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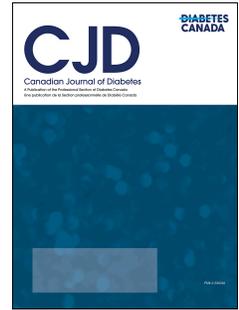
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Running Head: IDIOGRAPHIC INVESTIGATION OF DIABETIC ALERT DOGS

An idiographic investigation of diabetic alert dogs' ability to learn from a small sample set

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Key messages

- Dogs are trained to detect an odour signaling hypoglycemia in people with T1D
- No research examines the generalizability of this odour training in dogs
- This study shows that one dog transferred the odour of hypoglycemia across multiple breath samples from one person

Keywords: hypoglycemia, diabetic alert dog, canine olfaction

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Abstract

Objective: There is a growing market for diabetes alert dogs but little published regarding their ability to reliably detect hypoglycemia. We aimed to determine whether two dogs could detect hypoglycemic breath samples from people with type 1 diabetes (T1D) and then transfer detection to novel hypoglycemic breath samples.

Methods: Breath samples were collected from individuals with T1D during times of normo-, hypo- and hyperglycemia. Two dogs, previously trained (3 alternative forced choice) with breath samples from three different individuals with T1D, were presented with three breath samples from the same individual: one hypoglycemic, one normoglycemic, and one hyperglycemic, and trained to identify the hypoglycemic sample using a Yes/No procedure. The dogs' ability to transfer detection was then tested by presenting them with a novel sample set from the same individual. Then we tested whether one dog could transfer detection of the odour of hypoglycemia by presenting new samples from a different individual.

Results: One dog was able to transfer detection of the odour of hypoglycemia to samples from the same individual (Specificity 89%; Sensitivity 62%), but a second dog was not. Results were inconclusive regarding the ability of one dog to transfer detection of the odour of hypoglycemia across two individuals.

Conclusions: The results suggest that some dogs can be trained to detect hypoglycemic breath of an individual with T1D but detection may not transfer to novel samples from other individuals.

Results should be interpreted cautiously since the dogs were trained with a small number of breath samples before testing.

Introduction

Type 1 diabetes (T1D) is one of the most common chronic diseases in children [1]. Hypoglycemia is the most common acute complication of the disease, and is of particular concern for individuals lacking hypoglycemia awareness due to age or frequent hypoglycemic episodes [2]. If left untreated, severe hypoglycemic episodes can result in a loss of consciousness, a seizure, coma, or death [3]. Given the potential severity of hypoglycemia, individuals with T1D can develop a fear of hypoglycemia that negatively affects their management of diabetes, psychological well-being, and quality of life [4, 5]. Therefore, timely and accurate detection of hypoglycemia is imperative for individuals with T1D.

Individuals with T1D may self-monitor their blood sugar levels with home blood glucose meters or they may use newer technologies such as continuous glucose monitors (CGM). CGM may be linked to continuous subcutaneous insulin infusion (CSII) pumps with functionality to suspend insulin infusion functions during hypoglycemia [1, 6]. Use of these technologies can result in improved glycemic control but reports of their effect on the incidence of hypoglycemia are contradictory [6,7]. Some individuals choose not to use, or to discontinue use of these technologies because they are costly, can be uncomfortable to wear and may increase the likelihood of infection at insertion and tape sites [8,9]. Moreover, individuals using CSII may see these devices as a constant reminder of their disease, which can contribute to social impairments and a feeling of social stigmatization [10]. The limitations of current technologies have prompted research examining the efficacy of a potentially more user-friendly hypoglycemia detection

Abbreviations

CGM: Continuous Glucose Monitor

CSII: Continuous Subcutaneous Insulin Infusion

DADs: Diabetic Alert Dogs

PET: Poly(ethylene terephthalate)

PVC: Polyvinyl Chloride

T1D: Type 1 diabetes

VOCs: Volatile Organic Compounds

system: trained domestic dogs.

Reports of dogs behaving abnormally when their owners become hypoglycemic provide evidence that dogs may be able to detect hypoglycemia in their owners [11-13]. Following such reports, organizations throughout North America and the United Kingdom have begun to train Diabetic Alert Dogs (DADs). DAD owners report a decrease in the frequency of hypoglycemic episodes, less fear of hypoglycemia and a higher quality of life [14]. Despite the reported successes of DADs, empirical science elucidating how dogs detect hypoglycemia is minimal and inconclusive.

Given the incredible olfactory acuity of dogs [15, 16], it is believed that dogs who detect hypoglycemia are detecting volatile organic compounds (VOCs) corresponding with a physiological change in their owner. When human body cells undergo changes due to metabolic fluctuations, disease, or infection, they release compounds that become dissolved in the blood and are volatilized from the pulmonary circulation. VOCs are then emitted in breath and sweat [17] in concentrations of parts per billion (nmol/mol) and parts per trillion (pmol/mol) [18]. Analytical lab research shows that diabetic hypoglycemia may be correlated with a specific VOC profile [19-22] and since dogs have been shown to detect odours at concentrations of parts per trillion [23], it is possible that hypoglycemia-detecting dogs are detecting these changes in VOC profiles. Working under this premise, two research teams have tested dogs' ability to detect hypoglycemia in sweat and breath.

In 2013, Dehlinger et al. [24] tested whether three previously trained DADs could identify hypoglycemia in sweat samples from three individuals unknown to the dogs and found that none of the dogs could indicate the hypoglycemic samples at above chance levels. Hardin et al. [25] collected combined sweat and breath samples from four individuals with T1D during

hypoglycemic and normoglycemic states. After training six dogs to signal to the hypoglycemic samples, they tested the dogs' ability to locate the hypoglycemic samples in a seven-station line-up. The dogs could detect the hypoglycemic samples at above chance levels, most with a high degree of sensitivity and specificity. Importantly though, in this study dogs were presented with a small number of samples that they had previously been rewarded for indicating during training. Therefore, it is possible that they simply memorized the odour profile of the individual samples, rather than identifying a hypoglycemia-specific odour that is consistent across individuals.

Taken together, these results present inconclusive evidence as to whether dogs can be trained to detect hypoglycemia. The goal of this study, therefore, was to determine whether dogs could detect hypoglycemic breath samples from one person and then transfer detection to novel samples from the same person and a different person.

Methods

Participants

People with T1D were recruited through the Pediatric Diabetes Clinic at the IWK Health Centre in Halifax, NS, Canada and by word of mouth. Participants were required to have been diagnosed with T1D at least six months prior to participation, to have had no episodes of hypoglycemia unawareness in the past six months, and in the case of children, to have sufficient parental support to complete the procedures.

Families that participated in the study received \$15 for the initial visit during which consent was obtained, and kits and instruction on sample collection were provided. A \$10 gift card was issued following collection of record sheets and samples. Participants were given the opportunity to participate in a second phase of sample collection, after which they received a second gift card valued at \$10.

Breath samples

After in person training, participants were given sample collection kits that contained instructions for how to collect the samples. Participants were asked to collect six breath samples in total; during two different hypoglycemic events (defined here as a blood glucose of less than 4 mmol/L on their home glucose monitor with hypoglycemic symptoms), during two different hyperglycemic events (higher than 14 mmol/L), and during two different times of “normoglycemia” (between 5-10 mmol/L).

The breath collection tubes were PET [poly(ethylene terephthalate) cylindrical tubes (Uline.ca)], measuring 20.5 cm in length and 7 cm in diameter, inside which were silicone oil coated cotton balls, used to capture an increased variety and amount of breath VOCs stored over time [26]. Each breath collection tube was labeled for the sample type (e.g. hypoglycemic sample #1) and both ends of the tubes were capped with a tight-fitting plastic cap. Samples were stored between two and four weeks before presentation to the dogs.

Participants were instructed to collect breath samples after performing their regular blood glucose checks. Participants removed the plastic caps on both ends and exhaled two deep breaths through one side of the tube, an additional two deep breaths through the opposite end of the tube, and then replaced the caps and stored the collected breath sample at room temperature, inside an insulated bag provided. Previous work by Reeve et al. [26] demonstrates that samples stored using this procedure are detectable by dogs for at least four weeks. Afterward, participants completed the sample information sheet with the date, time, and their blood sugar level at the time the sample was collected. Completed sample sets were returned to the clinic or picked up by researchers. If participants collected and returned all six samples, they were then invited to

provide a further nine samples (3 hypoglycemic, 3 hyperglycemic, and 3 normoglycemic samples).

Approval was obtained from IWK Health Centre Research Ethics Board and Dalhousie's University Committee on Laboratory Animals.

Experiment 1: Can dogs generalize the odour of hypoglycemia across breath samples from the same person

Dogs

These studies utilized a small n (idiographic) approach [27, 28], whereby a small number of dogs were carefully selected for participation [29]. Two dogs were selected from a larger pool of dogs for their trainability, high work drive, and history of high performance in the lab: Nutella, a 4-year-old female border collie, and Koda, a 2-year-old male border collie mix. It is important to note that, as a result of the careful selection criteria, these dogs were considered to be inherently different than the general population of dogs. Therefore, the authors are not attempting to generalize the findings of the studies presented here to all dogs, but rather, to demonstrate the potential of a few carefully selected and highly trained dogs. Both Nutella and Koda had completed previous training and had shown high performance discriminating between breath samples donated by people with T1D at different glycaemic states (see Appendix). Dogs were brought by their owners once or twice a week for 2 to 3-hour work days. Only one dog worked in the lab at any given time.

Procedure

All procedures took place at Dalhousie University's Canid Behaviour Lab, which contains three adjoining rooms: Room 1 where the samples were prepared, Room 2, a small interior room connecting the other two rooms, and Room 3, where trials were completed.

Sample presentation. Breath samples were organized into sample sets, where one sample set consisted of three breath samples from the same individual: one hypoglycemic, one normoglycemic, and one hyperglycemic. Breath samples were prepared for presentation to the dogs by first removing the caps from the breath collection tubes and placing each of the tubes inside separate sample stations: wooden platforms holding black polyvinyl chloride (PVC) tubes upright (Figure 1). The breath collection tubes were shorter in length and smaller in diameter than the PVC tubes, such that they fit inside the PVC tube and sat an inch lower than the opening of the PVC tube. This prevented dogs from coming into direct contact with the breath collection tubes. Which sample stations the breath collection tubes were placed in was decided randomly each work day. To avoid potential contamination of the stations and contamination between dogs, researchers adhered to the following cleaning protocol: at the beginning and end of every work day, the sample stations were cleaned with a solution of 20% ethanol and water and dried. Furthermore, once a week the PVC tubes were soaked for one hour in a solution of bleach and water, the wooden platforms were wiped down with the bleach and water solution, and both components were left to air dry for 48 hours.

Shaping and Training. First, we shaped the dogs' indication behaviours for the given sample types. During shaping, Nutella and Koda were presented with sample sets from different individuals (e.g. Nutella was presented with a sample set from Participant A and Koda was presented with a sample set from Participant B). Breath samples were presented to the dogs inside the sample stations one at a time. When the dogs were presented with a hypoglycemic sample, they were encouraged to sniff the tube and provide a previously trained nose hold behaviour (Figure 1), holding their nose or chin against the top of the tube for five seconds (the "Yes" response). If the dog held the nose hold position for the full five seconds a secondary

reinforcer (a clicker) was used, followed by a food reward. When the dogs were presented with a normoglycemic or hyperglycemic breath sample, they were encouraged to sniff the tube and were asked to sit (the “No” response). Upon sitting, a secondary reinforcer was used (clicker), followed by a food reward. Once a dog spontaneously demonstrated the correct behaviour for a given sample type, shaping was complete and training trials began using the same samples used during shaping. Hypoglycemic samples were presented half of the time and normoglycemic or hyperglycemic samples were presented the other half of the time so that there was an equal distribution of “Yes” and “No” trials. Dogs began a work session by waiting with a handler inside Room 2 while the stimulus was prepared in Room 3 by a research assistant. When a trial began, the door to Room 3 was opened and the dog was led in by the handler who made a gesture towards the stimulus and uttered the phrase “what’s this?”. The dog smelled the stimulus and provided the nose-hold or sit behaviour. The study design was double-blind (the research assistant could not see the dog or the handler), therefore, if the dog provided the nose-hold behaviour, the handler uttered “one” and if the dog provided the sit behaviour, the handler uttered “two”. If the dog provided the correct behaviour for a given stimulus, the research assistant activated a secondary reinforcer (clicker) and then the handler gave the dog verbal praise and a food reward. If the dog provided an incorrect behaviour, the research assistant uttered a gentle “nope”, and the handler led the dog out of the room. While the dog and handler were in Room 2 between trials, the research assistant wiped the opening of the PVC tubes with a paper towel wet with a solution of 20% ethanol and water.

The dogs completed two work days of training within which as many trials as possible were completed given the amount of time the dog could spend at the lab. Koda completed 80 trials and Nutella completed 120 trials.

Testing. During testing the dogs were presented with the samples used during the training phase plus an additional sample set (one hypoglycemic, one normoglycemic, and one hyperglycemic) *from the same individual*. Therefore, the dogs could be presented with any one of six breath samples from the same individual, three of which they had previously been trained on, and three of which they had never smelled before. Hypoglycemic samples were presented half of the time and normoglycemic or hyperglycemic samples were presented the other half of the time so that there was an equal distribution of “Yes” and “No” trials. Samples from the first and second sample sets were presented in equal proportions. After all correct responses (hits and correct rejections), the clicker was activated and dogs received a small food reward. All trials were double blind such that researchers who knew the identity of the sample were not visible to the dog handler or the dog. As with the training phase, the dogs completed as many test trials as possible within two work days. Koda completed 120 trials and Nutella completed 160 trials (for both dogs, this was split over two work days, one week apart).

Experiment 2: Can a dog transfer detection of hypoglycemic odour across breath samples from different people

Procedure

Nutella was the only dog that participated in this experiment. She completed all of the trials for Experiment 2 after finishing the second half of the trials for Experiment 1 (one the same day, with a break between experiments). Both sample sets from Experiment 1 plus two additional sample sets from *a different person* were added to the pool of samples. This resulted in a total of 12 samples; 6 (2 hypoglycemic, 2 normoglycemic, and 2 hyperglycemic) that Nutella had smelled previously, and 6 (2 hypoglycemic, 2 normoglycemic, and 2 hyperglycemic) that were novel to her.

Nutella was presented with one of 12 potential samples at a time. Half of the time the sample presented was a hypoglycemic sample (a “yes” trial) and half of the time the sample was a normoglycemic or hyperglycemic sample (a “no” trial). Samples from the first and second individuals were presented in equal proportions. The trial procedure used was identical to that of the first experiment.

Analyses

The numbers of hits (true positives), misses (false negatives), false alarms (false positives), and correct rejections (true negatives) were documented for the training phase and testing phases. Each dog’s sensitivity, specificity, accuracy, and precision was calculated as: sensitivity = hits/(hits + misses); specificity = correct rejections/(correct rejections + false alarms); accuracy = (hits + correct rejections)/(hits + misses + correct rejections + false alarms); and precision = hit/(hits + false alarms). Signal Detection Theory was used to calculate a d' and a C (bias) value for each dog at each phase [26]. Binomial tests were conducted using the number of correct responses out of the total number of trials, 0.33 probability of success per trial, and an alpha level of 0.05.

Results

Participant demographics

Two individuals (female, $M_{\text{age}} = 32.5$) donated samples for the experiments presented here. Both participants provided informed consent and were given a sample collection kit. Both participants completed both sample collection phases, donating an initial six samples, followed by a further nine samples.

Training

The dog's distribution of hits, misses, false alarms, and correct rejections in the training phase is portrayed in Table 1. During the initial stages of training, both dogs obtained a performance of 100% accuracy within two 10-trial sessions, demonstrating little to no acquisition phase.

Therefore, for both dogs, all of their training data was included in analyses. Both dogs provided the correct indications for the samples at above chance levels, indicating that they could identify hypoglycemic samples from normo- and hyperglycemic samples from the same person.

Testing dogs' ability to transfer detection across multiple hypoglycemic samples from one person

Nutella and Koda completed 160 and 120 trials of testing, respectively. Their distribution of hits, misses, false alarms, and correct rejections can be seen in Table 2. Examination of the dogs' responses to the new samples alone revealed that Nutella's sensitivity was much higher than Koda's. Nutella correctly identified the first presentation of the new hypoglycemic sample, and further identified this new hypoglycemic sample on more than half of trials in which it was presented, suggesting that she detected some overlap in the VOC content between the first hypoglycemic sample and the second hypoglycemic sample from the same individual.

Furthermore, Nutella's high specificity value showed that she was accurate at indicating the new normoglycemic and hyperglycemic samples as well. Koda correctly identified the hypoglycemic sample from the original sample set on most presentations but was unable to identify the new hypoglycemic sample in any of its presentations. These results suggest that Koda likely memorized the odour of the hypoglycemic sample on which he was trained and then was unable to detect any overlapping VOCs between this trained sample and a second hypoglycemic sample from the same person. Koda's specificity value was very high only because he indicated "no" to all samples that were not the originally trained hypoglycemic sample. Nutella and Koda's

response profiles show that both dogs were conservative decision makers as indicated by their positive C (bias) values.

Testing a dog's ability to transfer detection across multiple samples from different people

Nutella completed a total of 80 trials. Her distribution of responses can be seen in Table 3. As illustrated by the data, her overall level of accuracy was 70%. However, a closer look reveals that she demonstrated a low level of sensitivity; she identified the new hypoglycemic samples in less than half of their presentations, as evidenced by the low ratio of hits to misses. Nutella also committed a miss on the first presentation of a new hypoglycemic sample, suggesting a lack of transferability. Interestingly, although Nutella had previously shown the ability to correctly identify the samples from the first sample set in Experiment 1, she identified less than half of these hypoglycemic samples here in Experiment 2. This resulted in equal performance identifying the hypoglycemic samples she was previously trained on and the new samples she had never smelled before. The authors hypothesize that the difficulty of the task led Nutella to become confused about the conditions of the reward, or that the number of samples was overwhelming, and she could not remember the odour of the original sample.

Nutella's degree of specificity was much better than her sensitivity. As shown by the high ratio of correct rejections to false alarms, Nutella was consistently able to identify the normoglycemic and hyperglycemic samples from both sample sets. Given the distribution of Nutella's responses across sample sets, however, it was difficult to assess whether she was able to transfer the detection of hypoglycemia from one previously known set of samples to new sample sets from a different individual. A more valid assessment would require more trials and would ideally include samples sets from additional people.

Discussion and Conclusions

The two experiments presented here examined two dogs' ability to detect hypoglycemia in breath samples from people with T1D and then transfer detection of a hypoglycemic odour across multiple breath samples from the same person or a different person, using a Yes/No task. Using an idiographic approach, two dogs, Nutella and Koda, were carefully selected for their work drive and previous performance on olfactory tasks.

The dogs' performance during training (including preliminary training, see Appendix) showed that they were both able to discriminate between breath samples donated by one person during different glycaemic states. This finding replicates those of Hardin et al. [25] in which dogs were tested for their ability to identify the same hypoglycemic samples they had already been trained to identify. This series of studies built on these findings and further tested two dogs' ability to transfer detection of a hypoglycemic odour to novel samples.

The results of the first experiment provided some evidence that one dog, Nutella, transferred detection of a hypoglycemic odour across two hypoglycemic samples from the same individual. When presented with a new sample set from the same person, Nutella accurately identified the first presentation of the new hypoglycemic sample, and, overall, accurately identified 62% of the new hypoglycemic samples. Across all samples, Nutella obtained 70% sensitivity and 88% specificity. To the best of our knowledge, no other empirical studies have explicitly tested dogs' ability to detect hypoglycemia across multiple samples from one individual, therefore it is difficult to determine how this level of performance should be interpreted. We do interpret these findings as promising, however, since Nutella showed some degree of success with a task arguably more difficult than that of Hardin et al. [25]. The second dog, Koda, rejected all new samples from the same person, including the new hypoglycemic

sample, suggesting that he was only able to identify the sample on which he had previously been trained. It is possible for dogs to memorize up to ten training odours [30], and therefore, as highlighted by Elliker et al. [31], this ability to memorize odours could be a confounder in studies of canine biomedical detection if novel samples are never presented. Indeed, Koda, in the first experiment, appears to have demonstrated this strategy, memorizing a specific sample, rather than recognizing an odour unique to hypoglycemia. A limitation of this experiment, however, is that the dogs were presented with only a small number different samples of hypoglycemia on which to learn (including samples from previous work, see Appendix). Therefore, it could be argued that Koda memorized an individual sample odour because he was not presented with sufficient samples to learn a putative odour of hypoglycemia that is consistent across hypoglycemic events.

In the second experiment we tested whether Nutella could transfer detection of a hypoglycemic odour between two different people by presenting her with the samples on which she had been trained and tested with in the first experiment, plus additional samples from a novel individual. The results provided inconclusive evidence as to whether Nutella could detect the odour of hypoglycemia across sample sets from two different individuals. Her level of sensitivity detecting the new hypoglycemic sample was only 45%, but her sensitivity detecting the hypoglycemic sample she had previously been trained to respond to also dropped to 45%. Therefore, her performance on each sample set was comparable, making interpretation of her performance difficult. Detecting a “hypoglycemic odour” across people is likely a difficult task because, although there appear to be some VOCs that may consistently signal hypoglycemia across different individuals [19-22], the enormous variability in the VOC’s contained in different people’s exhaled breath [30] may result in individual-specific VOC profiles emerging during

hypoglycemic events. Furthermore, as discussed above, the present study was only able to utilize a small number of training samples. Although different species require different numbers of exemplars for generalization [32], we are not aware of any research regarding the number of exemplars required for dogs to generalize stimuli (olfactory or otherwise). The complexity of the putative hypoglycemic odour, combined with a small number of training samples, could explain Nutella's performance here and may have also contributed to the poor performance observed by Dehlinger et al. [24], during which three DADs were unable to detect hypoglycemia in samples provided by individuals that were unknown to them. Furthermore, it is worth noting that during this experiment Nutella completed 80 consecutive trials in one work day. Although Nutella displayed motivation to continue working throughout the work day, she also began to show behaviours suggesting that she was tiring over time, such as lying down between trials. It is possible that, with further training over multiple work days, Nutella could have learned to detect the odour of hypoglycemia across samples from different people. As such, a limitation of the studies presented here is that we did not test whether the dogs could learn to identify the novel samples over time. Future research should examine whether an acquisition phase precedes reliable detection of new hypoglycemic samples. As discussed above, these results should be interpreted cautiously since the dogs were only presented with a small number of potential examples of hypoglycemic breath. Additional research should further examine whether dogs can detect a general odour of hypoglycemia across different people, utilizing a high number of breath samples. Finally, these studies only examined the role of odour cues in the detection of hypoglycemia; future work should explore the possibility of canine detection of behavioural cues signaling a change in physiological state.

The current study, combined with the findings of Dehlinger [24] and Hardin [25] contributes important findings to the literature pertaining to DADs and has notable implications. Dogs carefully selected and assessed for their motivation, trainability, and olfactory acuity [29] can be trained to detect VOCs associated with hypoglycemia from individuals with T1D, but, as our findings illustrate, dogs may have difficulties generalizing the odour of hypoglycemia, suggesting that the collective VOC profile of hypoglycemia may vary enough between individuals that learning to identify a universal “hypoglycemia profile” is a much harder task than identifying one sample. Applied to the training of DADs, the findings of the studies presented here suggest that dogs in training may be most successful if trained to detect hypoglycemia only with their “owner-to-be”, and conversely, that we should not assume that because a dog can identify hypoglycemia in samples provided by one individual in training that they will necessarily be able to transfer that activity to a new individual.

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Author Contributions

All authors were involved in research design and conceptualization. C.R. conducted the research, analyzed the results, and wrote the manuscript. E.C. oversaw sample collection with T1D participants, contributed to, reviewed, and edited the manuscript, E.M. contributed to, reviewed, and edited the manuscript, S.S. conducted research and reviewed the manuscript, and S.G. was the advisor of C.R. and the project supervisor.

C.R. and S.G. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

- [1] Acerini C. The rise of technology in diabetes care. Not all that is new is necessarily better. *Pediatr. Diabetes* 2016;17:168-173
- [2] Martin-Timon I, Javier del Canizo-Gomez, F. Mechanisms of hypoglycemia unawareness and implications in diabetic patients. *World J Diabetes* 2015;6:912-926.
- [3] Frier BM. Morbidity of hypoglycemia in type 1 diabetes. *Diabetes Res Clin Pract* 2004;65:S47-S52
- [4] Gonder-Frederick L, Nyer M, Shepard JA, Vajda K, Clarke W. Assessing fear of hypoglycemia in children with type 1 diabetes and their parents. *Diabetes Manag* 2011;1: 627-639
- [5] Wild D, von Maltzahn R, Brohan E, Christensen T, Clauson P, Gonder-Frederick L. A critical review of the literature on fear of hypoglycemia in diabetes: Implications for diabetes management and patient education. *Patient Educ Couns* 2007;68:10-15
- [6] Riemsma R, Ramos IC, Birnie R, Büyükaramikli N, Armstrong N, Ryder S et al. Integrated sensor-augmented pump therapy systems [the MiniMed® Paradigm™ Veo system and the Vibe™ and G4® PLATINUM CGM (continuous glucose monitoring) system] for managing blood glucose levels in type 1 diabetes: a systematic review and economic evaluation. *Health Technol Assess* 2016;20.
- [7] Jeitler K, Horvath K, Berghold A, Gratzner TW, Neeser K, Pieber TR et al. Continuous subcutaneous insulin infusion versus multiple daily insulin injections in patients with diabetes mellitus: systematic review and meta-analysis. *Diabetologia* 2008;51: 941-951
- [8] Aye T, Block J, Buckingham B. Toward closing the loop: an update on insulin pumps and continuous glucose monitoring systems. *Endocrinol Metab Clin North Am* 2010;39:609-624
- [9] Wong JC, Foster NC, Maahs DM, Raghinaru D, Bergenstal RM, Ahmann AJ et al. Real-time continuous glucose monitoring among participants in the T1D Exchange clinic registry. *Diabetes Care* 2014;37:2702-2709
- [10] Schabert J, Browne JL, Mosely K, Speight J. Social stigma in diabetes. *Patient* 2013;6:1-10
- [11] Chen M, Daly M, Natt, Susie, Candy, Williams G. Non-invasive detection of hypoglycaemia using a novel, fully biocompatible and patient friendly alarm system. *BMJ* 2000;321:1565-1566

- [12] Tauveron I, Delcourt I, Desbiez F, Somda F, Thiéblot P. Canine detection of hypoglycaemic episodes whilst driving. *Diabet Med* 2006;23:335-335
- [13] Wells DL, Lawson SW, Siriwardena AN. Canine responses to hypoglycemia in patients with type 1 diabetes. *J Altern Complement Med* 2008;14:1235-1241
- [14] Gonder-Frederick L, Rice P, Warren D, Vajda K, Shepard J. Diabetic alert dogs: a preliminary survey of current users. *Diabetes Care* 2013;36:e47-e47
- [15] Craven BA, Paterson EG, Settles GS. The fluid dynamics of canine olfaction: unique nasal airflow patterns as an explanation of macrosmia. *J R Soc Interface* 2009;7:933-943
- [16] Miklosi A. *Dog Behaviour, Evolution, and Cognition*. Oxford, UK, Oxford Univ. Press; 2007
- [17] Amann A, de Lacy Costello B, Miekisch W, Schubert J, Buszewski B, Pleil J et al. The human volatilome: volatile organic compounds (VOCs) in exhaled breath, skin emanations, urine, feces and saliva. *J Breath Res* 2014;8:1-17
- [18] Schmidt K, Podmore I. Current challenges in volatile organic compounds analysis as potential biomarkers of cancer. *J Biomark* 2015;2015:1-16
- [19] Minh TDC, Blake DR, Galassetti PR. The clinical potential of exhaled breath analysis for diabetes mellitus. *Diabetes Res Clin Pract* 2012;97:195-205
- [20] Neupane S, Peverall R, Richmond G, Blaikie TP, Taylor D, Hancock G, Evans ML. Exhaled breath isoprene rises during hypoglycemia in type 1 diabetes. *Diabetes Care* 2016;39:e97-e98
- [21] Smith D, Španěl P, Fryer AA, Hanna F, Ferns GA. Can volatile compounds in exhaled breath be used to monitor control in diabetes mellitus? *J Breath Res* 2011;5:022001
- [22] Siegel AP, Daneshkhah A, Hardin DS, Shrestha S, Varahramyan K, Agarwal M. Analyzing breath samples of hypoglycemic events in type 1 diabetes patients: towards developing an alternative to diabetes alert dogs. *J. Breath Res* 2017;11:026007
- [23] Walker DB, Walker JC, Cavnar PJ, Taylor JL, Pickel PJ, Hall SB et al. Naturalistic quantification of canine olfactory sensitivity. *Appl Anim Behav Sci* 2006;97:241-254
- [24] Dehlinger K, Tarnowski K, House JL, Los E, Hanavan K, Bustamante B et al. Can trained dogs detect a hypoglycemic scent in patients with type 1 diabetes? *Diabetes Care* 2013;36:e98-e99
- [25] Hardin DS, Anderson W, Cattet J. Dogs can be successfully trained to alert to hypoglycemia samples from patients with type 1 diabetes. *Diabetes Ther* 2015;6:509-517

- [26] Reeve C, Wentzell P, Wielens B, Jones C, Stehouwer K, Gadbois S. Assessing individual performance and maintaining breath sample integrity in biomedical detection dogs. *Behav Processes* 2012:online first access.
- [27] Morgan DL, Morgan RK. *Single Case Research Methods for the Behavioural and Health Sciences*. Thousand Oaks, CA, SAGE publications; 2008
- [28] Kazdin AE. *Single-Case Research Designs: Methods for Clinical and Applied Settings*. New York, NY, Oxford University Press; 2011
- [29] Gadbois S, Reeve C. The semiotic canine: scent processing dogs as research assistants in biomedical and environmental research. *Dog Behaviour* 2016;3-2016:26-32
- [30] Williams M, Johnston JM. Training and maintaining the performance of dogs (*Canis familiaris*) on an increasing number of odour discriminations in a controlled setting. *Appl Anim Behav Sci* 2002;78:55-65
- [31] Elliker KR, Sommerville BA, Broom DM, Neal DE, Armstrong S, Williams HC. Key considerations for the experimental training and evaluation of cancer odour detection dogs: lessons learnt from a double-blind, controlled trial of prostate cancer detection. *BMC Urol* 2014;14:22
- [32] Phillips M, Herrera J, Krishnan S, Zain M, Greenberg J, Cataneo RN. Variation in volatile organic compounds in the breath of normal humans. *J Chromatogr B Biomed Sci Appl* 1999;729:75-88
- [33] Wright AW, Katz JS. Generalization hypothesis of abstract-concept learning: Learning strategies and related issues in *Macaca mulatta*, *Cebus apella*, and *Columba livia*. *J. Comp. Psychol* 2007;121:387-397

Tables

Table 1

Nutella and Koda's performance on the training phase of a Yes/No task detecting hypoglycemia

	Nutella	Koda
Total number of trials	120	80
Hypoglycemic samples	60	40
Normoglycemic/hyperglycemic samples	60	40
Hits	0.75 (n=45)	0.70 (n=28)
Misses	0.25 (n=15)	0.30 (n=12)
Correct Rejections	0.77 (n=46)	0.85 (n=34)
False Alarms	0.23 (n=14)	0.15 (n=6)
d'^*	1.41	1.56
C^\dagger	0.03	0.26
Sensitivity [‡]	75%	70%
Specificity [§]	77%	85%
Accuracy	76%	78%
Precision [¶]	76%	82%
p value (correct responses) [¥]	$< 1 \times 10^{-6}$	$< 1 \times 10^{-6}$

* $d' = Z_{\text{hits}} - Z_{\text{false alarms}}$

† $C = - [(Z_{\text{hits}} + Z_{\text{false alarms}})]/2$

‡ sensitivity = hits/(hits + misses)

§ specificity = correct rejections/(correct rejections + false alarms)

|| accuracy = (hits + correct rejections)/(hits + misses + correct rejections + false alarms)

¶ precision = hit/(hits + false alarms).

¥ Based on binomial test with 0.5 probability of success

Table 2

Nutella and Koda's distribution of responses with new sample set from the same individual.

	Nutella			Koda		
	Original sample set	Second sample set from same individual	Overall	Original sample set	Second sample set from same individual	Overall
Total number of samples presented	81	79	160	60	60	120
Hypoglycemic samples	38	42	80	30	30	60
Normoglycemic/ Hyperglycemic samples	43	37	80	30	30	60
Hits	0.79 (n=30)	0.62 (n=26)	0.70 (n=56)	0.87 (n=26)	0 (n=0)	0.43 (n=26)
Misses	0.21 (n=8)	0.38 (n=16)	0.30 (n=24)	0.13 (n=4)	1 (n=30)	0.57 (n=34)
Correct Rejections	0.86 (n=37)	0.89 (n=33)	0.88 (n=70)	0.90 (n=27)	1 (n=30)	0.95 (n=57)
False Alarms	0.14 (n=6)	0.11 (n=4)	0.12 (n=10)	0.10 (n=3)	0 (n=0)	0.05 (n=3)
d'	1.89	1.51	1.68	2.41	--*	1.47
C	0.14	0.47	0.31	0.08	--*	0.91
Sensitivity	79%	62%	70%	87%	0.00 [†]	43%
Specificity	86%	89%	88%	90%	100% [†]	95%
Accuracy	83%	75%	79%	88%	50%	69%
Precision	83%	87%	85%	90%	--	90%
<i>p</i> value (correct responses)	< 1x10 ⁻⁶	4.3x10 ⁻⁶	< 1x10 ⁻⁶	< 1x10 ⁻⁶	= 0.103	=9.3x10 ⁻⁶

*d' and C cannot be calculated if zero hits are committed

†These are mathematically correct results, however, this result must be interpreted cautiously because Koda rejected all new samples, including the hypoglycemic sample, indicating that he could not accurately discern what was *not* a hypoglycemic sample

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Table 3

Nutella's distribution of responses when presented with new sample sets from a different individual

	Original sample sets	Sample sets from different individual	Overall
Total number of samples presented	40	40	80
Hypoglycemic samples	20	20	40
Normoglycemic/ Hyperglycemic samples	20	20	40
Hits	0.45 (n=9)	0.45 (n=9)	0.45 (n=18)
Misses	0.55 (n=11)	0.55 (n=11)	0.55 (n=22)
Correct Rejections	0.95 (n=19)	0.85 (n=17)	0.90 (n=36)
False Alarms	0.05 (n=1)	0.15 (n=3)	0.20 (n=4)
d'	1.52	0.91	0.72
C	0.89	0.58	0.48
Sensitivity	45%	45%	45%
Specificity	95%	85%	90%
Accuracy	70%	65%	68%
Precision	90%	75%	82%
<i>p</i> value (correct responses)	= 0.005	= 0.021	= 6.4×10^{-4}

Figures

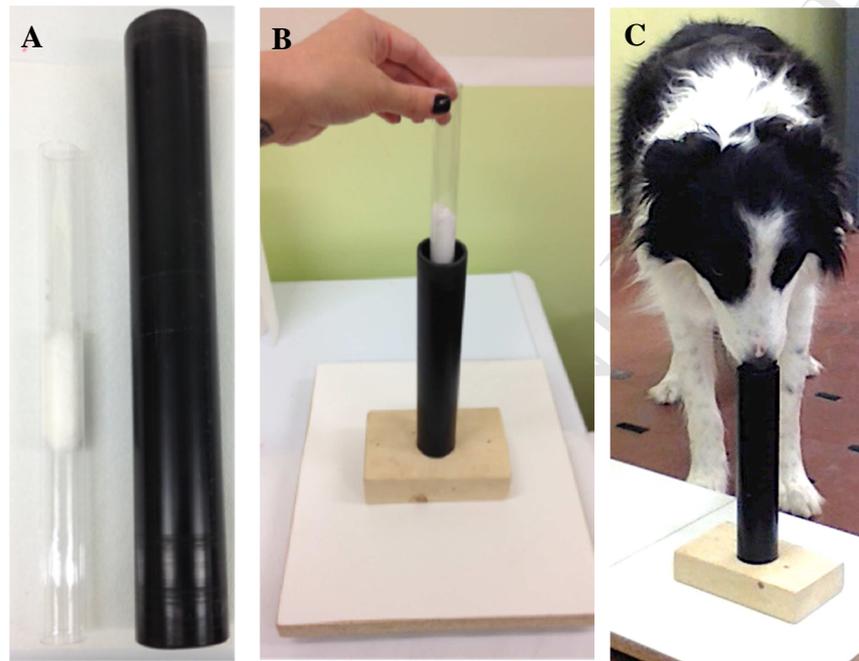


Figure 1. A. Illustrates that the breath sample tube is shorter than the black PVC pipe in which the breath sample tube is placed. B. The breath sample tube inside the black PVC pipe is held upright when placed inside the wooden stand. C. A dog signaling a “Yes” response using the “nose-hold” behaviour.

Appendix

Prior to the experiments presented here, Nutella and Koda were trained to discriminate between breath samples donated by people with T1D in different glycaemic states. Discrimination was demonstrated using a cued, three alternative forced choice procedure. For each session of this procedure, a dog was first presented with a cue odour in a stainless steel jar with holes in the lid: a hypoglycaemic breath sample donated at the same time as the to-be-identified hypoglycaemic sample (two cotton balls were in the hypoglycaemic breath collection tube). Then the dog was simultaneously presented with three breath samples that had been donated by the same person that donated the cue: the matching hypoglycaemic sample, one normoglycaemic sample, and one hyperglycaemic sample, and was required to identify the hypoglycaemic sample using the nose hold behaviour. Samples were presented to the dogs using the same sample stations described in Experiment 1. In an attempt to present the dogs with a variety of potential exemplars of “hypoglycaemia odour” along with the potential diversity of background odours, both dogs saw breath samples from three different people across different sessions. A larger number of samples was not feasible due to participant attrition. No samples were used more than once. The dogs’ performance discriminating between the samples was 100% correct across all tests with different samples ($n = 20$ trials per sample set, $p < 1.0 \times 10^{-6}$).

After successfully completing discrimination training, the dogs were transitioned from the alternative forced choice procedure to the Yes/No procedure used in the studies presented in the manuscript. The transition was accomplished by first presenting a new sample set to the dog using the three alternative forced choice procedure, and rewarding them for indicating the hypoglycaemic sample. Once the dog reached performance of 80% correct or

higher on 10 individual trials, researchers then commenced the “*Shaping and Training*” procedure described above.

Figures

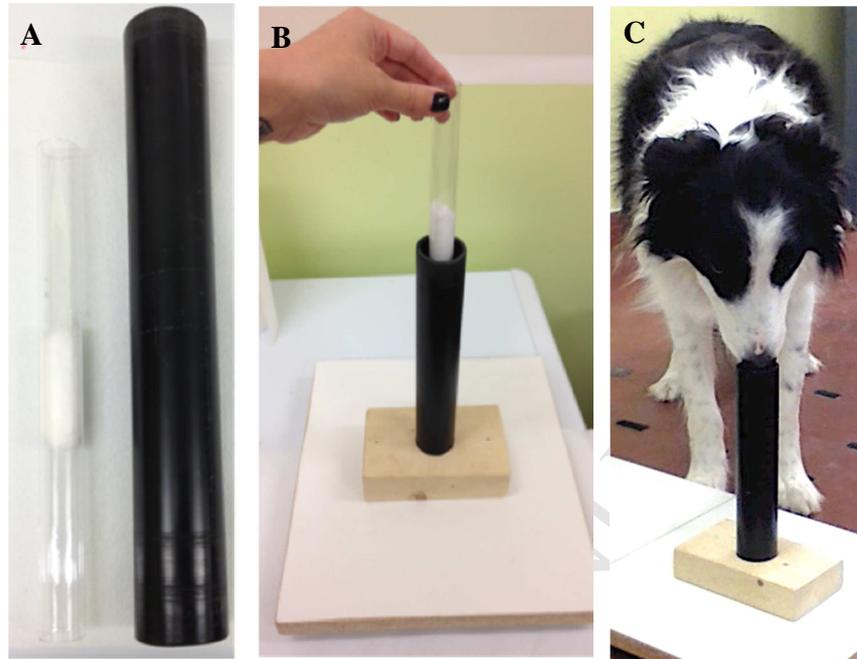


Figure 1. A. Illustrates that the breath sample tube is shorter than the black PVC pipe in which the breath sample tube is placed. B. The breath sample tube inside the black PVC pipe is held upright when placed inside the wooden stand. C. A dog signaling a “Yes” response using the “nose-hold” behaviour.