DIABETES, OBESITY AND METABOLISM
A JOURNAL OF PHARMACOLOGY AND THERAPEUTICS

Environmental effects of ambient temperature and relative humidity on insulin pharmacodynamics in adults with type 1 diabetes mellitus

Journal:	*Diabetes, Obesity and Metabolism*
Manuscript ID	DOM-18-0857-OP.R1
Manuscript Type:	Original Paper
Date Submitted by the Author:	20-Sep-2018
Complete List of Authors:	Al-Qaissi, Ahmed; Hull Royal Infirmary, Department of Academic Diabetes, Endocrinology and Metabolism Papageorgiou, Maria; Hull Royal Infirmary, Department of Academic Diabetes, Endocrinology and Metabolism Javed, Zeeshan; Pakistan Kidney and Liver Institute and Research Center, Department of Endocrinology and Diabetes Heise, Tim; Profil Institut für Stoffwechselforschung GmbH, Rigby, Alan; Hull York Medical School, University of Hull Garrett, Andrew; University of Hull, Department of Sport, Health and Exercise Science Hepburn, David; Hull and East Yorkshire Hospitals NHS Trust and Hull York Medical School, Department of Academic Diabetes, Endocrinology and Metabolism Kilpatrick, Eric; Sidra Medical and Research Center Atkin, Stephen; Weil Cornell Medical College, Diabetes, Endocrinology and Metabolism Sathyapalan, Thozhukat; Hull York Medical School, Department of Academic Diabetes, Endocrinology and Metabolism
Key Words:	type 1 diabetes, insulin analogues, pharmacodynamics

Environmental effects of ambient temperature and relative humidity on insulin pharmacodynamics in adults with type 1 diabetes mellitus

Short Title: Environmental effects in type 1 diabetes mellitus

Ahmed Al-Qaissi[1], Maria Papageorgiou[1], Zeeshan Javed[1,2], Tim Heise[3], Alan S Rigby[4], Andrew T Garrett[5], David Hepburn[6], Eric S Kilpatrick[7], Stephen L Atkin[8], Thozhukat Sathyapalan[1]

[1]Department of Academic Diabetes, Endocrinology and Metabolism, Hull York Medical School, University of Hull, Hull,UK; ahmedsattar1978@yahoo.com (A.A); M.Papageorgiou@hull.ac.uk (M.P.); zeeshan.javed@pkli.org.pk (Z.J.); Thozhukat.Sathyapalan@hyms.ac.uk (T.S.)

[2] Pakistan Kidney and Liver Institute and Research Center, DHA Phase 6, Lahore, Pakistan; Zeeshan.Javed@pkli.org.pk (Z.J.)

[3]Profil, Neuss, Germany; Tim.Heise@profil.com (T.H.)

[4] Department of Academic Cardiology, Hull York Medical School, University of Hull, Hull, UK; A.rigby@hull.ac.uk (A.R.)

[5]Department of Sport, Health and Exercise Science, University of Hull, Hull, UK; A.Garrett@hull.ac.uk (A.T.G.)

[6] Department of Academic Diabetes, Endocrinology and Metabolism, Hull and East Yorkshire Hospitals NHS Trust and Hull York Medical School, University of Hull, UK; David.Hepburn@hull.ac.uk

[7]Department of Pathology, Sidra Medicine, Doha, Qatar; ekilpatrick@sidra.org (E.S.K.)

[8]Weill Cornell Medicine in Qatar, Education City, P.O. Box 24144, Qatar; Sla2002@qatar-med.cornell.edu (S.L.A.)

Correspondence: Professor Thozhukat Sathyapalan, Department of Academic Diabetes, Endocrinology and Metabolism, Hull Medical School, University of Hull, Hull, UK, Email address: Thozhukat.Sathyapalan@hyms.ac.uk.

Abstract

Objective: This study aimed to explore the effects of ambient temperature and relative humidity on insulin pharmacodynamics in adults with type 1 diabetes.

Research Design: A 3-way, cross-over, randomised study was performed in adults with type 1 diabetes mellitus (n=10). The pharmacodynamics profile of a single dose of short-acting insulin (insulin lispro) was investigated under three environmental conditions: i) temperature: 15°C and humidity: 10%, ii) temperature: 30°C and humidity: 10%, and iii) temperature: 30°C and humidity: 60%, controlled in an environmental chamber. Euglycaemic glucose clamp technique ensured a constant blood glucose of 100 mg/dl (5.5 mmol/l). The following pharmacodynamic endpoints were calculated: maximum glucose infusion rate (GIR_{max}), time to GIR_{max} (t_{GIRmax}), total area under the curve (AUC) for GIR from 0-6 hours ($AUC_{GIR.0–6h}$), and partial AUCs ($AUC_{GIR.0-1h}$, $AUC_{GIR.0-2h}$ and $AUC_{GIR.2-6h}$).

Results: Higher temperature (30°C) under 10% fixed humidity resulted in a greater GIR_{max} (p=0.04), a later $t_{GIR.max}$ (p=0.049) compared to lower temperature (15°C). Humidity did not affect any pharmacodynamic parameter. When the combined effects of temperature and humidity were explored, $t_{GIR.max}$ (p=0.008) occurred earlier with a lower late insulin pharmacodynamic effect ($AUC_{GIR.2-6h}$, p=0.017) at temperature 15°C and humidity 10% compared to temperature 30°C and humidity 60%.

Conclusions: High ambient temperature resulted in greater insulin peak effect compared to low ambient temperature, with the contribution of high relative humidity only apparent at high ambient temperature. This suggests that patients with type 1 diabetes mellitus entering higher environmental temperatures with or without high humidity could experience more hypoglycaemic events.

Keywords: environmental conditions, ambient temperature, relative humidity, insulin pharmacodynamics, type 1 diabetes mellitus

Introduction

Type 1 diabetes mellitus is characterised by β-cell destruction and a lifelong requirement of exogenous insulin. Insulin requirements depend on insulin absorption from the injection site, the individual's insulin sensitivity, body composition, inflammatory processes and environmental factors (1, 2). Evidence from epidemiological research suggests seasonal differences in HbA1c (3-5) and clinical onset of diabetes (6), with warmer temperature (summer) favouring lower HbA1c and lower incidence of diabetes compared to cooler temperatures (winter). Conversely, there is paucity of recent, well-controlled experimental studies employing technological advancements, such as an environmental chamber (7) using the gold standard glucose clamp technique (8) to investigate the effects of ambient temperature on insulin action, which could provide evidence of a cause-effect relationship. Nevertheless, there is increasing evidence of the local effects of temperature on insulin pharmacodynamics and pharmacokinetics. For instance, local warming of the injection site as a result of local skin massage (9), application of an insulin infusion site heating device (InsuPatch^{TM}) (10-13), hot baths (14), or sauna exposure (15) has been shown to accelerate insulin absorption and improve insulin sensitivity in patients with diabetes, with these effects largely mediated by an increase in skin temperature, which results in an increased perfusion at the injection site.

The effects of relative humidity on insulin pharmacodynamics and pharmacokinetics are largely unexplored. An epidemiological study conducted in the Mediterranean area suggested an increased prevalence of diabetes among the elderly who lived in islands with high relative environmental humidity when adjusted for ambient temperature (16). Notably, high relative humidity often occurs in the presence of high ambient temperature, making it challenging to unravel their individual effects (17). Individuals with diabetes appear to tolerate moist, warm air above 50% humidity less well than adults without diabetes (18). This may be due to the fact that high humidity when combined with high temperature decreases the rate of cooling of the human body leading to tiredness, exhaustion, reduction in alertness and potentially heat stroke (17, 19, 20), which may also affect glycaemic control.

In order to assess the independent and combined effects of ambient temperature and relative humidity, this study evaluated the insulin pharmacodynamic profile following a single injection of a short-acting insulin analogue.

Research Design and Methods

A single-centre, open label, 3-way cross-over study was performed in the Diabetes Research Centre at Hull Royal Infirmary in adults with type 1 diabetes mellitus (n=10). All participants provided their written informed consent. The trial was approved by the Yorkshire & the Humber - Leeds West Research Ethics Committee (REC number: 14/YH/1129), registered at www.clinicaltrials.gov (NCT03102476) and conducted according to the Declaration of Helsinki. Individuals with type 1 diabetes mellitus were identified from the diabetes clinics and adverts placed in the Diabetes Centre at the Hull Royal Infirmary. Participants were included if they i) were males, ii) aged between 18-55 years, iii) had been diagnosed with type 1 diabetes mellitus, iv) had HbA1c ≤ 9.0 % (75 mmol/mol) and a total insulin dose of < 1.2 U/kg/day, and v) had a body mass index (BMI) between 18.0 and 28.0 kg/m^2. Exclusion criteria were: i) known or suspected allergy to insulin, ii) recurrent major hypoglycaemia or hypoglycaemic unawareness within the previous 6 months, iii) clinically significant diabetes neuropathy, iv) participation in clinical trials involving investigational drugs within 3 months prior to screening and v) supine blood pressure at screening outside the range of 90-140 mmHg for systolic blood pressure or 50-90 mmHg for diastolic blood pressure and/or resting supine heart rate outside the range 50 -90 beats per minute.

The pharmacodynamic profile of the short-acting insulin lispro dosed at 0.2 units/kg was investigated under three environmental conditions for each subject: i) temperature: 15°C and humidity: 10%, ii) temperature: 30°C and humidity: 10%, and iii) temperature: 30°C and humidity: 60%. Participants attended six visits (Visits 1, 2a, 2b, 3, 4 and 5). Visit 1, 2a and 5 were conducted in the Diabetes Centre, Hull Royal Infirmary, whereas Visits 2b-4 were performed at the environmental chamber (Type SSR 60-20H, Design and Manufacture of Environmental Test Chambers, Gwent, Wales) located at the Department of Sport, Health & Exercise, University of Hull. During Visit 1, potential participants were screened against inclusion and exclusion criteria by medical history and clinical examination, routine blood tests (*i.e.,* HbA1c) and an electrocardiogram (ECG). Visit 2a was performed >72 hours prior to Visit 2b to discuss and allow any arrangements in insulin regimens and lifestyle (diet, exercise). More specifically, participants were switched from insulin Lantus or Detemir to neutral protamine hagedorn (NPH) insulin 48 hours before Visit 2b. The NPH insulin was stopped 22 hours before Visit 2b-4, except for short acting insulin analogues, which were stopped 6-8 hours before that visit. Visits 2b-4 were the main experimental days, during which different environmental conditions were controlled

and the euglycaemic clamp was performed. Participants were weighed without shoes on a weighing scale (Marsden Weighing Machine Group Ltd, UK), height was taken barefoot using a wall-mounted stadiometer and BMI was calculated as body mass (kg) divided by the height squared (m^2). Blood pressure was measured using a sphygmomanometer (Datascope Duo Masimo Set, Mindray Ltd, UK). Blood glucose was continuously monitored pre-administration and for the duration of the clamp procedures. Standard safety parameters including blood pressure, heart rate and temperature were performed every 30 minutes throughout the study. Three to twenty-one days were allowed between Visit 2a, 3 and 4. Visit 5 was performed as a follow-up examination within 14 days of the last experimental day (Visit 2b, 3 or 4) and included physical examination and glycemic management review.

Euglycaemic glucose clamp procedure

Prior to the euglycaemic glucose clamp, all participants fasted overnight and for the duration of the 6-hour procedure. Water was allowed as required. In the clinic room, with the participant in a comfortable supine or semi-supine state, vital signs were recorded before two cannulas were inserted, one in the hand or forearm to be used for venous sampling, with the hand heated to 55°C throughout the clamp allowing arterialisation of the venous blood (21). The second cannula was inserted on the opposite arm situated in the cubital fossa to be used for a variable infusion of insulin [15 units of Humulin S in 49mL saline and 1mL of participants own blood] or glucose (20% in saline). The infusion was initiated with a target blood glucose level of 5.5mmol/L (100mg/dL) ± 20% for 30-60 min prior to the participant being relocated to the environmental chamber where baseline glucose levels were taken followed by the injection of insulin lispro (NovoFine 32G Tip etw 0.23/0.25 x 6mm, Novo Nordisk A/S, Denmark) on the left shoulder of the participants, equal to time 0.

In the environmental chamber, subjects were allowed to wear light clothes to mimic real life situations. The variable glucose infusion was used to maintain the target blood glucose level of 5.5mmol/L (100mg/dL) ± 20% guided by an algorithm (22) and the participants' measured blood glucose concentration in the preceding 5 min. The blood glucose concentrations were measured by a glucose analyser (HemoCue® glucose 201+) and

recorded together with the glucose infusion rate every 5–10 min throughout the clamp. Upon completion of the clamp procedure, vital signs were checked and lunch was provided before discharge.

Biochemical analysis

Venous blood samples were collected at Visit 1 as part of screening procedures. Plasma blood samples were centrifuged at 3,500×G for 15min at 5°C and analysed for HbA1c on a Menarini Diagnostics HB9210 premier (A.Menarini Diagnostics Ltd., Winnersh-Wokingham, UK).

Statistical analysis

The exogenous glucose infusion rate (GIR) was analysed every 5 to 10 minutes throughout the clamp. A weighted local regression technique (LOESS) with a smoothing factor (SF) of 0.1 for the calculation of time-related parameters and maximum GIR in accordance with previous studies that had investigated the pharmacodynamics of short-acting insulin (23). The pharmacodynamic endpoints calculated for each clamp study visit (Visit 2b, 3 and 4) were the maximum glucose infusion rate (GIR_{max}) and time to maximum glucose infusion rate (t_{GIRmax}). In addition to total area under the curve (AUC) for GIR from 0 to 6 hours min ($AUC_{GIR.0–6h}$), partial AUCs from 0-1 hour, 0-2 hours ($AUC_{GIR.0-1h}$), 0-6 hours ($AUC_{GIR.0-2h}$) and 2-6 hours ($AUC_{GIR.2-6h}$) following the insulin injection were also calculated to determine early and late insulin action. A two-way ANOVA with temperature, humidity and their interaction as fixed effects and the subject as random effect was used for $AUC_{GIR.0-1h}$, $AUC_{GIR.0-2h}$, $AUC_{GIR.0-6h}$, $AUC_{GIR.2-6h}$, $GIR_{max\ (SF=0.1)}$ and $t_{GIR.max(SF=0.1)}$. Data are presented as mean (1SD) and statistical significance was set at $p \leq 0.05$. For graphical presentation (Figure 1) a SF of 0.3 was used and 10 data points with GIR-values of nearly 40 mg/kg/min in one subject were excluded in order to minimise random GIR-fluctuations. Statistical analysis was conducted using SAS, version 9.4.

Results

The demographic and clinical characteristics of the adults with type 1 diabetes mellitus at baseline are presented in Table 1.

The independent effects of ambient temperature

As illustrated in Figure 1 and Table 2, at temperature 30°C and humidity 10% the time-action curve of insulin was shifted to the right, with a later $t_{GIR.max}$ (p=0.049) and a significantly greater GIR_{max} (p=0.04), compared to the condition at 15°C temperature and same level of humidity (10%). Although $AUC_{GIR.0\text{-}1h,}$ $AUC_{GIR.0\text{-}2h}$ and $AUC_{GIR.0\text{-}6h}$ did not differ significantly between the conditions with different temperatures, there was a trend towards a higher $AUC_{GIR\ 2\text{-}6h}$, when comparing temperature 30°C *vs.* temperature 15°C (p=0.08) (Table 2).

The independent effects of relative humidity

There was no effect of humidity on insulin pharmacodynamics, as indicated by no significant differences in GIR_{max}, $t_{GIR.max}$ and AUCs for the time-action profile between the condition at temperature 30°C and humidity 10% *vs.* the condition at temperature 30°C and humidity 60% (p values between 0.21 and 0.95) (Table 2).

The combined effects of ambient temperature and relative humidity

When exploring the combined effects of temperature and humidity, $t_{GIR.max\ (SF=0.1)}$ (p=0.008) occurred on average 44 min earlier ($AUC_{GIR.2\text{-}6h}$, p=0.017) at temperature 15°C and humidity 10% compared to temperature 30°C and humidity 60% (Figure 1, Table 2) with less glucose that needed to be infused at lower temperature and humidity, but no differences were seen for early ($AUC_{GIR.0\text{-}1h,}$ p=0.48, $AUC_{GIR.0\text{-}2h}$, p=0.87) and overall ($AUC_{GIR.0\text{-}6h,}$ p=0.48) effects on insulin action (Table 2).

Discussion

By using the glucose clamp technique, the present study demonstrated that sudden changes in environmental conditions affect short-acting insulin analogue (insulin lispro) pharmacodynamics in adult men with type 1 diabetes mellitus. In response to higher temperature (30°C *vs.* 15° C) under fixed humidity there was a greater GIR_{max} and a trend towards a greater $AUC_{GIR2\text{-}6h}$. High humidity affected insulin pharmacodynamics only when it was combined with high temperature. The mean time to GIR_{max} was prolonged under 30°C temperature and 10 or 60% humidity compared to 15° C and 10% humidity and the GIR_{max} and the late AUC ($AUC_{GIR2\text{-}6h}$) were greater, suggesting enhanced insulin absorption and peak effect.

A limited number of studies have simulated the effects of environmental conditions on insulin pharmacodynamics. Ronnemaa & Koivisto investigated the acute effects of ambient temperature (10° C *vs.* 30° C) with and without exercise on insulin absorption and postprandial glycaemia in patients with type 1 diabetes mellitus, but in a different experimental protocol without using a glucose clamp. They showed no significant effect of ambient temperature on total blood glucose AUC, calculated using glucose values from the time of insulin injection to the end of the study (195 min) (7), but significant effects were revealed for partial AUC from 80 min post injection to 195 min. These results are in accord with the results of the present study where there were no significant differences between the experimental conditions at 15°C and 30°C for $AUC_{GIR\ 0\text{-}6h}$, but a trend towards a greater $AUC_{GIR\ 0\text{-}6h}$ with a higher temperature. The same study (7) also assessed insulin pharmacokinetic parameters and showed a 3- to 5-fold higher AUC for plasma free insulin at 30°C than at 10°C, regardless of exercise. We cannot provide comparative data on these aspects, given that our study is limited to insulin pharcodynamics rather than its pharmacokinetic profile. Furthermore, it is more challenging to detect differences in pharmacodynamic parameters than in pharmacokinetic parameters, as pharmacodynamic parameters are often characterised by greater variability and, therefore, the pharmacokinetic results would be expected to be in line with the pharmacodynamic findings in our study.

Exposure to higher temperatures compared to the high temperature (30°C) investigated in this work has been shown to have favourable effects on time-action profiles of different types of insulin analogues. It is reported that sauna exposure (twice for 25 min at temperature 85°C and relative humidity 30-50%) accelerated insulin absorption by 110% (assessed by measuring the disappearance rate of ^{125}I-labelled rapid-acting insulin) compared with room temperature in 8 participants with diabetes (type 1 diabetes mellitus , n=7; type 2 diabetes mellitus, n=1) (15). Hot baths (water temperature ≥ 40°C) increased serum insulin levels 90 minutes after injection (14). Other studies have shown temperature effects on insulin pharmacodynamics, when heat is applied locally at the site of injection (10-13, 24). When a local heating device at the injection site ($InsuPatch_{TM}$) was utilised to achieve skin temperature of 38.5°C, the time to reach maximal action of a 0.2 U/kg bolus dose of insulin aspart decreased from 125 min to 90 min in adults with type 1 diabetes mellitus (13) and similarly at 40°C (12). Meal tolerance test studies showed that local heat resulted in significant reductions in the time to maximal insulin action and lower postprandial excursion in patients with type 1 diabetes mellitus (11, 24).

These data suggest that high ambient temperature increases subcutaneous insulin absorption due to effects on blood perfusion at the injection site. In line with these findings, we showed enhanced insulin action and a prolonged time to maximum infusion rate with higher temperature compared to lower temperature. The latter findings about the time to maximum infusion rate can be explained at least partially by a greater GIR_{max} observed in the condition with the higher temperature (*i.e.,* a greater GIR_{max} is expected to be reached later). The discrepancies between this and previous studies may be largely due to differences in the exposure to the heat (i.e., extent, locality and duration). Although measurements of skin temperature were not available in this study, these results are suggestive of a delayed thermoregulatory effect on subcutaneous tissue in the hotter environment (30^{o}C), which may explain the absence of earlier changes in the environment surrounding the insulin depot.

Conversely, we observed a shorter mean time to GIR_{max} under 15^{o}C and 10% humidity compared to 30^{o}C temperature and 10% humidity and a lower GIR_{max} and late AUC ($AUC_{GIR2\text{-}6h}$). These results are in agreement with a previous study by Vallerand et al. which showed that in response to an intravenous glucose tolerance test under nude exposure to cold (3h at 10^{o}C) plasma glucose area under the curve was lower and plasma glucose levels returned to baseline levels within an hour compared to 2h under warm exposure (3h at 29^{o}C) despite low insulin levels and enhanced carbohydrate metabolism (25). It is speculated that the marked effects of cold exposure may due to enhanced insulin sensitivity and/or increased responsiveness for glucose uptake in peripheral tissues such as skeletal muscles (25-27), although in the current study we cannot provide further insight into these mechanisms, given that subcutaneous insulin was used and therefore, other factors (e.g., visceral and subcutaneous tissues) may have differentially effect the pharmacodynamics parameters.

Short term exposure to different levels of relative humidity (10 and 60%) under fixed temperature had no effect on the insulin time-action profile. However, exposure to high relative humidity in combination with high ambient temperature resulted in a prolonged time to GIR_{max} and a greater insulin pharmacodynamic effect compared to the responses to the low temperature low humidity condition, suggesting that high humidity may augment the high temperature effect on enhanced insulin absorption from the injection site, but has little effect in its own right.

In conclusion, high ambient temperature resulted in greater insulin peak effect compared to low ambient temperature, with the contribution of high relative humidity to insulin absorption only apparent at high ambient temperature. This suggests that patients with type 1 diabetes mellitus entering environmental higher temperatures with or without high humidity could experience more hypoglycaemic events.

Acknowledgements

We thank all study participants for their commitment to this study. This study was supported by Hull and East Yorkshire Hospitals Charitable Funds. No potential conflict of interest relevant to this article was reported.

Authors' contributions

AA, ZJ, TH, ASR, ATG, DH, ESK, SLA and TS participated in study conception and design. AA performed the acquisition of data. AA, MP, TH, ASR, SLA and TS participated in analysis and/or interpretation of data. MP drafted the first draft of the paper; all authors reviewed and approved the final manuscript. TS is the guarantor of the study.

References

1. Heinemann L. Variability of insulin absorption and insulin action. Diabetes Technol Ther. 2002;4(5):673-82.
2. Freckmann G, Pleus S, Haug C, Bitton G, Nagar R. Increasing local blood flow by warming the application site: beneficial effects on postprandial glycemic excursions. J Diabetes Sci Technol. 2012;6(4):780-5.
3. Sohmiya M, Kanazawa I, Kato Y. Seasonal changes in body composition and blood HbA1c levels without weight change in male patients with type 2 diabetes treated with insulin. Diabetes Care. 2004;27(5):1238-9.
4. Ishii H, Suzuki H, Baba T, Nakamura K, Watanabe T. Seasonal variation of glycemic control in type 2 diabetic patients. Diabetes Care. 2001;24(8):1503-.
5. Tsujimoto T, Yamamoto-Honda R, Kajio H, Kishimoto M, Noto H, Hachiya R, et al. Seasonal Variations of Severe Hypoglycemia in Patients With Type 1 Diabetes Mellitus, Type 2 Diabetes Mellitus, and Non-diabetes Mellitus. Medicine. 2014;93(23).
6. Vazeou AG, Kordonouri O, Witsch M, Hermann JM, Forsander G, de Beaufort C, et al. Seasonality at the clinical onset of type 1 diabetes-Lessons from the SWEET database. Pediatr Diabetes. 2016;17:32-7.
7. Ronnemaa T, Koivisto VA. Combined effect of exercise and ambient temperature on insulin absorption and postprandial glycemia in type I patients. Diabetes Care. 1988;11(10):769-73.
8. Berglund L, Berne C, Svardsudd K, Garmo H, Melhus H, Zethelius B. Seasonal variations of insulin sensitivity from a euglycemic insulin clamp in elderly men. Ups J Med Sci. 2012;117(1):35-40.
9. Dillon RS. Improved serum insulin profiles in diabetic individuals who massaged their insulin injection sites. Diabetes Care. 1983;6(4):399-401.
10. Landau Z, Feldman D, Shusterman A, Wainstein J, Klonoff D, Nyberg I, et al. Improved pharmacokinetic and pharmacodynamic profile of insulin analogues using InsuPatch, a local heating device. Diabetologia. 2012;55:S54-S.
11. Raz I, Weiss R, Yegorchikov Y, Bitton G, Nagar R, Pesach B. Effect of a local heating device on insulin and glucose pharmacokinetic profiles in an open-label, randomized, two-period, one-way crossover study in patients with type 1 diabetes using continuous subcutaneous insulin infusion. Clin Ther. 2009;31(5):980-7.
12. Cengiz E, Weinzimer SA, Sherr JL, Tichy EM, Carria L, Cappiello D, et al. Faster in and faster out: accelerating insulin absorption and action by insulin infusion site warming. Diabetes Technol Ther. 2014;16(1):20-5.
13. Cengiz E, Weinzimer SA, Sherr JL, Tichy E, Martin M, Carria L, et al. Acceleration of insulin pharmacodynamic profile by a novel insulin infusion site warming device. Pediatr Diabetes. 2013;14(3):168-73.
14. Berger M, Cuppers HJ, Hegner H, Jorgens V, Berchtold P. Absorption Kinetics and Biologic Effects of Subcutaneously Injected Insulin Preparations. Diabetes Care. 1982;5(2):77-91.
15. Koivisto VA. Sauna-Induced Acceleration in Insulin Absorption from Subcutaneous Injection Site. Brit Med J. 1980;280(6229):1411-3.
16. Tyrovolas S, Chalkias C, Morena M, Kalogeropoulos K, Tsakountakis N, Zeimbekis A, et al. High relative environmental humidity is associated with diabetes among elders living in Mediterranean islands. J Diabetes Metab Disord. 2014;13(1):25.
17. Sharma VM, Pichan G, Panwar MR. Differential effects of hot-humid and hot-dry environments on mental functions. Int Arch Occup Environ Health. 1983;52(4):315-27.
18. Petrofsky J, Berk L, Alshammari F, Lee H, Hamdan A, Yim JE, et al. The effect of moist air on skin blood flow and temperature in subjects with and without diabetes. Diabetes Technol Ther. 2012;14(2):105-16.

19. Lim CL, Byrne C, Lee JK. Human thermoregulation and measurement of body temperature in exercise and clinical settings. Ann Acad Med Singapore. 2008;37(4):347-53.

20. Epstein Y, Roberts WO. The pathopysiology of heat stroke: an integrative view of the final common pathway. Scand J Med Sci Sports. 2011;21(6):742-8.

21. Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. Metabolism. 1981;30(9):936-40.

22. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. 1979;237(3):E214-23.

23. de la Pena A, Seger M, Soon D, Scott AJ, Reddy SR, Dobbins MA, et al. Bioequivalence and comparative pharmacodynamics of insulin lispro 200 U/mL relative to insulin lispro (Humalog(R)) 100 U/mL. Clin Pharmacol Drug Dev. 2016;5(1):69-75.

24. Freckmann G, Pleus S, Westhoff A, Krinelke LG, Buhr A, Jendrike N, et al. Clinical performance of a device that applies local heat to the insulin infusion site: a crossover study. J Diabetes Sci Technol. 2012;6(2):320-7.

25. Vallerand AL, Frim J, Kavanagh MF. Plasma-Glucose and Insulin Responses to Oral and Intravenous Glucose in Cold-Exposed Humans. J Appl Physiol. 1988;65(6):2395-9.

26. Vallerand AL, Zamecnik J, Jacobs I. Plasma-Glucose Turnover during Cold Stress in Humans. J Appl Physiol. 1995;78(4):1296-302.

27. Hanssen MJW, Hoeks J, Brans B, van der Lans AAJJ, Schaart G, van den Driessche JJ, et al. Short-term cold acclimation improves insulin sensitivity in patients with type 2 diabetes mellitus. Nat Med. 2015;21(8):863-5.

Figure Legends

Figure 1. Average glucose infusion rate (GIR) (mg/kg/min) values to maintain euglycaemia under different environmental conditions; T15/H10: temperature 15^{o}C and humidity 10%, T30/H10: temperature 30^{o}C and humidity 10% and T30/H60: temperature 30^{o}C and humidity 60%.

Table 1: Baseline characteristics of the participants (n=10).

	Adults with Type 1 diabetes (n=10)
Age (years)	28.3±7.1
Weight (kg)	74.1±12
Height (cm)	170.6±5.7
BMI (kg/m^2)	24.3±2.9
Systolic BP (mmHg)	124.2±9.4
Diastolic BP (mmHg)	75.6±7.5
Duration of diabetes (years)	18.8±7.7
HbA1c (%)	7.9±0.8
HbA1c (mmol/mol)	63±6.7

Data are presented as means ±1SD. BMI body mass index, BP blood pressure, HDL high density lipoprotein, LDL low density lipoprotein, HbA1C Haemoglobin A1c.

Table 2: AUC_{GIR} for 0-1h, 0-2h, 0-6h and 2-6 h, GIR_{max} and $t_{GIR.max}$

	$AUC_{GIR\cdot 0\text{-}1h}$ (mg/kg)		$AUC_{GIR\cdot 0\text{-}2h}$ (mg/kg)		$AUC_{GIR\cdot 0\text{-}6h}$ (mg/kg)	
	Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**	**Mean±SD**	**Min-Max**
T15/H10	136±251	0-835	347±496	12-1727	815±764	260-2823
T30/H10	94±65	0-180	314±162	29-537	825±453	37-1501
T30/H60	85±78	0-233	325±172	101-626	977±435	347-1606
P values	T15/H10 *vs.* T30/H10	0.56	T15/H10 *vs.* T30/H10	0.81	T15/H10 *vs.* T30/H10	0.96
	T15/H10 *vs.* T30/H60	0.48	T15/H10 *vs.* T30/H60	0.87	T15/H10 *vs.* T30/H60	0.48
	T30/H10 *vs.* T30/H60	0.90	T30/H10 *vs.* T30/H60	0.94	T30/H10 *vs.* T30/H60	0.51
	$AUC_{GIR.2\text{-}6h}$ (mg/kg)		GIR_{max} (mg/kg/min)		$t_{GIR.max}$ (min)	
	Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**	**Mean±SD**	**Min-Max**
T15/H10	467±319	114-1096	7.4±7.6	2.3-29	107±61.8	10-205
T30/H10	511±965	8-965	11.1±6.5	1.2-22	137±63	18-217
T30/H60	652±1196	140-1196	8.5±2.9	4-13	151±99	10-319
***p*-values**	T15/H10 *vs.* T30/H10	0.08	T15/H10 *vs.* T30/H10	***0.04***	T15/H10 *vs.* T30/H10	***0.049***
	T15/H10 *vs.* T30/H60	***0.008***	T15/H10 *vs.* T30/H60	0.57	T15/H10 *vs.* T30/H60	***0.017***
	T30/H10 *vs.* T30/H60	0.22	T30/H10 *vs.* T30/H60	0.21	T30/H10 *vs.* T30/H60	0.65

Data are presented as mean± 1SD, (min) and maximum(max) T15/H10: Temperature 15°C/Humidity 10%, T30/H10: Temperature 30° C/Humidity 10%, T30/H60: Temperature 30° C/Humidity 60%, AUC: area under the curve, GIR: glucose infusion rate, GIR_{max}: maximum glucose infusion rate, $t_{GIR.max}$: time to maximum glucose infusion rate. *P*-values <0.05 are indicated in bold italics. Statistical analysis was performed on the unsmoothed data.

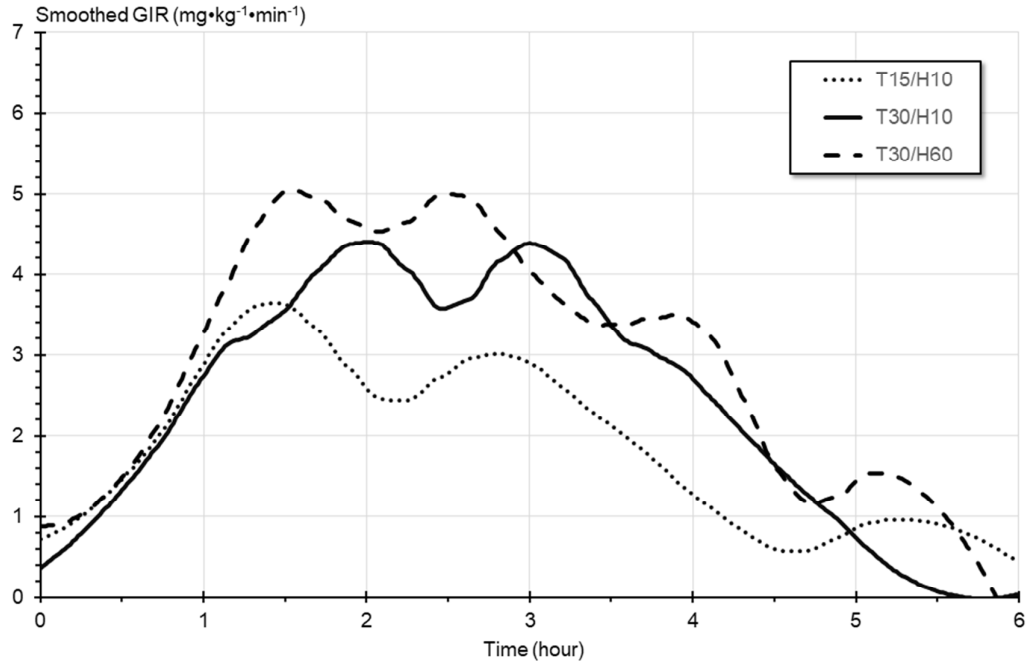

Figure 1.