First Millimeter-Wave Animal In Vivo Measurements of L-Glucose and D-Glucose: Further Steps Towards a Non-Invasive Glucometer

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***Abstract*—The authors present the first in vivo measurements of L-Glucose and D-Glucose concentrations using millimeter waves. Employing direct injection of solutions into the rat jugular vein, we have been able to correlate immediate changes in blood glucose readings with transmission loss through the ears of anesthetized rats at Ka band (27-40 GHz). The sensed changing D-glucose levels are shown to track blood values, but with a delay of approximately 10-15 minutes, which may be due to cellular glucose uptake. Surprisingly, levels of L-glucose, the non-bioactive enantiomer (L-isomer) are also tracked by millimeter-wave transmission through the ear, suggesting that we are looking at the direct chemical presence of glucose or products of its breakdown in the tissue, rather than metabolism. Both isomers of glucose are gradually removed from the bloodstream after injection of insulin, consistent with the observed changes in the millimeter-wave absorption. These results represent a major step towards the realization of a non-invasive glucose monitor based on millimeter-wave transceivers.**

I. Introduction

Non-Invasive glucose monitoring has been an elusive goal for both researchers and medical device companies for many years [1], [2]. Several groups, e.g. [3]-[10], have demonstrated that glucose levels in extracted blood samples can be correlated with measurable changes in the millimeter-wave dielectric constant (real and imaginary parts). Our group showed, *for the first time*, that these changes can be monitored in vivo, using a simple Ka band (27-40 GHz) transceiver [11], [12]. In those measurements, changes in the millimeter-wave transmission through the ear of anesthetized rats closely tracked changes of glucose in the blood, which were experimentally manipulated by *subcutaneous injections* of glucose and insulin. Here we present the first in vivo millimeter-wave measurements of both D-glucose and L-glucose, and correlate changes in radio frequency (RF) transmission through the tissue (ear of anesthetized rat) with blood glucose values *after direct infusion of these solutions into the bloodstream*. Evaluation of L-glucose is important because it is the non-bioactive isomer of D-glucose, and its detection in the tissue by millimeter-waves is a strong indication that the millimeter-wave transceiver is sensitive to the glucose (or products of its breakdown in tissue), rather than a general metabolic process activated by D-glucose.

II. Methods

All studies were done with IACUC approval. After induction of anesthesia, both jugular veins of Sprague-Dawley rats (Charles River Laboratories, Hollister, CA, USA) were cannulated: left for infusion and right for blood sampling (Fig. 1). A Ka band waveguide transceiver (Fig. 2, described in [11]) was magnetically clamped around the ear with spacers to protect tissue from crush injury (waveguide gap≈1mm). After approximately one hour, during which the RF transmission decreased rapidly and then plateaued, D-glucose (50% by weight, Teknova G9005, Hollister, CA, USA) and L-glucose (Alfa Aesar A17496, Ward Hill, MA, USA) were slowly infused intravenously using a syringe pump (Harvard Apparatus, Holliston, MA, USA). The RF transmission through the ear was continuously monitored at 14 discrete frequencies between 27 and 40 GHz with a 2 second dwell time at each step, repeating the cycle every 30 seconds. After glucose infusions, insulin was injected to induce a hypoglycemic state. Blood was collected at regular intervals (approximately 0.1ml) and glucose levels were measured with an Alpha Trak II pet glucose test strip calibrated for use in cats [13].

III. Results

Fig. 3. shows a full experimental run with millimeter-wave transmission vs. time at the 14 frequencies. Typical glucose levels in awake female and male rats (weights 260-320 gm) are expected to range from 85-130 mg/dl [14]. Note that following anesthetic administration at time=0 via an intra-peritoneal injection (ketamine (90 mg/kg) and xylazine (10 mg/kg)), there is a dramatic drop in RF signal level (detector voltage) with an eventual leveling off at around 30 minutes, when the first Alpha Trak II reading is taken. This rapid initial drop in the transmitted RF signal level was not observed in our earlier measurements [11], where isoflurane gas was employed as the anesthetic. Also note that the blood glucose reading before the start of the direct glucose infusion is much higher than the expected normal level (reaching 250-260 mg/dl at time=30 to 40 minutes). There is at least one report of significantly elevated glucose levels in rats (75-100% increase) after injection of a ketamine/xylazine anesthetic [15]. This finding was consistent across 6 animals.

Fig. 4, from another experiment, highlights the correlation of blood glucose levels with millimeter-wave transmission at a single frequency with a highly zoomed scale (all frequencies showed similar behavior). As also reported in [11] and [12], increasing glucose levels correlate with decreasing RF loss (higher transmission) in the tissue.

Figs. 3 and 4 both show tracking of glucose levels by the millimeter-wave signal change, but lagging the direct blood readings by about 10-15 minutes. This effect was not noticed in [11] because of the delay between the subcutaneous injections of glucose, and the later appearance of elevated glucose in the blood and tissue. Here we see an immediate rise in blood glucose after intravenous administration on the contralateral side, compared to a delay in the RF measured rise in the ear tissue. This suggests that the RF measurements reflect the glucose levels in the tissue rather than in the blood vessels of the ear.

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In an attempt to determine whether our RF system is directly measuring the concentration of glucose, or products of its breakdown in tissue, rather than a metabolic process activated by D-glucose, we substituted D-glucose with the non-bioactive enantiomer, L-glucose. The Alpha Trak II blood glucose strips are equally sensitive to both forms of glucose [13]. Interestingly, the millimeter-wave measurements showed similar responses to both L-glucose and D-glucose. The blood level readings are seen to rise in Fig. 5 as soon as the L-glucose or D-glucose enters the bloodstream. The RF signal also exhibits a rise after similar delays in both D-glucose and L-glucose infusions. The intravenous administration of insulin at 90-minutes post glucose results in decreased blood level readings as well as decreased RF signal transmission, reflecting a hypoglycemic state (both L- and D-glucose blood levels are reduced by insulin). Since L-glucose and D-glucose have the same effect on the millimeter-wave signal before and after insulin administration, but only D-glucose affects the general metabolism, this suggests that the RF is measuring glucose or products of its breakdown in the tissue, rather than other metabolic products.

IV. Discussion

The measurements reported in this paper support our prior work that shows normo-, hypo- and hyper-glycemic states can be tracked in vivo by millimeter-wave transmission through the tissue. In addition, we demonstrate that the RF signals respond to glucose levels in the tissue, rather than in the blood – at least in the ear of anesthetized rats. The fact that our RF transmission levels respond equally to both D-glucose, and the non-metabolically active enantiomer, L-glucose, suggests that we are indeed measuring the presence of glucose (or products of its breakdown in the tissue), rather than a general metabolic effect of glucose. These early, but positive results, point to the potential implementation of a non-invasive continuous reading glucometer based on millimeter-waves.

References

[1]. David D. Cunningham and Julie A. Stenken, "In Vivo Glucose Sensing," John Wiley and Sons, Hoboken, NJ, c. 2010, 476 pages.

[2]. John L. Smith, "The Pursuit of Noninvasive Glucose: 'Hunting the Deceitful Turkey'," Fourth Edition, 180 pages, on-line release in 2015 and currently available at: http://www.mendosa.com/The%20Pursuit%20of%20Noninvsive%20Glucose,%20Fourth%20Edition.pdf

[3]. M. Hofmann, G. Fischer, R. Weigel and D. Kissinger, "Microwave-based noninvasive concentration measurements for biomedical applications," IEEE. Trans. Microwave Theory and Tech., vol. 61, pp. 2195-2204, May 2013.

[5]. Y. Nikawa, and D. Someya. "Non-invasive measurement of blood sugar level by millimeter waves," IEEE Int. Mic. Sym. Digest, pp. 171-4, 2001.

[6]. J.M. Alison and R. Sheppard, "Dielectric properties of human blood at microwave frequencies," Phys. Med. in Biology, v. 38, pp. 971-78, 1993.

[7]. E. Topsakai, T. Karacolak and E.C. Moreland, "Glucose dependent dielectric properties of blood plasma," XXXth URSI Symposium, pp. 1-4, Istanbul, Turkey, Aug. 13-20, 2011.

[8]. T. Karacolak, E. Moreland and E. Topsakal, "Cole-Cole model for glucose-dependent dielectric properties of blood plasma for continuous glucose monitoring," Microwave and Optical Tech. Lett., v.55, pp.1160–64, May 2013.

[9]. M. Zhadobov, R. Augustine, R. Sauleau, S. Alekseev, et al., "Complex permittivity of representative biological solutions in the 2-67 GHz range," Bioelectromagnetics, vol. 33, pp. 346-355, May 2012.

[10]. V. V. Meriakri, E.E. Chigrai, D. Kim, I.P. Nikitin, L.I. Pangonis, M.P. Parkhomenko and J.H. Won, "Dielectric properties of glucose solutions in the millimeter-wave range and control of glucose content in blood," Measurement Science and Technology, v. 18, pp. 1-6, 2007.

[11]. P.H. Siegel, Y. Lee and V. Pikov, "Millimeter-wave non-invasive monitoring of glucose in anesthetized rats," 39th Int. Conf. on Infrared, Millimeter, and THz Waves (IRMMW-THz2014), T2_D8.10, Tucson, AZ, Sept. 14-19th, 2014.

[12]. Peter H. Siegel, Adrian Tang, Gabriel Virbila, Y. Kim, M.C.F. Chang and Victor Pikov, "Compact non-invasive millimeter-wave glucose sensor," 40th Int. Conf. on Infrared, Millimeter, and Terahertz Waves (IRMMW-THz2015), F1D, Hong Kong, China, Aug. 23-28, 2015.

[13]. Abbott Laboratories, Zoetis, Veterinary Division, private communications, Dec. 2015.

[14]. D.F. Kohn and C.B. Clifford, "Biology and diseases of rats," Ch. 4 of Laboratory Animal Medicine, 2nd Edition, J.G. Fox, L.C. Anderson, F.M. Lowe and F. Quimby, Editors, Academic Press, pp. 121-167, c. 2002.

[15]. E.D. Braslasu, C. Bradatan, M. Cornila, I. Savulescu, R. Cojmaleata, and M.C. Braslasu, "Normal blood glucose in white Wistar rat and its changes following anesthesia," Lucrări Stiintifice Medicina Veterinara, vol. Xl, Timisoara, pp. 120-123, 2007.

Figures

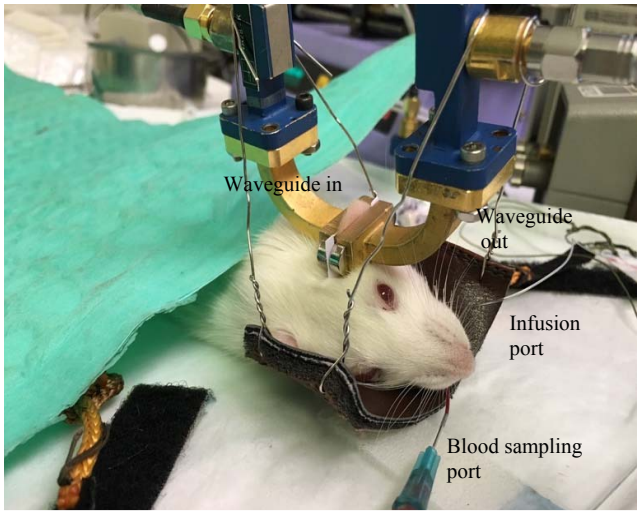

Fig. 2. Photo showing anesthetized rat with Ka band waveguide transmission system clamped around the ear. An IV port for blood sampling is shown on the lower center. An infusion port into the jugular vein is at the top right. Body temperature is constantly monitored and recorded using a rectal thermometer. Power level at the ear varies between 1 and 5mW over the frequency range 27-40 GHz. RF data is continually collected at 14 frequencies with a dwell time of 2 seconds with a repeating cycle of 30 seconds.

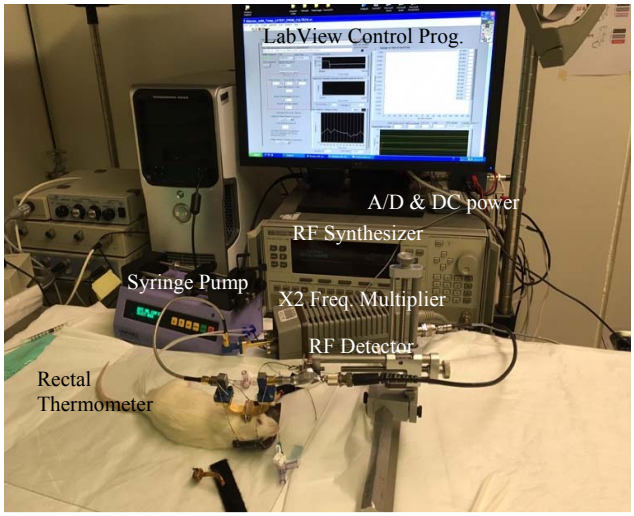

Fig. 2. Complete experimental set up showing anesthetized rat; HP83620A synthesized source (.1-20GHz) and HP83554A X2 multiplier; coax-to-waveguide transitions; a magnetically coupled Ka band waveguide transmission system; HP8474 broadband detector; data logging hardware (Measurement Computing USB1408) and control computer using LabView®.

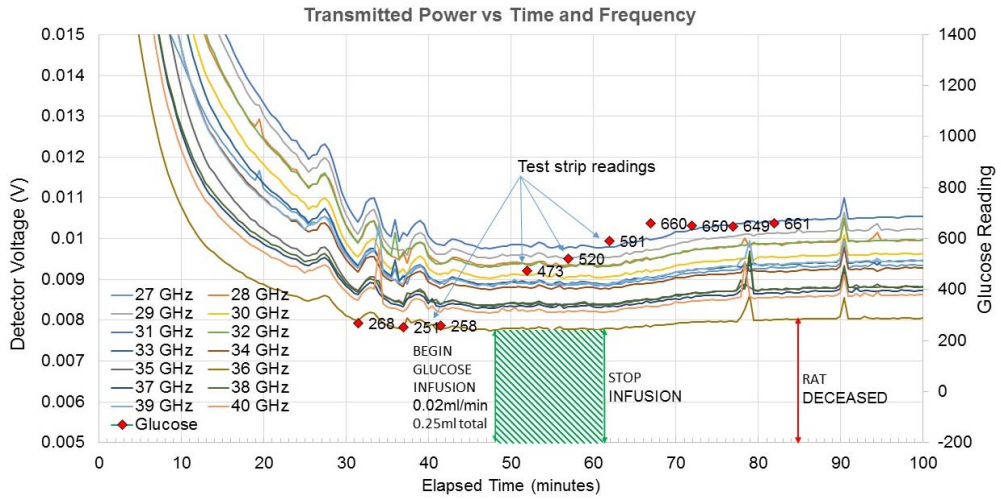

Fig. 3. Plot showing complete experimental run with transmission vs. time at all 14 frequencies tracked. Overlaid on the RF transmission curves are the measured blood glucose levels in mg/dl. Anesthetic is injected at time=0 and the rat expired at time=84 minutes. Glucose infusion (0.02ml/min) starts at t=49 minutes and extends for 12 minutes (0.25ml total). The immediate rise in blood glucose level (from 258 to 473 mg/dl) is evident as the glucose infusion begins, but the RF change (measured in the ear) does not occur for an additional 15 minutes (at around time=60). Peak glucose is 660 mg/dl (extremely abnormal).

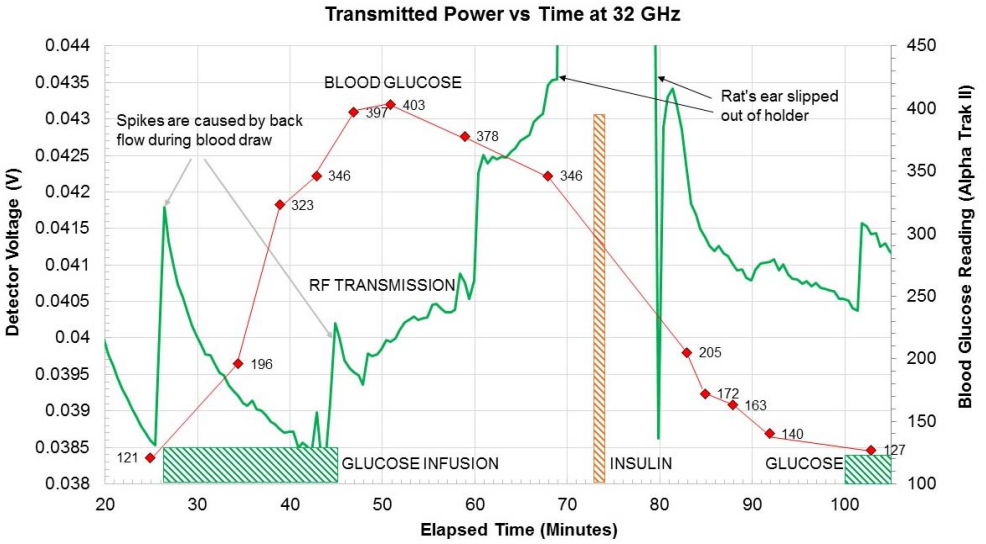

Fig. 4. Blood glucose levels plotted against millimeter-wave transmission at one representative frequency (32 GHz) after infusion of 50% glucose (0.2ml total) [time=26-45]; an IV injection of 0.4U insulin [time=73]; and a final infusion of 0.1ml glucose (rat expires at this point) [time=100]. The jumps in the millimeter-wave signal (green curve) at time=25 and 45 are due to inadvertent backflow of the blood sampling (collected from the same jugular vein in this experiment – we later used the vein on the opposite side to avoid this problem). The millimeter-wave readings between 70 and 80 minutes are inaccurate as the ear slipped out from the waveguide clamp. Note that the readings returned to prior levels when the ear was reinstalled between the waveguide clamps. ill effect. The spike in the millimeter-wave reading at t=104 minutes was when the rat expired.

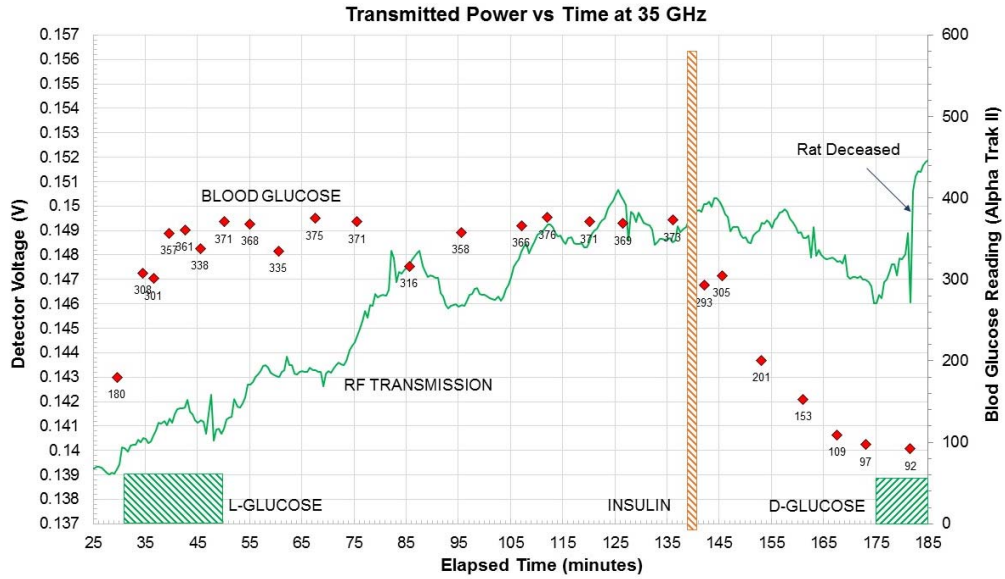

Fig. 5. Blood glucose levels plotted against millimeter-wave transmission at one representative frequency when 50% L-glucose, the non-bioactive isomer of D-glucose, is infused at 0.01ml/min for 20 minutes. Both the blood level readings and millimeter-wave signal increase and oscillate, reaching a steady state after some 90 minutes. After injection of insulin (0.4U) both readings decrease, with the millimeter-wave signal lagging the blood readings by 10-20 minutes. A last D-glucose infusion at 175 minutes causes both readings to break slope again, but the animal expired at 182 minutes. Note the sharp jump in millimeter-wave reading after death. This is consistently observed in all experiments to date and likely indicates blood pooling away from the ear via gravity when circulation ceases.