in group R developed hypophosphataemia which required phosphate supplementation.

Conclusions and relevance: IV CRI of lispro insulin has few side effects and appears to be as effective as IV CRI of regular insulin in the treatment of cats with DKA.

INTRODUCTION

Diabetic ketoacidosis (DKA) is the most common complication of naturally occurring diabetes mellitus (DM) and is characterised by a biochemical triad of hyperglycaemia, ketosis and acidosis.[1-5] Treatment of DKA comprises intravenous (IV) fluid resuscitation, correction of acid/base and electrolyte derangements, insulin therapy and targeted therapy for comorbid conditions.[5]

During DKA regular insulin is usually administered intramuscularly or intravenously in cats and dogs;[6] in humans, it is also injected subcutaneously.[7] Nevertheless, the dehydration and shock state of patients with DKA leads to erratic and inconstant absorption of intramuscular and subcutaneous (SC) insulin.[7] For this reason, IV infusion of regular insulin has been the mainstay of treatment of DKA as it causes a more predictable fall in blood glucose and it allows for rapid adjustments.[8]

Lispro insulin is a genetically engineered analogue of human insulin in which proline at position B28 and lysine at position B29 are inverted in their sequence, reducing the formation of insulin dimers and hexamers. This structural change ensures more rapid absorption and elimination from the SC injection site, resulting in the rapid onset and a short duration of hypoglycaemic activity.[9,10] Furthermore, one study in human medicine comparing the end-organ metabolic effects of IV lispro insulin, regular insulin and glulisine insulin showed that all these insulins have similar effects on the suppression of endogenous glucose production, glucose uptake and free fatty acid, glycerol and lactate levels.[11] The success of lispro insulin, as well as other insulin analogues, has gradually reduced the use of regular insulin, as demonstrated by Eli Lilly's financial report.[12] Assuming that the production of regular insulin may be discontinued, a valid alternative for treating DKA in dogs and cats should be found. Two studies demonstrated that IV continuous rate infusion (CRI) of lispro and aspart insulin is safe and appears to be as effective as an IV CRI of regular insulin for the treatment of canine DKA.[13,14]

The aim of this study was to evaluate the efficacy and safety of lispro insulin for the treatment of feline DKA by comparing the times to resolution of hyperglycaemia, ketosis and acidosis between cats treated with CRI of lispro insulin and cats treated with CRI of regular insulin.

MATERIALS AND METHODS

Client-owned cats admitted to the University Veterinary Hospital of Bologna (Italy) between May 2009 and March 2017 with naturally occurring DKA, either newly diagnosed with DM or with known DM, were considered for inclusion. The diagnosis of DKA involved the presence of at least two clinical signs consistent with DKA (e.g, polyuria/polydipsia, anorexia, severe lethargy, vomiting and dehydration), blood glucose concentration >13.9 mmol/l (>250 mg/dl), blood beta hydroxybutyrate (BHB) concentration >2.5 mmol/l and venous pH <7.3 or bicarbonate <15 mEq/l.[15] Cats with DKA, admitted between May 2009 and February 2012, and treated with a

protocol for insulin therapy adapted from a published protocol using IV CRI of regular insulin (Humulin R; Ely Lilly)[16] were used as part of the control group of this study. From March 2012 to April 2014 cats with DKA admitted to the University Veterinary Hospital were treated with the same insulin protocol, but using lispro insulin (Humalog; Eli lilly), until the number of cats was the same in both groups. Between May 2014 and March 2017, cats admitted for DKA were alternately treated with regular insulin or lispro insulin.

Cases were divided according to whether they received an IV CRI of regular insulin (group R) or IV CRI of lispro insulin (group L). Cats with multiple hospitalisations for DKA management during the study period were included in the analyses, with each hospitalisation event treated as a separate case.

Cases were excluded from the study if they had unavailable or missing medical records and if they died or were euthanased prior to administration of insulin therapy. The trial was approved by the Scientific Ethics Committee, University of Bologna, Italy. Owners signed the written informed consent before enrolment in the study.

At the time of admission to the hospital, history, physical examination findings and results of blood gas analysis, complete blood count, serum biochemistry profile, urinalysis and bacterial culture from urine collected via cystocentesis were performed in each cat in order to confirm DKA and identify any concurrent disorder. An abdominal ultrasound was performed in order to detect any abnormalities (e.g, acute pancreatitis, neoplasia). Thoracic radiographs or other diagnostic tests were also performed according to the clinician's discretion.

Definitions of 'resolution time', time of SC insulin administration and length of hospitalisation

The 'resolution time' for the variables hyperglycaemia, ketosis and acidosis was calculated starting from 'time zero', which was the time at which the IV CRI of insulin treatment was initiated. The time to resolution of pronounced hyperglycaemia was defined as the time interval between 'time zero' and the time at which the blood glucose concentration fell to <13.9 mmol/l (<250 mg/dl). The time to resolution of ketosis was defined as the time interval between 'time zero' until BHB was ≤1.0 mmol/l for two consecutive measurements 1 h apart. The time to resolution of acidosis was defined as the interval between 'time zero' and the time at which venous pH was ≥7.3 and/or bicarbonate ≥15 mEq/l. The time to resolution of ketoacidosis was defined as the time interval between 'time zero' and the time at which ketosis and acidosis had

both resolved. The IV CRI of insulin was stopped when ketosis and acidosis had resolved and the cat was eating well.

The time to SC insulin administration was defined as the time interval from the resolution of ketoacidosis (when the transition from the IV to the SC insulin administration occurs) up to the hospital discharge. The length of hospitalisation (LOH) was defined as the time interval between 'time zero' and discharge from the hospital.

Monitoring protocol

Blood glucose was monitored hourly during the first 24 h with a hand-held glucometer, previously validated for use in cats (Optium Xceed, Optium Glucose Test Strips; Abbott Laboratories),[17] and then every 2-3 h during the entire time that the cat received an IV CRI of insulin. Blood BHB was measured every 4 h using a portable ketometer, previously validated for cats (Optium Xceed, Optium β-ketone Test Strips; Abbott Laboratories)[17] until BHB was ≤1.0 mmol/l; in this case the BHB was measured 1 h later and if a BHB ≤1.0 mmol/l was confirmed then ketosis was deemed to be resolved. A blood gas analysis (including pH, base excess, serum bicarbonate, sodium, potassium, ionised calcium and lactate) was performed with a point-of-care analyser (Idexx VetStat; Idexx Laboratories) every 8 h during the first 24 h, and then every 12 h until ketosis was resolved.

Insulin-induced hypoglycaemia was defined as a blood glucose concentration <4.4 mmol/l (<80 mg/dl); hypokalaemia was defined as serum potassium <3.6 mEq/l. Hypophosphataemia was defined as serum phosphate <0.5 mmol/l (<1.5 mg/dl).

Treatment protocol

Upon admission, all cats were treated with IV crystalloids (Ringer's lactate or acetate or 0.9% NaCl) prior to and while receiving insulin treatment. The initial rate of fluid administration was determined by the attending clinician to meet the specific rehydration needs of each cat. IV insulin CRI was initiated from 2-8 h after fluid administration had been started, depending on the clinician's perception that severe dehydration had resolved. The insulin solution, which was administered in a separate line from the fluids, consisted of 48 ml 0.9% NaCl to which 1.1 units per kg body weight of lispro insulin or regular insulin were added.[16] To saturate binding of insulin to the IV tubing, the insulin solution was allowed to stand in the line for 30 mins and then run through the IV line.[18] At this point, the insulin solution was re-prepared and the infusion was started. The initial dosage for the insulin CRI was based on the cat's blood glucose concentration

when the CRI was started ('time zero') (Table 1).[6] The insulin CRI was adjusted every 1-2 h based on the cat's blood glucose. Adjustments to the insulin CRI and the addition of dextrose were implemented at each clinician's discretion based on previously published guidelines (Table 1).[6] Long-term insulin was initiated when ketoacidosis was resolved and the cat was eating and appropriately hydrated.

Table 1 Sliding scale for adjustment of intravenous continuous rate infusion of insulin treatment and dextrose supplementation for cats with diabetic ketoacidosis

Blood glucose concentration in mmol/l (mg/dl)	Fluids	Rate of administration of insulin solution (ml/h)*
>13.9 (>250)	0.9% NaCl or Ringer's	2
11.1–13.9 (200-250)	2.5% dextrose[a]	1.5
8.4-11.0 (150-199)	2.5% dextrose[a]	1.5
5.6-8.3 (100-149)	5% dextrose[b]	1
<5.5 (<100)	5% dextrose[b]	Stop insulin infusion

* Insulin solution composed of 1.1 U/kg of regular or lispro insulin added to 48 ml of 0.9% NaCl
[a] 2.5% dextrose composed of 25 ml dextrose 50% added to 475 ml 0.9% NaCl or Ringer's
[b] 5% dextrose composed of 50 ml dextrose 50% added to 450 ml 0.9% NaCl or Ringer's

Serum potassium concentration was corrected as previously described.[16] If serum phosphate concentration decreased to <0.5 mmol/l (<1.5 mg/dl) it was corrected by administration of an IV CRI of potassium phosphate at a rate of 0.01-0.03 mmol phosphate/kg/h for 6 h and then phosphataemia was re-evaluated. Supplementation with potassium was taken into account when giving potassium phosphate for correction of hypophosphataemia. Antimicrobials were administered to all cats for the duration of hospitalisation; additional drugs, including gastroprotectants, antiemetics and analgesics, were administered as deemed appropriate by the attending clinician according to the clinical condition and concurrent disorders.

Statistical analysis

Statistical analysis was performed using commercially available software (GraphPad Prism 5). Owing to the small number of cases in each group, continuous variables were considered to be non-parametric and descriptive statistics are reported as median (minimum-maximum). The Mann Whitney U-test was used to compare variables between the two insulin groups. The Wilcoxon signed rank test was used to compare changes from baseline of the continuous variables within each insulin group. A P value <0.05 was considered significant. In order to

compare the different variables between cats with newly diagnosed DM and cats with known DM, regardless of the type of insulin used, the Mann Whitney U-test was used.

RESULTS

Twenty-four DKA cases were evaluated in the study period. Four cases were excluded because cats died or were euthanased before initiating insulin CRI therapy, and two cases were excluded because of incomplete medical records. A total of 18 cases in 15 cats were included in the study; one cat had three DKA events (in two events it received lispro insulin and in another event received regular insulin) and another cat had two DKA events during the study period (one received lispro insulin and the other received regular insulin). Nine cases were managed with lispro insulin (group L) and nine cases were managed with regular insulin (group R). In 15 cases cats were discharged from the hospital; one cat from group L died and two cats from group R were euthanased; these three cats were newly diagnosed with DM.

History, clinical signs and physical examination findings

There was no significant difference between groups with regard to median age, body weight, breed and sexual status (neutered or intact; Table 2). The median age among all 18 cases was 10.4 years (range 7.7-16.5 years). The median body weight of all 18 cases was 4.5 kg (range 2.6-8 kg) and median body condition score was 6.5 (range 2-8). All 15 cats included in the study were European shorthair cats except one Persian cat in group R and one Birman cat in group L. Eight cats were neutered males, five were neutered females, one was an intact male and one an intact female. In 10 cases cats were newly diagnosed with DM at the time of enrolment into the study. In eight cases (seven cats), cats had previously been diagnosed with DM (five in group L and three in group R), a median of 8 months (range 1-12 months) prior to enrolment into the study and they were all receiving glargine insulin (Lantus, 100 U/ml glargine; Aventis Pharmaceuticals). Insulin dosage at the time of enrolment into the study was 0.5 U q12h in one cat, 1 U q12h in four cats, 2 U q12h in two cats and 2.5 U q12h in one cat.

Clinical signs observed by the owners prior to admission into the hospital included lethargy (observed in 15/18 [83%]), anorexia (15/18; 83%), polyuria and polydipsia (10/18; 56%), vomiting (6/18; 33%), weight loss (5/18; 28%), asthenia (4/18; 22%) and diarrhoea (3/18; 17%). Medications administered to the cats at the time of admission into the hospital included

insulin (7/18; 39%), tylosin (2/18; 11%), methimazole (Tapazole; 1/18; 6%), marbofloxacin (Aristos; 1/18; 6%) and enrofloxacin (Baytril; 1/18; 6%).

Table 2 Baseline data and blood glucose and beta hydroxybutyrate (BHB) concentration at 'time zero' in cats with diabetic ketoacidosis treated with an intravenous (IV) continuous rate infusion (CRI) of lispro insulin (group L) and treated with an IV CRI of regular insulin (group R)

	Group L	Group R	*P* value
Age (years)	10.5 (8.25-14.2)	10.25 (7.75-16.5)	0.8
Body weight (kg)	4.8 (2.8-5.7)	4.2 (2.6-8)	0.27
Male : female (n)	4:5	5:4	1
Spayed	5/9	3/9	
Castrated	4/9	4/9	
Female	0/9	1/9	
Male	0/9	1/9	
Blood glucose concentration (mmol/l) (RI 4.1-8.8 mmol/l)	20.8 (12.4-35)	22.9 (12.4-41.3)	0.69
Blood glucose concentration (mmol/l) at 'time zero' (RI 4.1-8.8 mmol/l)	22.5 (11-27.8)	21.7 (13.4-27.8)	0.86
BHB concentration (mmol/l) (RI <2.5 mmol/l)	6.2 (3.7-8)	7.2 (4.9-8)	0.42
BHB concentration (mmol/l) at 'time zero' (RI < 2.5 mmol/l)	5.4 (4.2-7.8)	7.2 (4.7-8)	0.13
Serum bicarbonate (mmol/l) (RI 18-23.2 mmol/l)	12.9 (8.2-30.2)	12.1 (7.8-17.6)	0.89
Venous pH (RI <7.3)	7.16 (7.02-7.24)	7.15 (7.06-7.28)	0.69
CO_2 (mmol/l) (RI 32.7-44.7 mmol/l)	29.8 (25.2-40.6)	37.6 (22.6-43.1)	0.3
Sodium (mmol/l) (RI 141-155 mmol/l)	148 (131-154)	148 (135-165)	0.51
Potassium (mmol/l) (RI 3.6-5.8 mmol/l)	3.3 (2.2-4.4)	3.9 (2.5-4.6)	0.4
Chloride (mmol/l) (RI 119-132 mmol/l)	116 (92-122)	109 (83-126)	0.59
Creatinine (µmol/l) (RI 70.7-159.1 µmol/l)	119 (80-273)	156 (93-254)	0.56
Phosphate (mmol/l) (RI 0.94-2.69 mmol/l)	1.4 (1.2-1.8)	1.6 (1.1-2.4)	0.42
Calcium (mmol/l) (RI 1.5-2.63 mmol/l)	2.38 (2-2.48)	2.38 (1.93-2.53)	1
Total protein (g/l) (RI 60-80 g/l)	74.4 (57.5-93.5)	77.1 (52.8-85.8)	1
Albumin (g/l) (RI 21-33 g/l)	32.2 (24.4-39.3)	31 (22.8-34.7)	0.54
AST (U/l) (RI 14-41 U/l)	154 (48-1849)	113 (18-291)	0.41
ALT (U/l) (RI 22-45 U/l)	216 (98-1478)	154 (34-237)	0.14
ALP (U/l) (RI 0-120 U/l)	76 (32-193)	40 (32-82)	0.06
GGT (U/l) (RI 0-3 U/l)	0.4 (0.1-1.2)	0.1 (0.1-2)	0.68
Total bilirubin (µmol/l) (RI 0-11.98 µmol/l)	4.4 (2.2-65.1)	9.6 (3.8-64.9)	0.31
Cholesterol (mmol/l) (RI 1.65-5.94 mmol/l)	5.9 (3.3-8.0)	8.5 (1.8-11.1)	0.18

Data are median (range) unless otherwise indicated. Data were compared with the Mann-Whitney U-test
RI = reference interval for healthy cats; AST = aspartate transaminase; ALT = alanine transaminase; ALP = alkaline phosphatase; GGT = gamma glutamyltransferase

At the time of admission, the most common abnormalities included some degree of dehydration (observed in 17/18 [94%]), dull or depressed mentation (17/18; 94%), hypothermia (8/18; 44%), overweight body condition (7/18; 39%), underweight body condition (5/18; 28%), pale mucous membranes (5/18; 28%), jaundice (4/18; 22%), muscle atrophy (3/18; 17%), heart murmur (2/18; 11%) and palpable thyroid nodule (1/18; 6%).

Clinicopathological findings

At the time of admission into the hospital, median blood glucose concentration, BHB concentration, venous pH and serum bicarbonate concentration did not differ significantly between the lispro insulin and regular insulin-treated group (Table 2).

The median blood glucose concentration in group L and group R was 20.8 mmol/l (range 12.4-35 mmol/l; 374 mg/dl [224-630 mg/dl]) and 22.9 mmol/l (range 12.4-41.3 mmol/l; 413 mg/dl [224-744 mg/dl]), respectively. At 'time zero', the median blood glucose concentration was 22.5 mmol/l (range 11-27.8 mmol/l; 405 mg/dl [198-500 mg/dl]) and 21.7 mmol/l (range 13.4-27.8 mmol/l; 391 mg/dl [241-500 mg/dl]) in group L and group R, respectively. No significant differences were detected in blood glucose concentration between the two treatment groups at the time of admission ($P = 0.69$) or at 'time zero' ($P = 0.86$). The rate of decrease in blood glucose concentration was <5.6 mmol/l/h (100 mg/dl/h) in all 18 cases during the entire study.

The median BHB concentration in group L and group R was 6.2 mmol/l (range 3.7-8 mmol/l) and 7.2 mmol/l (range 4.9-8 mmol/l), respectively. At 'time zero', the median BHB concentration was 5.4 mmol/l (range 4.2-7.8 mmol/l) and 7.2 mmol/l (range 4.7-8 mmol/l) in group L and group R, respectively. No significant differences were detected in BHB concentration between the two treatment groups at the time of admission ($P = 0.42$) or at 'time zero' ($P = 0.13$).

At the time of admission, there were also no significant differences between the two treatment groups with respect to any of the biochemical parameters analysed (Table 2).

The median time interval between the time at which fluid therapy was initiated until 'time zero' was 4 h in the lispro insulin group (range 2-8 h) and 4.5 h (range 1-8 h) in the regular insulin group; there was no significant difference between the two groups ($P = 0.62$).

Adverse drug reactions

No local or systemic adverse effects associated with IV insulin administration were noted in either group. Two cats in group R developed hypoglycaemia during the CRI of insulin (4.39 mmol/l [79 mg/dl] and 2.22 mmol/l [40 mg/dl], respectively), but these cats did not show

clinical signs compatible with hypoglycaemia. In all 18 cases, cats developed transient hypokalaemia during the study. Median minimum potassium concentrations did not differ significantly between the lispro (2.8 mmol/l; range 2.2-3.7 mmol/l) and regular (2.6 mmol/l; range 2.2-3.5 mmol/l) insulin treatment groups (P = 0.82). One cat in group L and three cats in group R developed hypophosphataemia which required supplementation during the study.

Resolution time of hyperglycaemia, acidosis and ketosis, time of SC insulin administration and LOH

Severe hyperglycaemia resolved in all 18 cases, acidosis resolved in 15 cases (seven in group L and eight in group R) and ketosis resolved in 16 cases (eight in group L and eight in group R). One cat in group L died prior to resolution of acidosis and ketosis. Acidosis did not resolve in one cat in group L and one cat in group R that had suffered an acute kidney injury at the time of admission; ketosis did not resolve in one other cat in group R, possibly owing to the insulin resistance secondary to a concurrent carcinoma.

There were no significant differences in the median time to resolution of three variables (hyperglycaemia, ketosis and acidosis) between the two groups when evaluated separately; there was no significant difference in the median time to resolution of ketoacidosis (Table 3).

Table 3 Time to resolution of hyperglycaemia, ketosis, acidosis and ketoacidosis, time of subcutaneous (SC) insulin administration and length of hospitalisation in cats with diabetic ketoacidosis treated with an intravenous (IV) continuous rate infusion (CRI) of lispro insulin (group L) and treated with an IV CRI of regular insulin (group R)

	Group L	Group R	*P* value
Resolution time of hyperglycaemia (h)	8 (0-25)	9 (0-24)	0.72
Resolution time of ketosis (h)	29 (16-94)	26.5 (21-53)	0.83
Resolution time of acidosis (h)	8 (8-32)	20 (8-48)	0.26
Resolution time of ketoacidosis (h)	33 (16-94)	28 (21-53)	1
Time of SC insulin administration (h)	76 (34-168)	89 (48-244)	0.25
Length of hospitalization (h)	110.5 (74-268)	146 (94-294)	0.18

Data are expressed as median (range)

The median times to resolution of severe hyperglycaemia in group L and group R were 8 h (range 0-25 h) and 9 h (range 0-24 h), respectively (P = 0.72). Median time to resolution of ketosis was 29 h (range 16-94 h) in group L and 26.5 h (range 21-53 h) in group R (P = 0.83).

Median time to resolution of acidosis in group L and group R was 8 h (range 8-32 h) and 20 h (range 8-48 h), respectively (P = 0.26). Median time to resolution of ketoacidosis in group L and group R was 33 h (range 16-94 h) and 28 h (range 21-53 h), respectively (P = 1).

There were no significant differences between newly diagnosed and previously diagnosed diabetic cats with respect to median time to resolution of hyperglycaemia and ketosis (analysed separately), and ketoacidosis (Table 4). However, the median time to resolution of acidosis in the newly diagnosed diabetics (12 h; range 8-24 h) was significantly shorter than in previously diagnosed diabetics (24 h; range 8-48 h; P = 0.02).

Table 4 Time to resolution of hyperglycaemia, ketosis, acidosis and ketoacidosis, time of subcutaneous (SC) insulin administration and length of hospitalisation in cats with diabetic ketoacidosis, comparing cats with newly diagnosed diabetes mellitus (DM) and cats with known DM

	Newly diagnosed DM	Known DM	*P*-value
Resolution time of hyperglyacemia (h)	9 (0-15)	10 (4-25)	0.37
Resolution time of ketosis (h)	25 (21-51)	40 (16-94)	0.96
Resolution time of acidosis (h)	12 (8-24)	24 (8-48)	**0.02**
Resolution time of ketoacidosis (h)	26.5 (21-51)	42 (16-94)	0.48
Time of SC insulin administration (h)	70.5 (34-244)	87 (48-177)	0.3
Length of hospitalization (h)	97 (74-292)	137 (81-294)	0.19

Data are expressed as median (range)
Bold indicates statistical significance

Venous pH decreased during the first hours of treatment, before it began to rise, in 5/18 cases (one case in group L and four cases in group R). The lowest pH for these five cases was reached at a median of 8 h (range 8-16 h) from the time at which fluid infusion had begun. The median lowest pH for the five cases in which this initial decline occurred was 7.07 (range 6.94-7.25) and did not differ significantly (P = 0.06) from the pH of the same patients at admission (median 7.15; range 7.02-7.28). Also, there was no significant difference in LOH between cases in which pH decreased before it began to rise and cases in which this did not happen.

The median time to administration of SC insulin, in the 15 cases that were discharged, did not differ significantly between group L (76 h; range 34-168 h) and group R (89 h; range 48-244 h; P = 0.25). Likewise, the median duration of hospitalisation for these 15 cases did not differ significantly between group L (110.5 h; range 74-268 h) and group R (146 h; range 94-294 h; P = 0.18) (Table 3).

No significant differences were found in median time to administration of SC insulin and in median duration of hospitalisation between newly diagnosed and previously diagnosed diabetic cats (Table 4).

The lispro insulin-treated cat that died had been hospitalised for 45 h at the time of death; the two cats treated with regular insulin that were euthanased had been hospitalised for 106 and 129 h, respectively, at the time of euthanasia.

Evaluation for presence of concurrent disorders

Based on the diagnostic protocol, concurrent disorders were identified in 11 cases (five in group L and six in group R). In group L, two cases had inflammatory bowel disease, one cat had pancreatitis, one cat had concurrent pancreatitis, lipidosis and acute kidney injury, and one cat had pulmonary neoplasia. In group R, one cat was diagnosed with a bacterial urinary tract infection (based on urinary culture), one cat was diagnosed with pancreatitis and inflammatory bowel disease, one cat had pancreatitis and polycystic kidney disease, one cat had hyperthyroidism and herpesvirus infection, one cat had chronic kidney disease and one cat had a giant cell tumor.

The diagnosis of pancreatitis was based on abdominal ultrasound (enlarged, irregular, hypoechoic pancreas surrounded by hyperechoic mesentery, and mild-to-moderate ascites) and positivity to a feline pancreatic lipase immunoreactivity test.

DISCUSSION

Lispro insulin was developed to resolve the problems associated with the use of regular human insulin (peak of activity reached too late, hypoglycaemic effect possibly lasting too long) by SC injection.[19] The major difference between lispro insulin and regular insulin is the rate of self-disassociation, which causes differences in the rate of absorption from the injection site. However, this difference may not exist with IV administration. A study on rabbits showed that the hypoglycaemic response profiles after IV administration of lispro insulin and regular human insulin were very similar in pattern and confirmed that their biological activities are equivalent.[20]

The aim of this study was to evaluate the efficacy and safety of lispro insulin for the treatment of feline DKA.

The need to test a new insulin, which could provide an alternative to regular insulin, has arisen because insulin analogues are widely used for the management of DM and treatment of uncomplicated DKA in human medicine, although some patients with severe comorbidities still require intensive care and IV insulin administration.[8,21-24] It is possible that the production of regular insulin may be discontinued in the future, and as only regular insulin is currently indicated for the treatment of DKA with the constant low-dose IV insulin infusion technique in cats, a viable alternative to regular insulin is needed to manage these patients.

In a prospective randomised study, Sears et al compared the efficacy and safety of an IV CRI of lispro insulin with that of regular insulin in a population of 12 dogs with DKA.[13] They observed comparable improvement in glycaemia, ketosis and acidosis between the two groups and the time to resolution of ketoacidosis was significantly shorter in the lispro insulin group, although the LOH did not differ significantly. They concluded that an IV CRI of lispro insulin is safe and appears to be as effective as an IV CRI of regular insulin for the treatment of canine DKA.

In our study on feline DKA, the time of resolution of hyperglycaemia, ketosis, acidosis and ketoacidosis was similar in cases treated with lispro insulin and those treated with regular insulin; the time of SC insulin administration and the LOH also did not differ significantly between the two groups. Nevertheless, the time to resolution of acidosis and the LOH were both shorter in the group of cases treated with lispro insulin, although these differences were not significant. A number of variables, including concurrent disorders, may have contributed to these findings, and it is reasonable to suppose that studying a larger group of cases in the future could reveal that each of these times is significantly shorter with lispro insulin treatment.

The median time to resolution of acidosis in newly diagnosed diabetic cats was significantly shorter than in previously diagnosed diabetics; this result may reflect a different efficiency in the buffering system and a difference in acid-base status between newly and previously diagnosed diabetic cats.

In the first hours after the onset of insulin therapy, venous pH decreased before it began to rise in only one cat in group L and in four cats in group R; these results could be attributed to the more rapid action of lispro insulin compared with regular insulin. Furthermore, hyperglycaemia resolved in all 18 cases, acidosis resolved in 7/9 cases in group L and in 8/9 cases in group L, and ketosis resolved in 8/9 cases in group L and group R. On the basis of these results an IV CRI of lispro insulin appears to be as effective as an IV CRI of regular insulin for the treatment of cats with DKA.

With regards to safety, in our results, side effects were seen less frequently in cases treated with lispro insulin than in cases treated with regular insulin; in fact, in group R on two occasions cats developed hypoglycaemia and three cats developed hypophosphataemia, whereas only one cat in group L developed hypophosphataemia. Despite supplementation, transient hypokalaemia occurred in all cats during the IV infusion of insulin, regardless of the type of insulin used. Although hypokalaemia that develops during DKA rarely becomes symptomatic, in our opinion it would be more appropriate to use higher rates of supplementation than those normally reported in textbooks during the first hours of insulin therapy; this was also suggested by Nelson,[5] but only in those patients with normal urinary production and if frequent assessments of kalaemia are possible.

In this study there were no significant differences in blood glucose concentration or BHB concentration between the two treatment groups at the time of admission or at 'time zero', when insulin therapy began. On the contrary, a decrease in blood glucose concentration during the first hours of fluid therapy has been reported in human medicine and in some studies in veterinary medicine, and has been attributed to rehydration-induced renal excretion of glucose, decreased concentrations of the counter-regulatory hormones, or improved perfusion and delivery of endogenous insulin.[13-14,25,26] This reduction was not observed during our study, probably owing to the conservative fluid therapy, which was not too 'aggressive'. On that note, one of the current study's limitations is the lack of standardised criteria for the evaluation of the degree of dehydration (determined subjectively) to determine when to start insulin therapy.

Another important limitation is the small number of cases enrolled, which influenced the power of statistics. It is likely that some differences between groups were not detected because of this bias. Furthermore, some cats were included more than once and this is also a possible bias; in fact, it is possible that a cat responds in a similar manner with repeated treatment as a different cat, or that a cat can be more severely affected with subsequent visits because of progression of concurrent illness or even less severely affected because owners recognised the signs earlier.

Finally, further limitations are the absence of randomisation and the heterogeneity of the population with regard to the presence of concurrent disorders. However, our population's characteristics were very similar to those in other studies of feline DKA,[27-31] suggesting that this small population is representative of cats with spontaneous DKA.

CONCLUSIONS

The results demonstrate that an IV CRI of lispro insulin treatment did not show severe side effects in cats of this study and appears to be as effective as an IV CRI of regular insulin treatment in managing cats with DKA.

3. Use of lispro insulin for the treatment of diabetic ketoacidosis

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Al termine di questo studio, è doveroso puntualizzare che gli analoghi insulinici sono stati concepiti al fine di ottenere delle molecole che, somministrate per via sottocutanea, avessero delle qualità ed un'efficacia paragonabili a quelle dell'insulina cristallina regolare per via endovenosa (assorbimento ed eliminazione rapidi, attività ipoglicemizzante tempestiva ma di breve durata). In medicina umana, l'impiego di tali analoghi ha permesso di semplificare di molto la gestione dei pazienti chetoacidotici, con notevoli risparmi anche sul fronte economico ovviando alla necessità di ricoverare i pazienti, quanto meno quelli con DKA lieve/moderata, nel reparto di terapia intensiva. È evidente, quindi, che i pregi di queste molecole sono proprio da ricercarsi nel loro impiego per via sottocutanea.

Il nostro studio, come già specificato, ha avuto la finalità di trovare, in tempi rapidi, una valida alternativa all'insulina cristallina regolare qualora la sua produzione fosse interrotta, ignorando la finalità per la quale queste molecole sono state progettate.

A questo proposito, vogliamo specificare che abbiamo intrapreso uno studio che prevede l'impiego dell'insulina Lispro per via intramuscolare/sottocutanea in cani e gatti in DKA. Ci aspettiamo che le caratteristiche molecolari dell'insulina Lispro siano riproducibili anche in queste due specie e che, pertanto, tale insulina possa consentire una gestione più semplice ed economica, ma altrettanto efficace e sicura rispetto all'infusione continua endovenosa di insulina cristallina regolare.

Capitolo 4

EVALUATION OF ONE PORTABLE BLOOD GLUCOSE METER AND ONE PORTABLE GLUCOSE-KETONES METER IN DOGS

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Abstract premiato come miglior poster (a parimerito)
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4. Evaluation of one portable blood glucose meter and one portable glucose-ketones meter in dogs

BACKGROUND

Nowadays only few portable blood glucose meters (PBGMs) have been developed specifically for use in dogs and cats. Recently one glucometer (Gluco Calea, WellionVet; GC) and one glucose-ketones meter (Belua, WellionVet; BE) have been developed for use in veterinary medicine (Table 1).

	GLUCOCALEA	BELUA	
		Glucometer	Ketometer
Test principle	Glucose oxidase	Glucose dehydrogenase	3- Hydroxybutyrate dehydrogenase
Detecting range	20 – 600 mg/dL	20 – 600 mg/dL	0.1 – 8 mmol/L
Test time	5 seconds	6 seconds	8 seconds
Sample volume	0.5 µl	0.8 µl	0.8 µl
Blood sample	Capillary blood	Capillary or venous blood	Capillary or venous blood
HCT range	35% – 55%	10% - 70%	30% - 60%

Table 1: Specification of WellionVet® GLUCO CALEA and BELUA.

OBJECTIVES

The aims of this study were to assess the accuracy and precision of these devices in canine venous and capillary blood samples based on ISO 15197:2013 and to evaluate packed cell volume (PCV) interferences.

MATERIALS AND METHODS

Samples were obtained from 45 non anemic dogs (PCV 37-54%) and 10 anemic dogs (PCV<37%) divided into three glycemic ranges: high (>140 mg/dL), medium (90-139 mg/dL), and low (<90 mg/dL). Paired measurements of glucose and 3-ß-hydroxybutyrate (3-HB) from capillary and venous blood samples were determined using the two devices and compared with the results of reference methods (enzymatic hexokinase and 3-HB-dehydrogenase, respectively) obtained by an automated chemistry analyzer (Beckman-Coulter AU480). Linear regression, Bland-Altman plots and the Parkes error grid analysis (EG) were used to assess the accuracy.[1]

PCV interferences for glucose measurement were assessed comparing the differences between PBGMs readings and reference method values in anemic and non-anemic dogs. To assess within-run precision, glucose concentrations obtained from 12 samples, belonging to the three glycemic ranges, were measured 10 times within 10 minutes. Between-day precision was assessed by testing each manufacturer's glucose control solution over 10 consecutive days. P <0.05 was considered significant.

RESULTS

Mean differences (mg/dL) between measurements of each PBGM on venous and capillary blood and values measured by the reference method in patients with normal PCV were: GC 44.1±27.2, 37.8±24.2, BE 10.2±25.1 and 20.4±28.6, respectively. A positive significant correlation between all paired samples was found for both devices (r>0.89) (Table 2).

HAND-HELD METER	REFERENCE METHOD	MEAN BIAS (mg/dL)		CORRELATION (r)	
		Venous blood	Capillary blood	Venous blood	Capillary blood
Wellion vet GLUCO CALEA	Hexokinase	44.1 ± 27.2	37.8 ± 24.2	0.94	0.93
Wellion vet BELUA GLUCOMETER	Hexokinase	10.2 ± 25.1	20.4 ± 28.6	0.93	0.89
Wellion vet BELUA KETOMETER	3-HB-dehydrogenase	-0.07 ± 0.79	0.05 ± 0.57	0.59	0.48

Table 2: Mean differences (mg/dL) and correlations between measurements of Gluco Calea and Belua on venous and capillary blood and values measured by the reference method.

However neither PBGMs fulfilled ISO requirements: 82.21% and 84.08% of glucose values measured respectively on capillary and venous blood using GC fell in zone A+B of EG; 86.7% and 97.8% of glucose values measured respectively on capillary and venous by BE fell in zone A+B of EG (Figure 1, Figure 2).

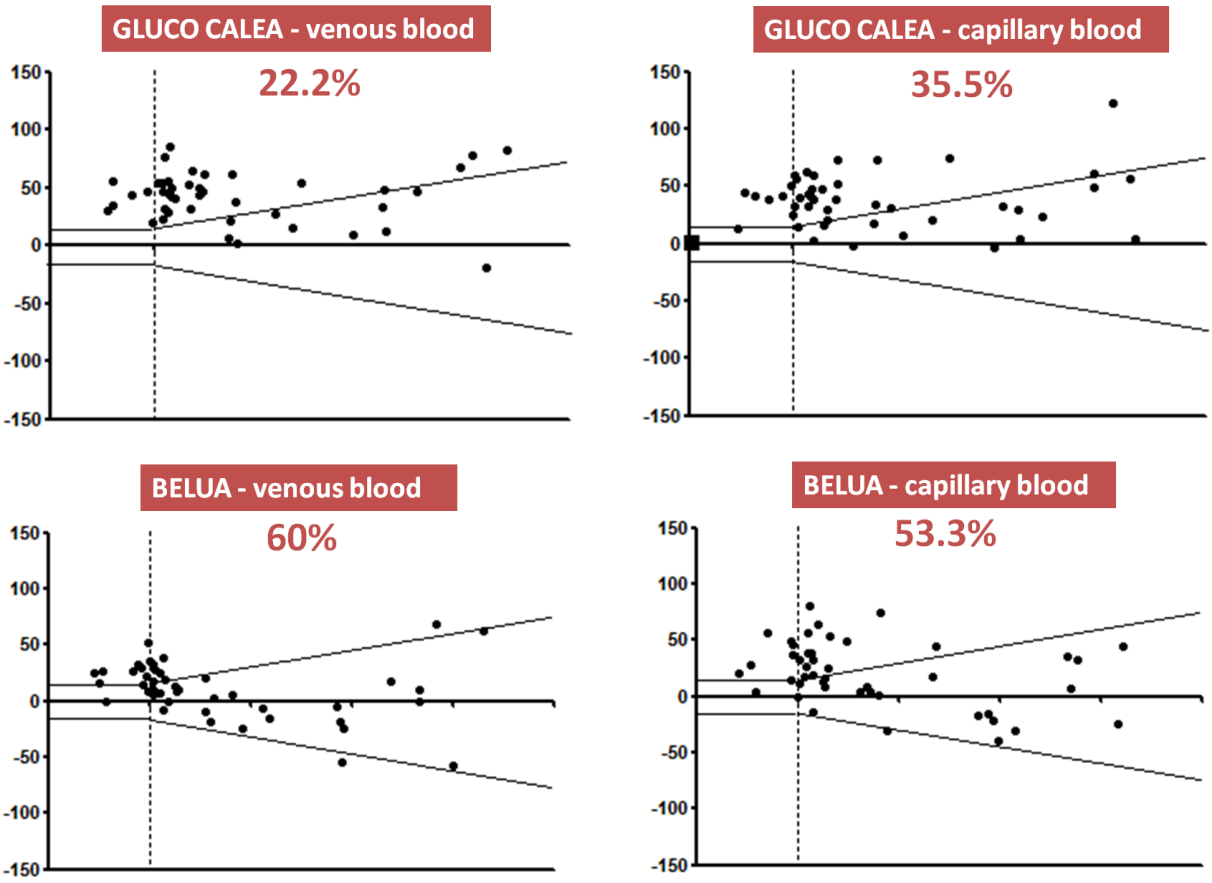

Figure 1: Bland-Altman plots represent the difference between blood glucose measurements obtained by the use of the two glucometer, Gluco Calea and Belua, versus blood glucose concentrations obtained by the reference method (enzymatic hexokinase; Beckman-Coulter AU480). On the x axis are the reference glucose values, plotted against the absolute errors for each corresponding value. The requirements established by ISO 15197:2013 criteria are represented by the 2 solid symmetrical lines: at ±15 mg/dL from the reference value for glucose <100 mg/dL and at ±15% from the reference for glucose ≥100 mg/dL. At the top, are reported the percentages of samples within limits for the total number of measurements.

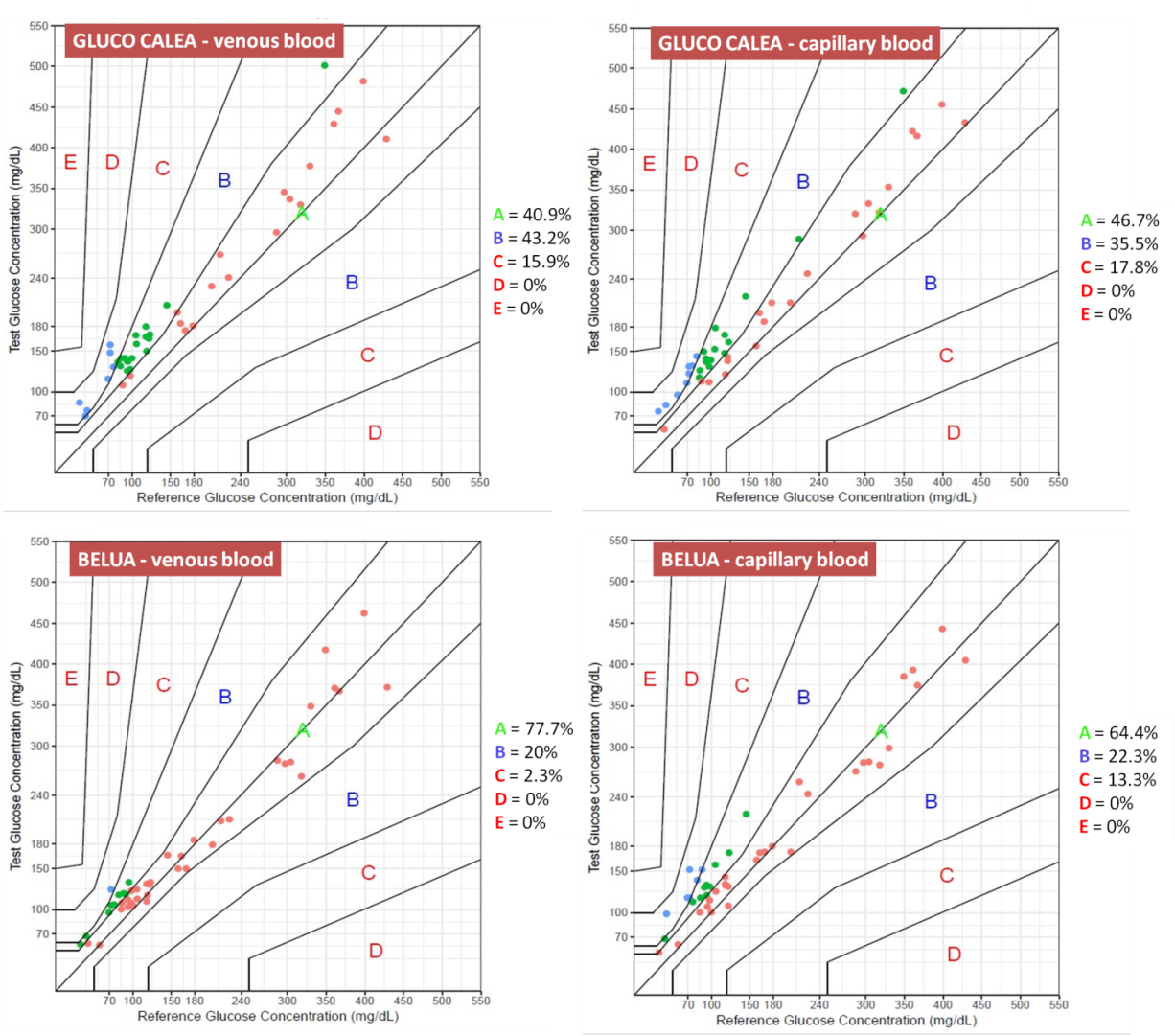

Figure 2: Parkes Consensus Error Grid Analysis representations for venous and capillary blood for each device with the percentages of values within different zones. The reference glucose values (blood glucose obtained by the reference method), on the x axis, are plotted against the blood glucose measurements obtained the two glucometers, Gluco Calea and Belua, on the y axis. The different zones designate the magnitude of risk: no effect on clinical action (zone A), altered clinical action - little or no effect on clinical outcome (zone B), altered clinical action – likely to affect clinical outcome (zone C), altered clinical action – could have significant medical risk (zone D) and altered clinical action – could have dangerous consequences (zone E). ISO 15197:2013 requires that 99% of the values fall within zones A+B for a device to be considered accurate.

Results of within-run and between-day precision are shown in Table 3.

HAND-HELD METER	WRP (mean±SD)	BDP (mean±SD)
Wellion vet GLUCO CALEA	5.66 ± 5.41	13.59 ± 21.17
Wellion vet BELUA GLUCOMETER	2.77 ± 1.34	9.40 ± 12.83
Wellion vet BELUA KETOMETER	4.85 ± 5.04	/

Table 3: Within-run precision (WRP) and between-day precision (BDP) of Gluco Calea glucometer and Belua gluco-ketometer.

The effect of PCV was significant and higher results with lower PCV were observed (Table 4).

HAND-HELD METER	MEAN BIAS (mg/dL) VENOUS BLOOD		P	MEAN BIAS (mg/dL) CAPILLARY BLOOD		P
	Non anemic dogs	Anemic dogs		Non anemic dogs	Anemic dogs	
Wellion vet GLUCO CALEA	44.0	60.0	0.0141	39.0	69.5	0.0029
Wellion vet BELUA GLUCOMETER	10.0	55.5	0.0003	19.0	74.5	0.0008

Table 4: Median of the mean bias on venous and capillary blood for Gluco Calea and Belua glucometers in non anemic and anemic dogs.

The correlations between capillary and venous 3-HB and reference 3-HB were r=0.48 and r=0.59, respectively. Mean differences between capillary and venous 3-HB and reference method were 0.05 (±0.57) and -0.07 (±0.79) respectively (Table 2); within-run precision is shown in Table 3.

DISCUSSION

Our results show that both GC and BE glucometers are not sufficiently accurate and safe for clinical use in dogs. BE ketometer has proven to be less accurate compared with results of other studies in which correlations of 0.96 and 0.97 were found.[2,3] However, to date, there are not specific guidelines for quality assurance for ketometers.

4. Evaluation of one portable blood glucose meter and one portable glucose-ketones meter in dogs

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Capitolo 5

EVALUATION OF ONE PORTABLE BLOOD GLUCOSE METER AND ONE PORTABLE GLUCOSE-KETONES METER IN CATS

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5. Evaluation of one portable blood glucose meter and one portable glucose-ketones meter in cats

BACKGROUND

Numerous portable blood glucose meters (PBGMs) have been developed during the last decade, the majority of which is designed for use in humans. Recently one glucometer (Gluco Calea, WellionVet; GC) and one glucose-ketones meter (Belua, WellionVet; BE) have been developed for use in veterinary medicine (Table 1).

	GLUCOCALEA	BELUA	
		Glucometer	Ketometer
Test principle	Glucose oxidase	Glucose dehydrogenase	3- Hydroxybutyrate dehydrogenase
Detecting range	20 – 600 mg/dL	20 – 600 mg/dL	0.1 – 8 mmol/L
Test time	5 seconds	6 seconds	8 seconds
Sample volume	0.5 µl	0.8 µl	0.8 µl
Blood sample	Capillary blood	Capillary or venous blood	Capillary or venous blood
HCT range	35% – 55%	10% - 70%	30% - 60%

Table 1: Specification of Wellion® GLUCO CALEA and BELUA.

OBJECTIVES

The aims of this study were to assess the accuracy and precision of these devices in feline venous and capillary blood samples based on ISO 15197:2013 and to evaluate packed cell volume (PCV) interferences.

MATERIALS AND METHODS

Samples were obtained from 29 non anemic cats (PCV 30-47%) and 18 anemic cats (PCV<30%) divided into three glycemic ranges: high (>140 mg/dL), medium (90-139 mg/dL), and low (<90 mg/dL). Paired measurements of glucose and 3-ß-hydroxybutyrate (3-HB) from capillary and venous blood samples were determined using the two devices and compared with the results of reference methods (enzymatic hexokinase and 3-HB-dehydrogenase, respectively) obtained by an automated chemistry analyzer (Beckman-Coulter AU480). Linear regression, Bland-Altman

plots and the Parkes error grid analysis (EG) were used to assess the accuracy.[1] PCV interferences for glucose measurement were assessed comparing the differences between PBGMs readings and reference method values in anemic and non-anemic cats. To assess within-run precision, glucose concentrations obtained from 14 samples, belonging to the three glycemic ranges, were measured 10 times within 10 minutes. Between-day precision was assessed by testing each manufacturer's glucose control solution over 10 consecutive days. $P<0.05$ was considered significant.

RESULTS

Mean differences (mg/dL) between measurements of each PBGM on venous and capillary blood and values measured by the reference method in cats with normal PCV were: GC 35.6±40.5, 30.7±35.4, BE 15.0±24.1 and 15.5±35.5, respectively. A positive significant correlation between all paired samples was found for both devices ($r>0.89$) (Table 2).

HAND-HELD METER	REFERENCE METHOD	MEAN BIAS (mg/dL)		CORRELATION (r)	
		Venous blood	Capillary blood	Venous blood	Capillary blood
Wellion vet GLUCO CALEA	Hexokinase	35.6 ± 40.5	30.7 ± 35.4	0.89	0.9
Wellion vet BELUA GLUCOMETER	Hexokinase	15.0 ± 24.1	15.5 ± 35.5	0.94	0.9
Wellion vet BELUA KETOMETER	3-HB-dehydrogenase	-0.30 ± 1.48	0.07 ± 1.15	0.82	0.66

Table 2: Mean differences (mg/dL) and correlations between measurements of Gluco Calea and Belua on venous and capillary blood and values measured by the reference method.

However neither PBGMs totally fulfilled ISO requirements, but 100% of glucose values measured on venous blood using BE fell in zone A+B of EG (Figure 1, Figure 2).

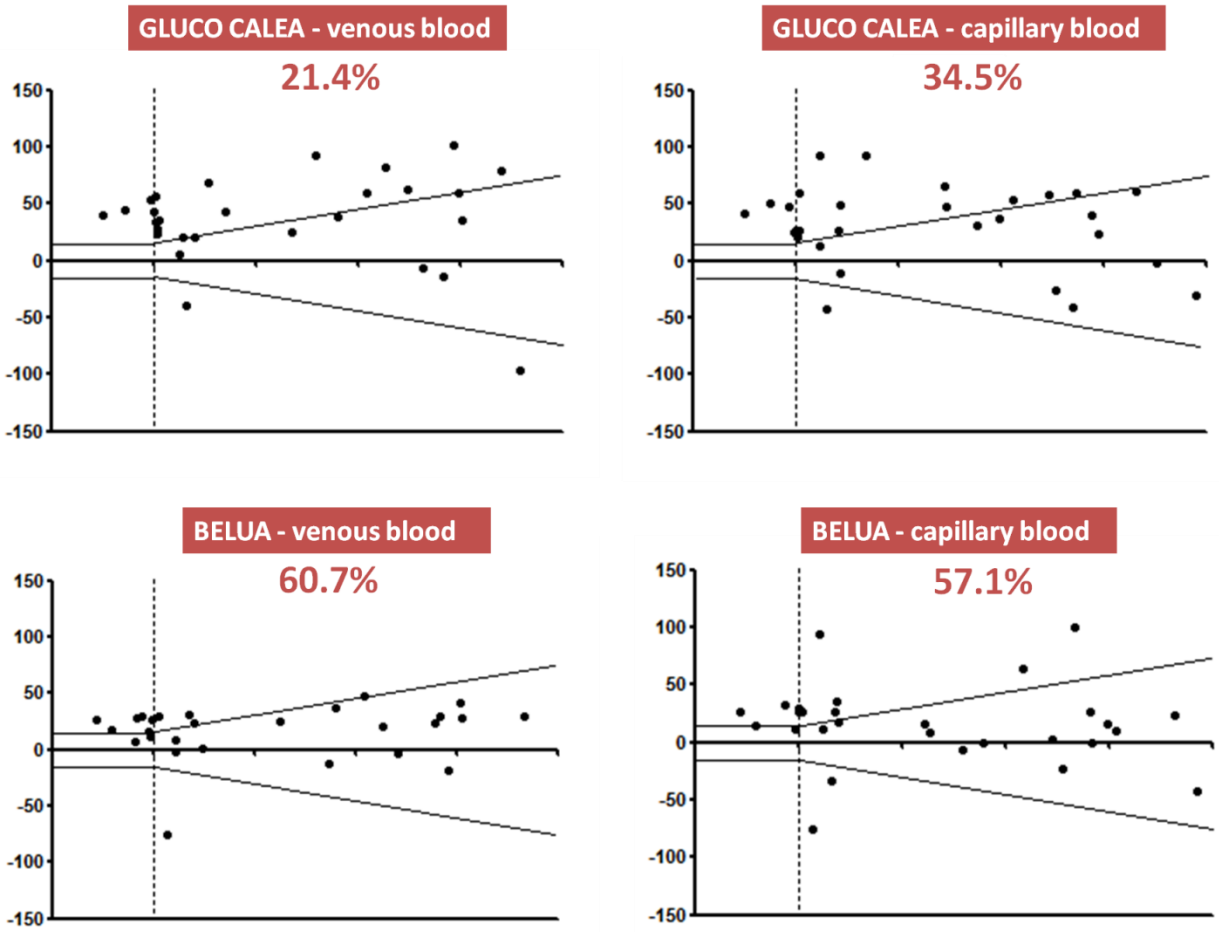

Figure 1: Bland-Altman plots represent the difference between blood glucose measurements obtained by the use of the two glucometer, Gluco Calea and Belua, versus blood glucose concentrations obtained by the reference method (enzymatic hexokinase; Beckman-Coulter AU480). On the x axis are the reference glucose values, plotted against the absolute errors for each corresponding value. The requirements established by ISO 15197:2013 criteria are represented by the 2 solid symmetrical lines: at ±15 mg/dL from the reference value for glucose <100 mg/dL and at ±15% from the reference for glucose ≥100 mg/dL. At the top, are reported the percentages of samples within limits for the total number of measurements.

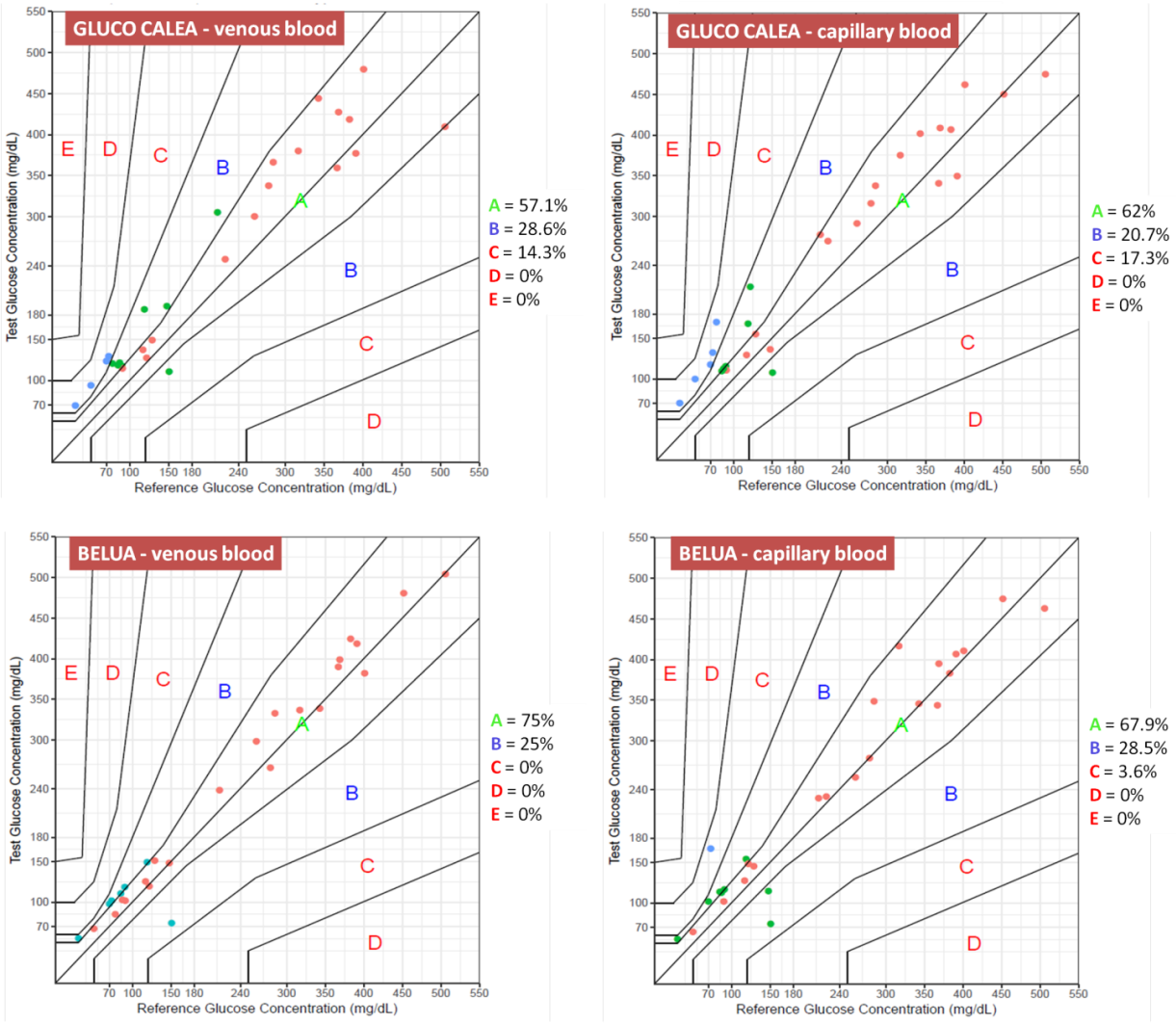

Figure 2: Parkes Consensus Error Grid Analysis representations for venous and capillary blood for each device with the percentages of values within different zones. The reference glucose values (blood glucose obtained by the reference method), on the x axis, are plotted against the blood glucose measurements obtained the two glucometers, Gluco Calea and Belua, on the y axis. The different zones designate the magnitude of risk: no effect on clinical action (zone A), altered clinical action - little or no effect on clinical outcome (zone B), altered clinical action – likely to affect clinical outcome (zone C), altered clinical action – could have significant medical risk (zone D) and altered clinical action – could have dangerous consequences (zone E). ISO 15197:2013 requires that 99% of the values fall within zones A+B for a device to be considered accurate.

Within-run and between-day precision were adequate and results are shown in Table 3.

HAND-HELD METER	WRP (mean±SD)	BDP (mean±SD)
Wellion vet GLUCO CALEA	4.78 ± 2.62	13.47 ± 17.68
Wellion vet BELUA GLUCOMETER	2.72 ± 1.07	10.0 ± 11.52
Wellion vet BELUA KETOMETER	5.41 ± 6.18	/

Table 3: Within-run precision (WRP) and between-day precision (BDP) of Gluco Calea glucometer and Belua gluco-ketometer.

The effect of PCV was significant (higher results with lower PCV) only for BE (Table 4).

HAND-HELD METER	MEAN BIAS (mg/dL) VENOUS BLOOD		P	MEAN BIAS (mg/dL) CAPILLARY BLOOD		P
	Non anemic cats	Anemic cats		Non anemic cats	Anemic cats	
Wellion vet GLUCO CALEA	39.5	46	0.22	37	45	0.19
Wellion vet BELUA GLUCOMETER	23	37.5	**0.0003**	16	34	**0.0007**

Table 4: Median of the mean bias on venous and capillary blood for Gluco Calea and Belua glucometers in non anemic and anemic cats.

The correlations between capillary and venous 3-HB and reference 3-HB were r=0.66 and r=0.82, respectively. Mean differences between capillary and venous 3-HB and reference method were -0.07 (±1.15) and -0.30 (±1.48) respectively (Table 2); within-run precision was adequate and result is shown in Table 3.

DISCUSSION

Our results show that GC glucometer is not sufficiently accurate while the superior performances of BE glucometer support its clinical use in cats.

BE ketometer has proven to be less accurate compared with results of other studies.[2,3] However, to date, there are not specific guidelines for quality assurance for ketometers.

5. Evaluation of one portable blood glucose meter and one portable glucose-ketones meter in cats

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Capitolo 6

ACCURACY OF A FLASH GLUCOSE MONITORING SYSTEM IN DOGS WITH DIABETIC KETOACIDOSIS

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ABSTRACT

***Background*:** A factory-calibrated flash glucose monitoring system (FGMS) (FreeStyle Libre, Abbott, UK) was recently evaluated in dogs with uncomplicated diabetes mellitus. It is not known if this system is reliable during diabetic ketoacidosis (DKA).

***Objectives*:** To assess the performance of the FGMS in dogs with DKA, and to determine the effect of body condition score (BCS), lactate level, severity of ketosis, and acidosis in terms of the accuracy of the device.

***Animals*:** Fourteen client-owned dogs with DKA.

***Methods*:** The interstitial glucose (IG) measurements were compared with blood glucose (BG) measurements, obtained using a validated portable glucometer. Accuracy was determined by fulfilment of ISO 15197:2013 criteria. The effect of BCS and the influence of changes in metabolic variables (lactate, β-hydroxybutyrate, pH and bicarbonate) on sensor performance were evaluated.

***Results*:** Good agreement between IG measurements and BG was obtained (r = 0.86). Analytical accuracy was not obtained, whereas clinical accuracy was demonstrated with 99.8% of results in zones A and B of the Parkes Consensus Error Grid analysis. A significant inter-patient variability in accuracy was observed; the FGMS tends to overestimate the glucose level in dogs with BCS ≤ 3 and to underestimate in dogs with BCS ≥ 7. Variations in metabolic variables do not affect sensor performance.

***Conclusions and clinical importance*:** Despite the ISO 15197:2013 requirements being only partially fulfilled, the FGMS provides clinically accurate estimates of BG in dogs with DKA. The effect of BCS on sensor performance requires further investigation; otherwise the accuracy was apparently unaffected by metabolic variables.

INTRODUCTION

Diabetic ketoacidosis (DKA) is the most common life-threatening complication of diabetes mellitus, involving extreme alterations of metabolic variables. The syndrome is characterized by a biochemical triad of hyperglycemia, ketosis, and acidosis.[1-5] Treatment of DKA involves intravenous fluid resuscitation, correction of acid/base and electrolyte derangements, insulin therapy, identification and treatment of any concurrent illness.[5] Insulin therapy aims to support cellular glucose uptake, decrease hepatic glucose production, interrupt the process of ketogenesis, and promote ketone metabolism and clearance.[6,7] Rapid changes in blood glucose (BG) concentration requiring frequent monitoring of glycemia result from insulin administration, glucose supplementation, and compromised homeostatic mechanisms that are characteristic of ketoacidotic patients. Nowadays, hospitalized ketoacidotic patients are usually monitored by measuring BG concentration using a portable blood glucose meter (PBGM). The main limitations of the use of these devices include repeated venipuncture or placement of a second or central catheter for blood sampling, increasing the risk of catheter-related complications, including infection and phlebitis.[8-10] In addition, such BG monitoring methods, apart from allowing only intermittent BG measurements (usually every 1–2 hours), can increase patient stress, owner expense, and nursing workload, and can lead to anemia, especially in small patients.[11] For these reasons, research is being directed toward less invasive methods to monitor BG concentrations continuously in patients with DKA.

In the past two decades, there has been great interest in devices measuring interstitial glucose (IG), which has been shown to reflect BG concentrations in several species, including humans, dogs, rats, and rabbits.[12] The first generation systems offered only retrospective analysis of glucose concentrations after disconnecting the sensor and uploading the data (continuous glucose monitoring system, CGMS), while the second generation measured and displayed the data immediately, allowing direct intervention (real-time CGMS).[13] However, the weak point of these devices was that they required frequent calibration, which still involved repeated capillary blood sampling.[13,14] A novel factory-calibrated flash glucose monitoring system (FGMS, FreeStyle Libre, abbott, UK) has been licensed for use in people (CE mark, August 2014). The system consists of a small, round, disposable, water-resistant sensor, which continuously measures glucose in the interstitial fluid through a small (5 mm long x .4 mm wide) filament inserted subcutaneously. The FGMS generates information every minute and the readings are

automatically stored in 15-minutes intervals for up to 14 days. IG levels are displayed when the sensor is "flashed" with a reader device on demand. The reader device will then display the past 8-hours of glucose information, including current glucose, a trend graph, and a trend arrow that indicates the direction and velocity of the patient's current glucose level. The FGMS has recently been evaluated in diabetic dogs without DKA,[15] but not in dogs with DKA, which typically have significant metabolic alterations that could affect the accuracy of the device.

The aims of this study were to assess the performance of the FGMS in dogs with DKA compared with BG measurements obtained with a PBGM, and to determine the effect of BCS, lactate level, severity of ketosis, and acidosis on the accuracy of the device.

MATERIALS AND METHODS

Dogs

Client-owned dogs admitted to the University Veterinary Teaching Hospital of Bologna between April 2015 and July 2017 with naturally occurring DKA were enrolled in the study. The diagnosis of DKA was based on the presence of at least two clinical signs consistent with DKA (e.g., polyuria/polydipsia, anorexia, severe lethargy, vomiting, and dehydration), BG concentration > 250 mg/dL, blood β-hydroxybutyrate (BHB) concentration > 3.8 mmol/L,[16] and venous pH < 7.3 and/or bicarbonate < 15 mEq/L. The dogs were treated with a modified previously published protocol,[6] using IV continuous rate infusion of regular insulin (Humulin R, Ely Lilly and Co, Indianapolis, IN).

The Scientific Ethics Committee of the University of Bologna approved this study.

Data collection

Once the diagnosis of DKA was confirmed, the FGMS was placed on a clipped and cleaned area of the dorsal part of the neck, and adherence to the skin was further ensured by an additional tape and a bandage applied around the neck (Fig 1).[15] The IG measurements were compared with BG measurements, obtained by a PBGM (Optium Xceed, Abbott, UK), validated for use in dogs.[17] Venous or capillary BG concentrations were measured every 1–2 hours from admission to the resolution of DKA, and then less frequently, at the discretion of clinicians, until discharge.

Figure 1: FreeStyle Libre is composed of the reader (A) and the sensor (B), which is placed on the dorsal part of the neck of the dog (C), secured by an additional tape (D) and a bandage applied around the neck (E). The sensor has to be scanned by the reader, which instantaneously shows the interstitial glucose value (F). The reader shows "HI" and "LO" when the IG concentration is ≥ 500 mg/dL and ≤ 20 mg/dL, respectively.

BCS was recorded at admission using a 9-point scoring system. The patient's metabolic status (pH and bicarbonate) and lactate concentration (the most frequently used marker of tissue perfusion in human medicine)[18-19] were assessed by blood gas analysis, performed with a blood gas analyzer (ABL 800 Flex, Radiometer Medical ApS, Brønshøj, DK), every 8–12 hours until the resolution of DKA. The degree of ketosis was quantified every 4 hours by measuring blood BHB using the same PBGM (using ketone test strips), previously validated for dogs.[16]

Accuracy of the FGMS

Analytical and clinical accuracy was evaluated by comparing the results of the PBGM measurements and those obtained using the FGMS.

Analytical accuracy was determined by calculating the mean *absolute relative* difference (MARD), median absolute relative difference (mARD), mean relative difference (MRD), and mean absolute difference (MAD). All these are measures of the average difference between sensor and reference values. MARD and mARD measure the size but not the direction (higher/lower) of the differences compared with the reference (*absolute*) as a percentage of the reference value

(*relative*). MAD is similar, but just reports the size of the difference (it is not reported as a percentage), and is commonly used to assess accuracy at low glucose levels (BG < 100 mg/dL). MRD measures the size and direction of the difference compared with the reference as a percentage of the reference value.[20] MARD has traditionally been used to assess the accuracy of CGMSs, representing it as a single numeric value.[21] MARD or mARD should be < 14%; a value > 18% is considered to represent poor accuracy.[22]

Secondly, analytical accuracy was estimated based on ISO 15197:2013 criteria, which state that at least 95% of results have to be within ± 15 mg/dL of the BG value for glucose <100 mg/dL and within ± 15% of the BG value for glucose ≥ 100 mg/dL.

Clinical accuracy was evaluated using ISO 15197:2013 criteria, which state that 99% of the measured glucose values should fall within zones A and B of the Parkes Consensus Error Grid analysis for type 1 DM.[23]

The effect of BCS and influence of changes in metabolic variables (lactate, β-hydroxybutyrate, pH, and bicarbonate) on sensor performance were evaluated to investigate whether specific patient metabolic parameters influenced the accuracy of the device during the resolution of DKA.

Statistical analysis

Normality was assessed with the Shapiro–Wilk test and non-parametric tests were used accordingly. The correlations between IG measured by the FGMS and BG measured by the PBGM were evaluated with Spearman's rank correlation; the differences were illustrated in Bland–Altman plots. The effect of BCS on the accuracy of the device was assessed using the Kruskal–Wallis test by dividing the population into 3 groups: BCS ≤ 3, BCS 4–6, and BCS ≥ 7. Sensor performance during changes in metabolic variables (lactate, β-hydroxybutyrate, pH, and bicarbonate) was evaluated using Spearman's rank correlation. Statistical analysis was performed using commercially available software (GraphPad Prism 5, GraphPad Software Inc., San Diego, CA), and a *P*-value < 0.05 was considered significant.

RESULTS

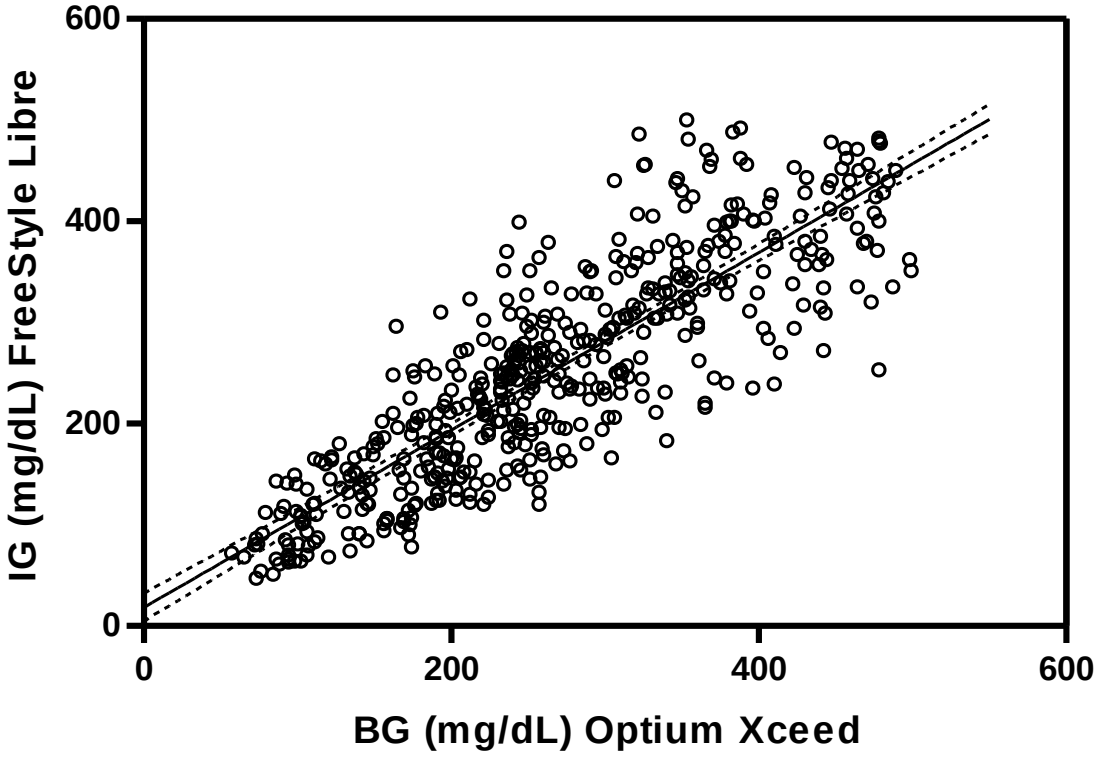
Fourteen dogs were included in the study. The application of the FGMS was carried out within 14 hours from the presentation of each patient, and it appeared to be painless, was easy to

measure, and was well tolerated by all dogs. The sensor has a 1-hour period of initialization and in all dogs the sensor read the IG concentrations after 60 minutes of application, as reported by the manufacturer. There were no relevant adverse events recorded during the use of the FGMS; only in one dog was a mild erythema noted at the site of the application of the sensor at the end of the wearing period, which spontaneously resolved within the next 24 hours.

Data were collected from each patient for a minimum of 3 days and up to 14 days (median, 5.5 days). Considering all the samples obtained, the median pH and bicarbonate values during DKA were 7.27 (range, 7.03-7.4) and 14.9 mmol/L (7.8-23.3 mmol/L), respectively; the median lactate value was 1.2 mmol/L (0.5-2.8 mmol/L). The median BHB value throughout the hospitalization was 1.7 mmol/L (0.1-7.5 mmol/L).

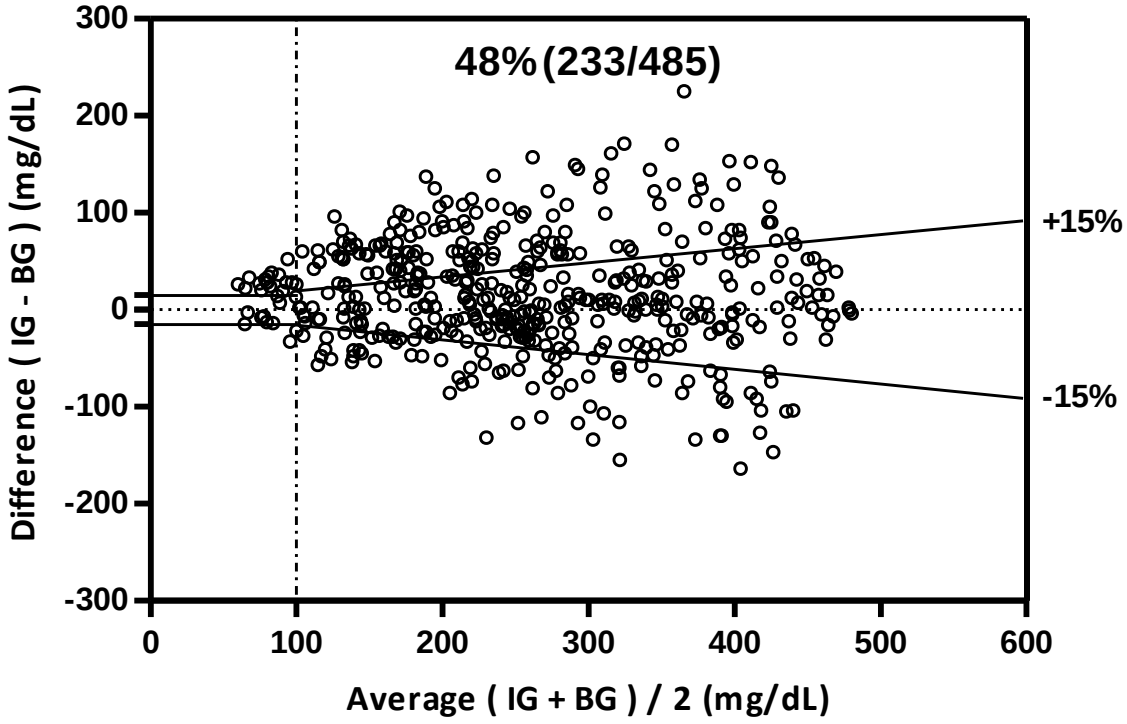
A total of 485 paired glucose measurements were available for analysis. The median BG value measured by the PBGM was 252 mg/dL (57-499 mg/dL); the median IG value measured by the FGMS was 245 mg/dL (47-500 mg/dL). Good agreement between IG measurements and BG was obtained (r = 0.86; slope = 0.88, intercept =18.37 mg/dL, r^2 = 0.72), as shown in Fig 2.

Figure 2: Linear regression. Solid line = regression line; dashed lines = 95% CI. BG = blood glucose measurements obtained by the portable glucometer; IG = interstitial glucose measurements obtained by the flash glucose monitoring system (FGMS).



Overall MARD was 18.9%, mARD was 16.6%, MRD was -4.4%. In the low glucose range (BG < 100 mg/dL, *n* = 26), MAD was 24.9 mg/dL; in the higher glucose range (BG ≥ 100 mg/dL, *n* = 459), MARD was 18.4%. The percentage of values within ± 15 mg/dL of the BG value for glucose < 100 mg/dL and within ± 15% of the BG value for glucose ≥ 100 mg/dL was 48% (Fig 3).

Figure 3: The Bland–Altman plot represents the difference between interstitial glucose (IG) measurements obtained by the use of the FGMS versus blood glucose (BG) concentrations obtained by a portable glucometer (Optium Xceed, Abbott, UK). The requirements established by ISO 15197:2013 criteria are represented by the two solid symmetrical lines: at ± 15 mg/dL from the reference value for glucose < 100 mg/dL and at ± 15% from the reference for glucose ≥ 100 mg/dL. The percentage at the top expresses the number of samples within limits for the total number of measurements.



The clinical accuracy of the FGMS was demonstrated, with 63.9% of results in zone A and 99.8% of results in zones A and B (Fig 4).

Figure 4: Parkes consensus error grid analysis (EGA) representation with the percentage of values within different zones. The reference glucose values (BG obtained by a portable glucometer), on the x axis, are plotted against the IG measurements obtained by the FGMS, on the y axis. The different zones designate the magnitude of risk: no effect on clinical action (zone A), altered clinical action—little or no effect on the clinical outcome (zone B), altered clinical action—likely to affect the clinical outcome (zone C), altered clinical action–could have a significant medical risk (zone D), and altered clinical action—could have dangerous consequences (zone E). ISO 15197:2013 requires that 99% of the values fall within zones A+B for a device to be considered accurate.

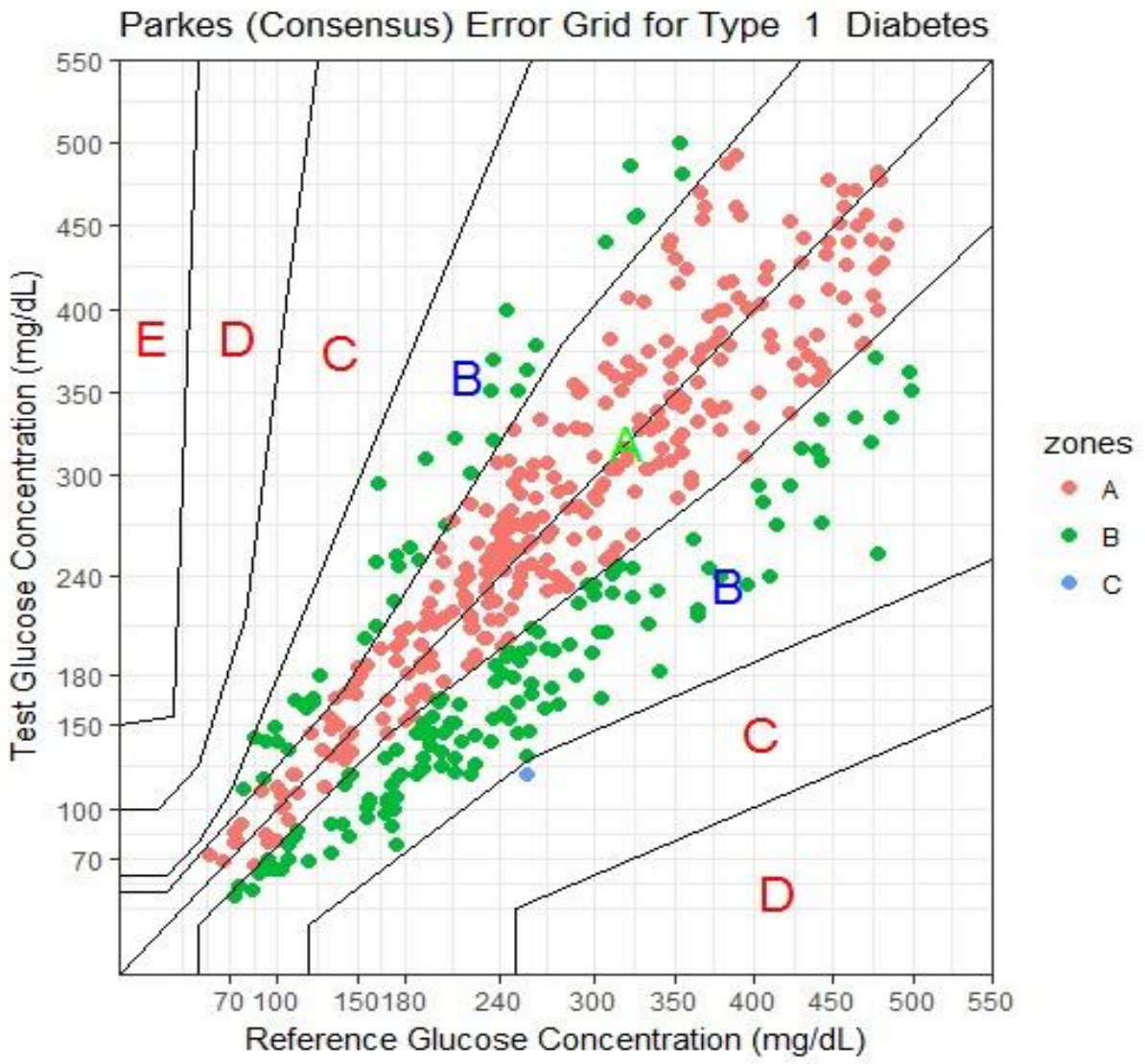


Figure 5 shows the distributions of the differences between IG measurements obtained with the FGMS and BG obtained with the PBGM for each patient. A significant inter-patient variability in the accuracy of the device was observed (Kruskal–Wallis test, $P < 0.0001$), suggesting that in some patients the device was more accurate than in others. The FGMS tends to overestimate the glucose level in dogs with BCS ≤ 3 and to underestimate in dogs with BCS ≥ 7 (Fig 6).

Figure 5: Interpatient variability (D = dog). Each patient is represented on the x axis with box and whisker plot. The y axis represents the relative difference defined as (IG – BG). IG = interstitial glucose; BG = blood glucose.

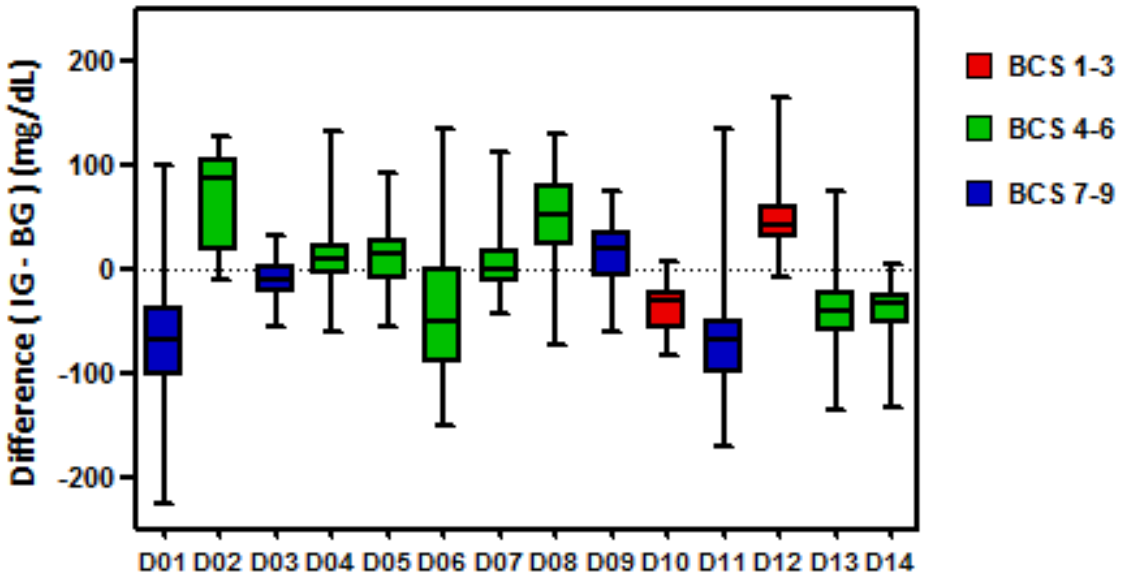
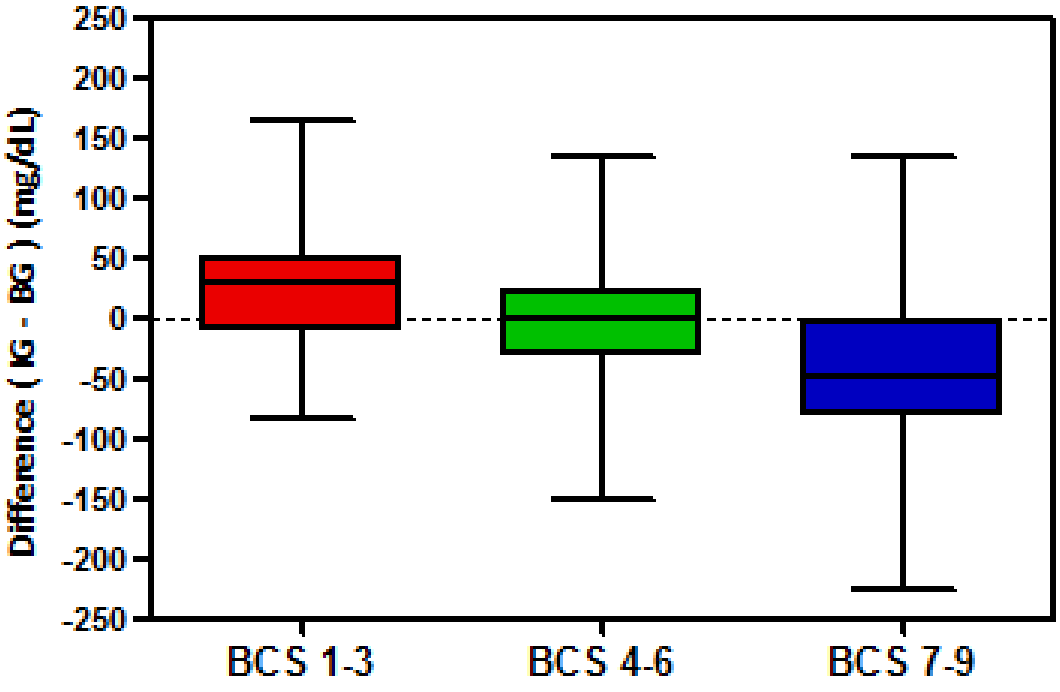


Figure 6: Kruskal–Wallis box plots comparing the effect of BCS on the accuracy of the FGMS. The x axis represents patients divided into three groups: BCS ≤ 3 (red); BCS 4–6 (green), and BCS ≥ 7 (blue). The y axis represents the relative difference defined as (IG – BG). IG = interstitial glucose; BG = blood glucose.



Variations in lactate, β-hydroxybutyrate, pH, and bicarbonate that occurred during the resolution of DKA did not affect sensor performance (Table 1).

Table 1: Spearman's rank correlation results regarding the effect of changes in metabolic variables on the accuracy of the FGMS.

Metabolic variable	*n*	Spearman's *r*	95% CI	*P*
Lactate	52	-0.044	-0.321 to 0.239	0.76
β-hydroxybutyrate	135	-0.131	-0.298 to 0.044	0.13
pH	53	0.239	-0.042 to 0.484	0.09
Bicarbonate	53	0.192	-0.091 to 0.446	0.17

DISCUSSION

This study is the first to evaluate the clinical use and performance of the FGMS in ketoacidotic dogs and has shown that its use results in the accurate measurements of IG, reflecting BG concentration.

DKA is an endocrine emergency that has a high mortality rate if improperly managed. Complications induced by its treatment are common, and usually result from overly aggressive therapy, inadequate animal monitoring, and failure to reevaluate biochemical parameters in a timely manner.[5] Glycemic monitoring is a cornerstone for the management of treatment of DKA, and currently is performed using PBGMs. The main limitations include the cost of test strips and the requirement for repeated capillary blood sampling, which can be a source of stress and pain in some dogs, and therefore only provides single snapshots of glucose concentrations. In particular nocturnal and asymptomatic episodes can be missed as well as dynamics in BG concentrations may be missed and not factored into treatment decisions.

In ketoacidotic patients, accurate continuous glucose monitoring is the best way to avoid a rapid decrease in the BG concentration (which can result in cerebral edema and hypoglycemia), allowing correct management of insulin therapy. To our knowledge, there are no studies in human medicine that have investigated the influence of changes in metabolic variables during DKA on the accuracy of a CGMS. However, the results from studies investigating continuous glucose monitoring performance during intensive care have indicated that low pH, elevated lactate levels, and the use of vasoactive drugs do not compromise the agreement between BG and IG measurements.[24-26] A prospective study in veterinary medicine, that evaluated the effects

of hydration, body condition score (BCS), measures of perfusion (Doppler blood pressure, lactate and rectal-axillary temperature difference), and severity of ketosis on the performance of a continuous IG monitoring system (CGMS Gold, Medtronic Minimed, CA) in dogs and cats with DKA, found only a weak association between hydration and the accuracy of the measurements, with the device being more accurate in more hydrated patients.[11] The results suggest that this device is a clinically useful tool for monitoring BG concentration in critically ill patients, but it has a number of disadvantages, including the initial cost of the device, the cost of the sensor, the need to obtain blood samples for calibrations every 8–12 hours. The most significant limitation is that glucose measurements are only available retrospectively, after downloading the data onto a personal computer, thereby limiting its clinical usefulness in the management of hospitalized patients.[11] The factory-calibrated FGMS overcomes these limitations, and at the same time provides similar accuracy to systems requiring calibration. Indeed, in our study, the correlation between IG and BG concentrations (r = 0.86) was the same as that obtained by Reineke et al. (2010) in their study of the performance of a CGMS.[11] Our results compare favorably with an earlier veterinary study of the accuracy of the FGMS in stable diabetic dogs, which found a slightly stronger correlation, with peripheral glucose measured by the hexokinase method (r = 0.94).[15]

Parkes EGA showed acceptable clinical accuracy, with 99.8% of the FGMS readings in zones A and B, similar to reported rates of 99.5% in a human study using the FGMS to monitor critically ill patients with diabetes,[27] and of 99% in a previous veterinary study evaluating the use of a CGMS in dogs and cats with DKA.[11] In the latter study, the only measure of the average difference between sensor and reference values calculated was the median absolute percent difference (APD), which was the same as the mARD in our study; we found a mARD of 16.6%, while Reineke et al. (2010) found a median APD of 9.5%.[11] The percentage of results within ± 15 mg/dL of the BG value for glucose < 100 mg/dL and within ± 15% of the BG value for glucose ≥ 100 mg/dL was 48%, and therefore analytical accuracy, based on ISO 15197:2013 requirements, was not obtained.

In our study, we did not evaluate if the accuracy of the FGMS was stable over the duration of the wearing period because most of the dogs were discharged too soon to allow this comparison to be made. In stable diabetic dogs, Corradini et al. (2016) found a significant variation in the mean difference between 3 time periods (days 1–2, days 6–7, and days 13–14).[15] The authors attributed part of this difference to the inflammatory response to sensor insertion, which affects

glucose concentrations in interstitial fluid.[28] In our study, one dog showed mild erythema at the site of the sensor, which could be related to the patch used to ensure the adhesion of the device to the cutaneous surface; however an allergic contact sensitization caused by the device cannot be ruled out.[29] In a study of humans, mild skin issues, including itching, erythema, edema, rash, induration, bruising, and bleeding, were observed in less than 9% of cases.[30]

Fig 5 indicates that there was a significant inter-patient variability in the accuracy of the FGMS, as observed in studies evaluating CGMS in stable diabetic dogs, and in dogs and cats with DKA.[11,31] To investigate if specific patient factors or metabolic variables could account for the difference noted in the accuracy of the FGMS estimates of BG concentration, we evaluated BCS, lactate level, severity of ketosis, and acidosis in each subject. The variables lactate, β-hydroxybutyrate, pH, and bicarbonate did not appear to be associated with the accuracy of the measurements; for BCS, a tendency to overestimate the glucose level in dogs with BCS ≤ 3 and to underestimate in dogs with BCS ≥ 7 was noted. In humans, an increasing clinical accuracy for the FreeStyle Navigator (Abbott, UK), which uses a sensor with the same wired enzyme technology, was found for participants who had a body mass index (BMI) > 30 kg/m^2 (84.4% in zone A) compared to participants with BMI < 25 kg/m^2 (78.8% in zone A). The authors supposed these findings could be attributed to differences in blood flow relative to subcutaneous adipose tissue thickness.[32] Conversely, another recent study that evaluated the FreeStyle Libre did not find any correlation with BMI.[30] In this study, only 2/14 and 4/14 dogs had BCS ≤ 3 and BCS ≥ 7, respectively; with such low numbers, a statistical comparison was not valid.

In our study, the thickness of the skin at the site of application of the sensor was not evaluated, making it impossible to assess its influence on the accuracy of the device. Other limitations include the use of a single sensor for each dog, so the precision of the FGMS was not investigated. The sensor was placed at a single body site (the dorsal part of the neck, an area not particularly subject to tractions and traumas), not allowing evaluation of the application site as a variable that might influence the accuracy of the device.

In our study, capillary and venous BG values, obtained by a validated PBGM and not with the classical reference method (hexokinase), were used as a reference to evaluate the accuracy of the FGMS. FreeStyle Libre is an interstitial glucose monitoring system, intended to be a replacement for the capillary BG measurement, and therefore capillary BG may be considered an appropriate comparison in evaluating the performance and accuracy of this factory-calibrated

system. In addition, capillary BG provides more reference points and represents real-life accuracy during clinical practice.[30]

The FreeStyle Libre is unique among existing interstitial glucose monitoring technologies in that the wired enzyme factory-calibrated sensor has a wearing time of up to 14 days without additional calibration, which represents a potential advantage as errors in PBGM could possibly lead to glucose monitoring errors. Moreover, the FGMS provides results across a wide range and numerous readings during a 24-hour period that can be used to evaluate glucose patterns and trends as the hand-held reader displays the previous 8-hour history. This ability to foresee and avoid impending hyperglycemic and hypoglycemic events in critically ill ketoacidotic patients could potentially improve both morbidity and mortality in patients. The maximum upper range of 500 mg/dL is appropriate for dogs with DKA, in which intensive glycemic control (glycemia around 250 mg/dL) is usually the goal of treatment.

In conclusion, although the ISO15197:2013 requirements were only partially fulfilled, the novel FGMS provides accurate estimates of BG compared with PBGM, and represents a clinically useful device to monitor BG concentration in critically ill hospitalized dogs with DKA. Imbalances of the acid-base status and lactate level seem to exert no influence on the accuracy of the sensor, making it suitable not only for stable diabetic dogs, but also for dogs with DKA. Future studies evaluating the effect of BCS on the performance of the FGMS are warranted.

6. Accuracy of a Flash Glucose Monitoring System in dogs with diabetic ketoacidosis

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Capitolo 7

USE OF 3-β-HYDROXYBUTYRATE IN THE TREATMENT OF CANINE DIABETIC KETOACIDOSIS

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7. Use of 3-β-hydroxybutyrate in the treatment of canine diabetic ketoacidosis

BACKGROUND

Diabetic ketoacidosis (DKA) is a severe life-threatening complication of diabetes mellitus. To date, in veterinary medicine, urinary acetoacetate (AcAc) is the most commonly used parameter for monitoring dogs with DKA. Urine dipstick test provides a semiquantitative measure of urinary AcAc but does not register the presence of urinary 3-beta-hydroxybutyrate (3-HB), the predominant ketone body. In human medicine, several studies have demonstrated that 3-HB is oxidized back to AcAc during resolution of DKA. As a result, ketonuria may remain positive once ketosis has been reverted and it may give the misleading impression that ketosis is not improving. Another study in human diabetic patients demonstrated that the use of 3-HB as end-point for the intravenous insulin therapy is simple and earlier compared to the use of AcAc (our current end-point).[1]

OBJECTIVES

The aim of this study was to evaluate a new end-point for intravenous insulin therapy in the treatment of DKA in dogs.

MATERIALS AND METHODS

Dogs with DKA presented at the Veterinary Teaching Hospital of University of Bologna between June 2011 and April 2016 were prospectively enrolled in the study. The inclusion criteria for DKA were the following: blood glucose ≥250 mg/dL, ketonemia (3-HB ≥3.0 mmol/L) and/or ketonuria (urinary AcAc ≥1+), metabolic acidosis (pH <7.3 or bicarbonate <15 mEq/L) and at least two clinical signs consistent with DKA. All patients were treated with fluid therapy, a continuous rate infusion (CRI) of low dose of regular insulin[2] and miscellaneous treatments for concurrent disorders. Each patient was monitored closely (Table 1). Dogs were divided into two groups, 3-HB group and AcAc group, and two different end-points for intravenous insulin therapy were used. In the 3-HB group the CRI of insulin was stopped when pH >7.3 and two 3-HB measurements (evaluated in one hour apart) were <1 mmol/L; while in the AcAc group the CRI of insulin was stopped when pH >7.3 and absence of ketonuria has been recorded. Statistical analysis was performed using non parametric tests. A p value <0.05 was considered significant.

Parameter	Time interval in the first 24 h	Time interval in the next 24 h and until the end-point	Time interval after the end-point
Capillary blood glucose	Every h	Every 2 h	Every 2-3-4 h
Capillary 3-HB	Every 4 h	Every 4 h	Every 4-12 h
Urinary AcAc	Every 6 h	Every 6 h	Every 6-12 h
Blood gas analysis	Every 8 h	Every 12 h	Every 24 h

Table 1: Monitoring protocol used for diabetic ketoacidosis.

RESULTS

Twenty dogs met the inclusion criteria; ten were included in the 3-HB group and ten in the AcAc group. The two groups resulted homogeneous regarding breed, sex, age, body weight, glucose concentration, 3-HB concentration, urinary AcAc, pH, bicarbonate concentration, anion-gap and presence or absence of concomitant disorders at the time of diagnosis. The median time of CRI of insulin in the 3-HB group was 44 h, in the AcAc group was 36 h (Figure 1).

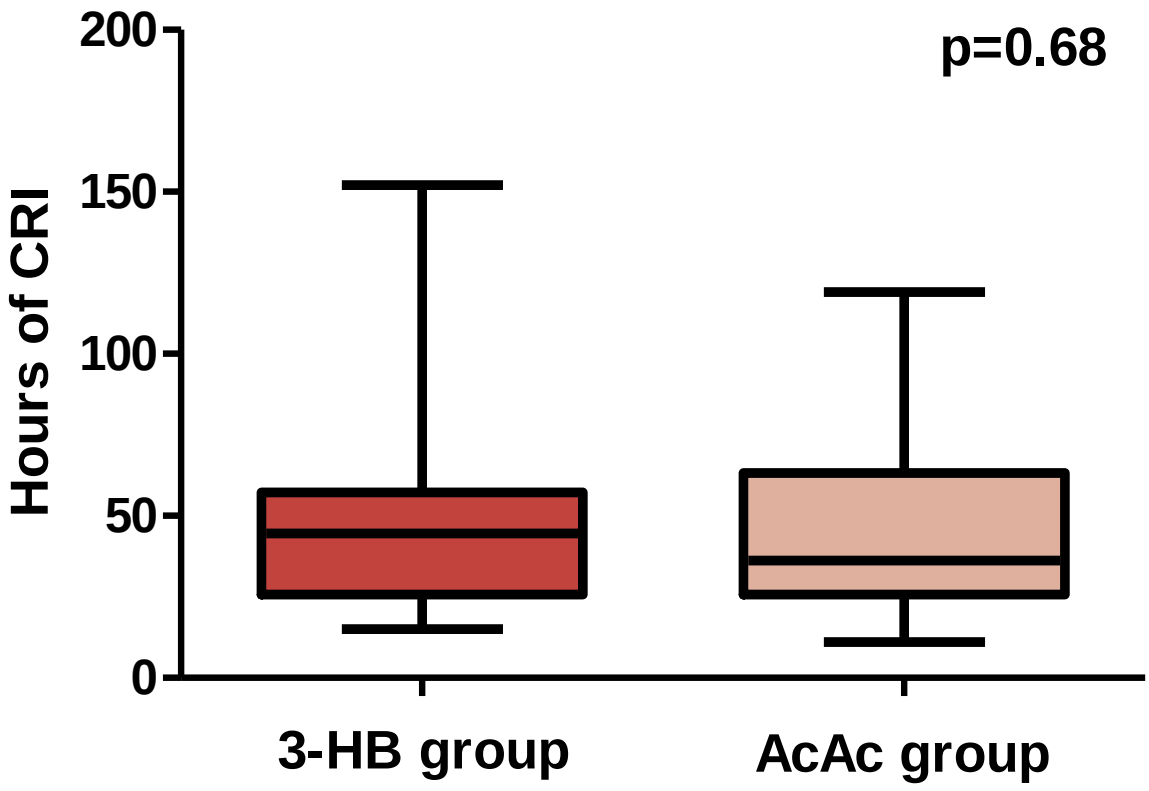

Figure 1: Box plots comparing the median time of constant rate infusion (CRI) of insulin between 3-HB group and AcAc group. The horizontal lines of the box represent the 25th, 50th (median) and the 75th percentiles. Outlying horizontal lines of the box represent minimum and maximum values.

The median time of hospitalization was 144 h in the 3-HB group, and 156 h in the AcAc group (Figure 2). The differences were not significantly different.

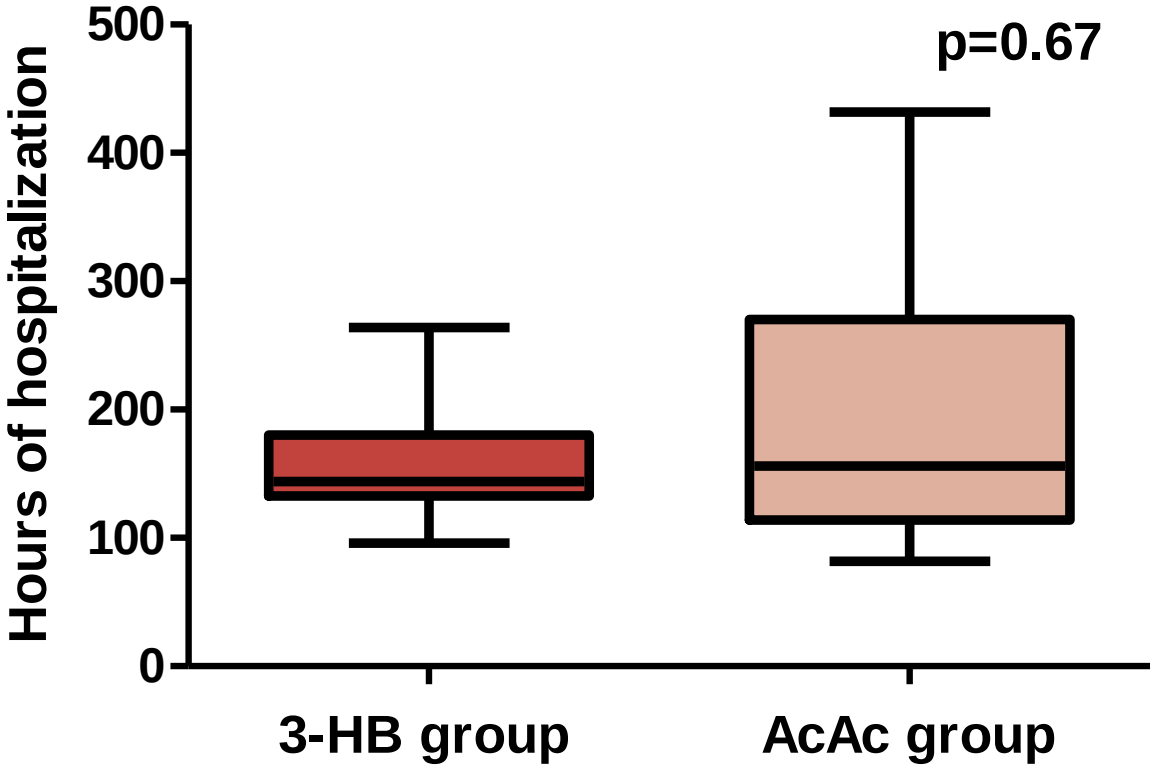

Figure 2: Box plots comparing the median time of hospitalization between 3-HB group and AcAc group. The horizontal lines of the box represent the 25th, 50th (median) and the 75th percentiles. Outlying horizontal lines of the box represent minimum and maximum values.

DISCUSSION

The results show that the use of 3-HB in monitoring dogs with DKA does not reduce the hours of CRI of insulin if compared with urinary AcAc. However, the measure of 3-HB is a quick and easy procedure for samples collection and therefore, although not substantiated by the study results, the authors recommend the use of 3-HB in monitoring dogs with DKA.

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Capitolo 8

DISCUSSIONE E CONCLUSIONI

La DKA è una emergenza endocrina che, quando inappropriatamente gestita, può associarsi ad un elevato rischio di mortalità per l'intervenire di complicazioni in genere conseguenti ad una terapia troppo aggressiva, ad un monitoraggio clinico inadeguato, oppure all'impossibilità di rivalutare sistematicamente alcuni parametri laboratoristici.

Nel **Capitolo 3** è riportato lo studio effettuato allo scopo di confrontare l'efficacia e la sicurezza dell'infusione endovenosa lenta di insulina cristallina regolare (gruppo R) o di insulina Lispro (gruppo L) nei gatti con DKA. Non sono state evidenziate differenze statisticamente significative nei tempi mediani di risoluzione dell'iperglicemia, della chetosi e dell'acidosi metabolica tra i due gruppi di trattamento. Durante l'infusione endovenosa di insulina, due gatti appartenenti al gruppo R avevano subito un episodio di ipoglicemia asintomatica; un gatto nel gruppo L e tre gatti nel gruppo R avevano sviluppato una grave ipofosfatemia, che aveva richiesto una supplementazione endovenosa di fosfati. I risultati di questo studio dimostrano che l'impiego dell'insulina Lispro in infusione endovenosa nei gatti in DKA è associato a minori effetti collaterali e alla stessa efficacia rispetto all'insulina cristallina regolare.

È importante ribadire che l'insulina Lispro, così come gli altri analoghi insulinici, sono stati concepiti per l'impiego per via sottocutanea, essendo questa la via di somministrazione che consente di sfruttarne le qualità e i vantaggi derivanti dalla loro struttura molecolare. Pertanto, abbiamo previsto che, a questo nostro studio preliminare, faccia seguito un progetto che valuti l'efficacia e la sicurezza di un nuovo protocollo insulinico basato sull'impiego dell'insulina Lispro per via sottocutanea/intramuscolare (in funzione dello stato di idratazione del paziente) in cani e gatti con DKA.

L'efficacia della terapia insulinica è dimostrata da un buon controllo della glicemia e dalla risoluzione dello stato di chetosi. Il monitoraggio di questi parametri è fondamentale per effettuare le opportune modifiche al protocollo insulinico e per ottenere il successo terapeutico. Nei **Capitoli 4** e **5** sono riportati due studi che indagano l'accuratezza e la precisione di un glucometro (Gluco Calea, WellionVet; GC) e di un glucometro/chetometro (Belua, WellionVet; BE) ad uso umano nelle specie canina e felina, valutando anche l'interferenza esercitata dal packed cell volume (PCV). Sono stati impiegati campioni appartenenti a soggetti non anemici (cane PCV 37-54%; gatto PCV 30-47%) e anemici (cane PCV< 37%; gatto PCV< 30%) classificati in 3 range glicemici: alto (>140 mg/dL), medio (90-139 mg/dL) e basso (<90 mg/dL). I valori di glicemia e di 3-β-idrossibutirrato (3-HB) ottenuti con l'impiego dei due dispositivi, sia da sangue capillare che venoso, sono stati comparati col

corrispondente valore ottenuto con la metodica di riferimento. La precisione è stata valutata esaminando la ripetibilità del risultato *within-run* e *between-day*. Sia nel cane che nel gatto è stata individuata una correlazione significativa tra i valori di glicemia ottenuti con ciascuno dei due glucometri e la metodica di riferimento (r >0,89); inoltre è stato dimostrato che il PCV esercita un'influenza significativa sull'accuratezza dei dispositivi, misurando valori di glicemia più elevati quando il PCV è più basso; infine, la precisione è risultata adeguata per entrambi i dispositivi. Tuttavia, nella specie canina, nessuno dei due dispositivi soddisfaceva pienamente i requisiti della norma ISO, con una percentuale di valori che cadevano all'interno delle zone A+B della Parkes error grid analysis tra l'82,2% e il 97,8%. La comparazione tra i valori di 3-HB ottenuti con il chetometro BE, da sangue capillare e periferico, e quelli ottenuti con la metodica di riferimento ha dato luogo a delle correlazioni rispettivamente di r=0,48 e r=0,59. In conclusione, nessuno dei due dispositivi è sufficientemente accurato da consentirne un utilizzo sicuro nel cane **(Capitolo 4)**.

Per quanto concerne la specie felina, anche in questo caso, nessuno dei due dispositivi soddisfaceva pienamente i requisiti della norma ISO, nonostante il 100% delle misurazioni ottenute con il BE da sangue periferico cadesse all'interno delle zone A+B della Parkes error grid analysis. La comparazione tra i valori di 3-HB ottenuti con il chetometro BE, da sangue capillare e periferico, e quelli ottenuti con la metodica di riferimento ha dato luogo a delle correlazioni rispettivamente di r=0,66 e r=0,82. In conclusione, il glucometro GC non è sufficientemente accurato; diversamente il BE ha mostrato delle performance superiori che ne supportano l'impiego clinico nel gatto **(Capitolo 5)**.

Nel **Capitolo 6** è riportato lo studio che valuta le performance di un dispositivo che misura il glucosio interstiziale in maniera continuativa, il FreeStyle Libre, nei cani con DKA, indagando l'interferenza esercitata dal body condition score (BCS), dalla lattatemia, dalla gravità della chetosi e dell'acidosi metabolica sull'accuratezza dello strumento. Dalla comparazione tra i valori di glucosio interstiziale misurati con il FreeStyle e i relativi valori di glicemia ottenuti mediante l'utilizzo di un glucometro portatile (Optium Xceed, Abbott, UK) si è ottenuta una buona correlazione (r=0.86). Tuttavia, secondo i criteri stabiliti dalla normativa ISO 15197:2013, il dispositivo non ha un'accuratezza analitica sufficiente, mentre ne è stata dimostrata l'accuratezza clinica, con il 99,8% dei risultati all'interno delle zone A e B della Parkes Consensus Error Grid. L'analisi dei dati raccolti ha evidenziato una significativa variabilità dell'accuratezza tra i pazienti, mostrando una tendenza dello strumento a sovrastimare i valori di glucosio nei cani con BCS ≤3 ed a sottostimarli nei pazienti con BCS ≥7. Diversamente, le modificazioni nel tempo delle variabili metaboliche non hanno interferito sulle performance del

dispositivo. In conclusione, sebbene il FreeStyle non abbia rispettato pienamente i criteri ISO, la sua accuratezza clinica, non compromessa dalle variabili metaboliche, ne supporta l'impiego nei cani con DKA, anche se l'effetto esercitato dal BCS sulle performance merita ulteriori indagini.

La scelta del criterio da utilizzare per decidere quando interrompere l'infusione endovenosa di insulina è ancora oggetto di controversie in medicina veterinaria. Lo studio che mette a confronto l'acetoacetato (AcAc) urinario e il 3-beta-idrossibuttirato (3-HB) ematico come endpoint della terapia insulinica nei cani con DKA è riportato nel **Capitolo 7.** I risultati delle analisi statistiche effettuate non hanno evidenziato differenze statisticamente significative nella durata mediana dell'infusione endovenosa di insulina (P=0,68) e dell'ospedalizzazione (P=0,67) tra i due gruppi. Il nostro studio dimostra che l'impiego del 3-HB ematico come endpoint della terapia insulinica nei cani in DKA, comparato con l'AcAc urinario, non riduce la durata dell'infusione e dell'ospedalizzazione. Tuttavia, trattandosi di un parametro più veloce e semplice da monitorare, ne raccomandiamo comunque l'impiego come strumento di monitoraggio della terapia della DKA in sostituzione all'AcAc urinario **(Capitolo 7)**.