# Interstitial glucose profile associated with symptoms attributed to hypoglycemia by otherwise healthy women

Elizabeth J Simpson*, Michelle Holdsworth [1], Ian A Macdonald

*University of Nottingham, School of Biomedical Sciences, Queen's Medical Centre, Nottingham, NG7 2UH, UK and* [1]*Division of Nutritional Sciences, Sutton Bonington campus, Loughborough, LE12 5RD, UK.*

*Corresponding Author ; EJ Simpson. Biomedical Sciences, E Floor Medical School, QMC, Nottingham. NG7 2UH, UK. Tel: +44 115 8230128, Fax : +44 115 8230142. E-mail: liz.simpson@nottingham.ac.uk

This study was funded by The Harold Hyam Wingate Foundation. London UK

Running header: IG profile associated with hypoglycemic symptoms

**Abstract**

**Background:** Reporting postprandial symptoms, attributed to 'hypoglycemia' by otherwise healthy individuals, appears to be a relatively common phenomenon in UK women. Whether these symptoms are related to blood glucose is a contentious issue, which periodic ambulatory blood glucose measurement has failed to resolve.

**Objective:** To investigate, using continuous glucose monitoring technology (Medtronic, MiniMed CGMS), whether postprandial symptoms are associated with interstitial glucose concentrations (IG) in the hypoglycemic range, or a prior fall in IG.

**Design:** Thirty non-obese, healthy women (20-48yr) reporting symptoms they attributed to hypoglycemia, and 20 non-symptomatic controls wore a subcutaneous CGMS probe in abdominal fat for 4-7d (median 5d), and kept a diet and activity diary during this time.

**Results:** Twenty women reported postprandial symptoms, with 41 episodes recorded. When symptomatic, IG was ≤3.3mmol/l in 5% of cases. A significant fall in IG over the preceding 60min was observed prior to autonomic symptoms (*P<0.005)*. The proportion of total energy intake derived from dietary fat in the symptomatic group, was higher than in controls (*P<0.05)*. The proportion of total sugars was similar between groups, however, the meal preceding symptoms showed a higher percentage of energy derived from total sugars when compared with the individuals' diet over the study period (*P<0.05*).

**Conclusions:** The majority of symptoms attributed to hypoglycemia were not associated with an IG in the hypoglycemic range. A prior fall in IG may be implicated in the aetiology of autonomic symptoms, with the consumption of meals high in sugars potentially playing a role in symptom initiation.

**Key words: Reactive Hypoglycemia, Women, Continuous glucose monitoring, Free-living, UK**

## Introduction

Postprandial or Reactive Hypoglycemia (RH) is a condition that has been popularised in the media and lay literature, particularly those targeting women, over the past 30 years (1). These sources and anecdotal evidence claim that many people, who are otherwise healthy, experience periodic symptoms such as faintness, irritability, tremor, hunger and anxiety, which can be attributed to low blood glucose concentration (BG) (2, 3). Indeed, in several countries, the number being referred to medical agencies for this condition had reached epidemic proportions (4, 5), although this is considered to have been less marked in the UK (6). A recent survey of randomly selected women from Nottinghamshire supports this, with only 0.5% of those reporting symptoms that they attribute to hypoglycaemia, having sought medical help (7). However, it does not appear that there is an absence of the phenomenon in the UK. This survey in Nottinghamshire revealed that more than one third reported symptoms that they attribute to a 'low blood sugar', with 18% reporting these symptomatic episodes more than once a week (8).

It has long been contentious whether symptoms are actually related to low BG (9-13), as biochemical hypoglycemia (defined by symptom thresholds from hyperinsulinemic clamp studies (14, 15)) is not commonly observed in symptomatic individuals when symptoms are experienced (6). However, periodic, ambulatory blood sampling, which has been used previously to investigate this condition, simply provides a 'snapshot' of postprandial BG, and it has been proposed that by the time symptoms are recognised and a blood sample taken, glucose may already be rising under the action of counter-regulatory hormones, such that true nadirs are being missed (1). Moreover, periodic, ambulatory blood sampling does not provide information on the BG profile prior to symptoms and thus can not elicit whether symptoms are associated with a prior fall in BG.

The MiniMed System Gold™ (Northridge, California USA) continuous glucose monitoring system (CGMS) is comprised of an electrochemical sensor attached via a wire to a monitor which is worn by the individual. The electrical current generated at the sensor electrode is proportional to the concentration of glucose in the interstitial fluid (16). Interstitial glucose concentration (IG) is measured every 10 seconds with these readings then averaged over 5mins by software in the monitor (17-19). CGMS therefore allows the researcher to study both the IG when symptoms are reported, and the glucose profile prior to developing symptoms, which addresses some of the limitations of periodic ambulatory sampling.

The primary purpose of this current study was to investigate whether women who report symptoms which suggest RH, but have not been referred for a clinical problem, have a concomitant IG in the hypoglycemic range when symptomatic, or demonstrate a fall in IG prior to symptoms.

## Methods

*Protocol*

Thirty non-obese, healthy women (aged 18-50yr), who reported symptoms that they attribute to hypoglycemia more than once a week, and 20 non-symptomatic controls, were recruited onto the study. All volunteers were asked to complete a medical screening form prior to recruitment, to ensure that they were healthy and satisfied inclusion criteria. Volunteers subsequently attended the laboratory in the morning, after an overnight fast. A venous sample for HbA1c, whole blood glucose and serum insulin determination, was taken from each individual, and analysed by reversed-phase cation exchange chromatography (Menarini Diagnostics, Florence, Italy), the glucose oxidase method (YSI inc, Yellow Springs, USA) and radio-immunoassay (Euro DPC, Caernarfon, UK) respectively. Basal insulin sensitivity was then calculated from fasting glucose and insulin measures, using the homeostasis model assessment for insulin resistance (HOMA-IR) (20) and quantitative insulin-sensitivity check index (QUICKI) (20).

BMI was calculated from measured height and weight.

Following training and familiarisation with the measurement techniques to be used, a small (23G) subcutaneous probe, for continuous IG monitoring, was inserted into subcutaneous fat in the abdomen at the level of the umbilicus. This probe was attached to the CGMS monitor, which was worn continuously for the active life span of the probe. Volunteers were asked to push an event marker button on the monitor each time they ate and if applicable, to enter a different marker when they were symptomatic. They were also asked not to change their normal eating or activity patterns during the study and to keep a food and activity diary during this time. Volunteers were blinded to their IG during the recording period.

The study was carried out in accordance with the Helsinki Declaration of 1975 (revised 1983) and was approved by the Nottingham University Medical School Ethics Committee.

*CGMS Calibration*

Capillary glucose concentration was measured, by the volunteer, four times a day from finger prick blood samples taken using a 'Penlet II' and analysed using either a 'One Touch Profile' (Lifescan, High Wycombe, UK) or a 'Glucotrend 2' pocket glucose analyser (Roche, Basel, Switzerland). Readings were then entered into the CGMS monitor to calibrate the subcutaneous probe. Data were downloaded from the monitor at the end of the study period and the mean absolute difference between the capillary BG and the corresponding IG was calculated by MiniMed Solutions$^{TM}$ software (MMT-7310, version 3.0.128). If the mean absolute difference was >15%, the CGMS data were not used for that day. In addition, only days with complete data over the 24hr period (12 midnight to 12 midnight) were included in the analysis. As a consequence the first and last day of recording were discarded.

Prior to use, all 3 pocket glucose analysers used in the current study were validated against the glucose oxidase method (YSI inc, Yellow Springs, USA) in a separate study, using a hyperinsulinemic clamp (21) to generate 40 blood samples between 2.5 and 7.0 mmol/l. $R^2$ of the glucose oxidase method vs. the pocket monitors was 0.991, 0.995 and 0.997 respectively.

*Symptoms of hypoglycemia*

To avoid biasing the responses, symptoms of hypoglycemia were not defined for participants and any experiences that an individual attributed to a low blood 'sugar' were

taken as a symptomatic event. All such events were noted in a diary with time of occurrence and symptoms experienced being recorded.

In subsequent analysis of the diaries, symptomatic episodes were sub-divided into those associated with neuroglycopenia ('neurogenic' symptoms) and those associated with the release of epinephrine ('autonomic' symptoms), according to definitions described by Brun *et al* and others (1, 14, 22, 23), with those episodes associated with a combination of autonomic and neurogenic symptoms being classified as 'mixed'. These authors defined symptoms such as fatigue, headache, dizziness and 'difficulty in thinking' as neurogenic, with palpitations, sweating, anxiety and tremor described as autonomic. This allowed the more non-specific symptoms associated with the 'neurogenic' category to be investigated separately from the more specific ones associated with the 'autonomic' category. Where individuals experienced more than one symptomatic episode, each was considered separately and only those episodes that occurred <4hrs after eating were classified as postprandial.

*Interstitial glucose concentration recording*

The 'symptom' event marker was defined as t = 0 and IG at this time was taken as the symptomatic value. The concentration of interstitial glucose when symptomatic was classified as 'hypoglycemic' if ≤3.3mmol/l, according to definitions from symptom threshold studies (14) and those previously used in literature relating to reactive hypoglycemia (6, 24). IG data from 60mins preceding t = 0 and 30mins after this point were extracted for analysis. Symptomatic events were defined as being associated with a prior fall in IG if the glucose concentration decreased by ≥0.5mmol/l over the preceding 30min ('rate of fall' equivalent to 1mmol/l.hr). This arbitrary value for the decrease in IG was applied to the data as it has been used previously in studies investigating the

relationship between 'rate of blood glucose fall' and symptom thresholds in studies using insulin induced hypoglycemia (25, 26).

To investigate evidence of counter-regulation occurring after t = 0, it was necessary to select only those symptomatic events where volunteers did not eat, as food consumption would make it difficult to determine whether any increase in glucose concentration observed after the event marker reflected a postprandial rise, or a response to hypoglycemia. The majority of volunteers (78%) ate within 30min of experiencing symptoms, with 56% and 36% eating within 20min and 15min of symptoms respectively. To maximise the number of suitable data points and enable statistical analysis to be carried out, the IG profile from t=0 to t=15min was studied in the 64% of volunteers who had not eaten during this time.

To provide a comparator for t = 0 in the control group, the IG at 2hr 25mins (2.42hrs) after breakfast, lunch and dinner on the first complete day of recording was selected. If another eating episode occurred within 2.42hrs of consuming the selected meal, the equivalent meal on the next recording day was used. Corresponding IG data for non-symptomatic occasions were also extracted from the recordings made in the symptomatic group. If symptoms occurred within the time period, the equivalent meal on the next recording day was used, provided it was a non-symptomatic occasion. IG data from the 3 meals were then averaged to provide a postprandial IG profile for each individual, with individual data then combined to provide mean group profiles. The 2.42hr time point was chosen as this was the closest IG reading to the mean time that postprandial symptoms were reported by the symptomatic subjects in the current study.

For further comparison of symptomatic individuals with controls, characteristics of IG over every complete 24h period were averaged to obtain mean values in each subject, with these individual values then used to calculate group mean data. Similarly, 24h IG data from the

symptomatic group were subsequently subdivided to obtain mean values in each subject for days when symptoms were experienced (symptomatic days). These individual values were then used to calculate group mean data for symptomatic days. Fasting IG was determined from the average concentration in the 30min prior to waking, with time of waking documented by the volunteer in their diary. IG values 2hr post all meals and 3hr after eating breakfast were extracted from the data to use as comparators between the symptomatic and control group. IG values after breakfast were chosen as this meal showed the least variability in terms of energy intake, between subjects.

*Diet and Activity diaries*

Volunteers were requested to keep a diet diary for the duration of the study, with all food intake, including snacks and drinks, documented using household measures to estimate portion size. Participants were also asked to record all activities of daily living and sleep periods in the diary. This included all aspects of activity, not just formal exercise periods. Diaries were subsequently analysed using a food composition and activity database (WISP V2, Tinuviel Software, Anglesey, UK).

To calculate diet composition, individual macronutrient intake over all days of the recording period, were expressed as a percentage of total energy intake. Individual data were then combined to provide mean diet composition for each group. In the symptomatic group, the composition of the meal preceding symptoms was calculated, being expressed as a percentage of total energy of that meal.

Activity over the duration of the study was calculated as multiples of resting energy expenditure (METs) (27). Resting energy expenditure (REE) for each subject was estimated using the Schofield Equation (28), and mean daily energy expenditure was calculated by

multiplying this estimate of REE by the individual's mean MET value. Individual data were then used to derive mean activity levels for each group.

*Statistical analysis*

All data were coded and analysed using SPSS version 14.0 (Statistical Package for the Social Sciences 2005. SPSS Inc. Chicago, Illinois. USA).

Normally distributed group data are expressed as means with the standard deviation shown in parentheses, whilst non-parametric data are summarised in terms of the group median and range. For normally distributed mean data, comparisons between two groups were analysed using either unpaired or paired samples t-test where appropriate, with the Mann-Whitney test being employed where data were not normally distributed.

Comparison of mean data between more than 2 groups and analysing the change in group IG profiles over time, employed one-way ANOVA, and comparison of symptomatic IG profiles over time between groups was carried out using 2-way ANOVA (with repeated measures). Relationships were considered significant when $P < 0.05$.

## Results

*Subjects*

The test and control group were matched for age and BMI (*P=0.221* and *P= 0.189* respectively), with HbA1c, and fasting BG, being statistically similar between groups (*P=0.746*, *P=0.569,* respectively) **(Table 1)**. However, there was a trend for a greater fasting serum insulin concentration (*P=0.064)* and lower insulin sensitivity/higher insulin resistance measures (Quicki: *P=0.072*, HOMA-IR: *P=0.091*) in the control group, although all values were in the normal range. Of the 30 participants recruited in the test group, 22 experienced a symptomatic episode during the recording period, with 20 experiencing symptoms <4hrs after eating. This symptomatic subset of 20 were representative of the original cohort with respect to age and BMI (*P=0.160* and *P=0.289* respectively) and, as before, did not differ statistically from controls with respect to HbA1c (*P=0.764*), or fasting blood glucose (*P=0.192*). The serum insulin concentration, QUICKI and HOMA-IR measures in this subset were not different from the overall test group, but there was no longer a statistical trend for them to differ from the control group.

***Table 1 here***

*24hr IG Data*

Participants wore the subcutaneous probe for 4-7 (median 5) days. Comparison of the 24hr IG profiles, in terms of the variables shown in **Table 2**, showed no significant differences between the symptomatic group and controls, or any indication of differences in terms of trends. There was no statistical distinction seen between the 2hr postprandial (*P=0.684*) or 3hr post-breakfast (*P=0.792*) IG 'comparator' of each group. Moreover, all the 24hr IG variables, recorded on days when symptoms were reported, were statistically similar to control data.

**Figure 1a** shows the mean time course of IG (for 3 meals) over a 90min period around the 2.42hrs postprandial time point in the symptomatic group when no symptoms were reported and in controls. There were no significant changes in IG over this time period for either group and no statistical differences observed between the groups (*P=0.807*).

***Table 2 here***

*Hypoglycemic episodes*

In total, 50 symptomatic episodes were recorded. At the time symptoms were reported in the CGMS (t=0), mean IG was 4.45 (0.83) mmol/l (range 3.0 - 6.2), with 2 (4%) of the 50 'hypoglycemic' episodes being ≤ 3.3mmol/l (both being 3.0mmol/l). Mean time symptoms were experienced after eating was 2.88(1.32) hr (2hr 53min (SD 1hr 19min). Fifteen episodes were classified as 'autonomic', 25 as 'neurogenic' and 10 as 'mixed'. However 1 autonomic (4.92hr), 3 mixed (4.25-6.0hr) and 5 neurogenic (4.17-5.42hr) episodes were experienced >4hr after eating. None of these 'non-reactive' symptomatic episodes were associated with a prior fall in IG, or a glucose concentration of ≤3.3mmol/l and were omitted from further analysis, leaving 41 symptomatic episodes (**Table 3**). Two (5%) of these 41 postprandial episodes recorded an IG of ≤3.3mmol/l at t=0. At the corresponding postprandial time point (2.42hr) in the control group, there were no IG values ≤3.3mmol/l (Figure 1b).

***Table 3 here***

When episodes were sub-divided by symptom type, mean IG and the mean time after eating when symptoms were reported, were not statistically different among the three groups (*P=*

*0.363* and *P=0.785* respectively). Where individuals contributed more than one symptomatic episode to a group, the n used for SEM calculations was the number of subjects, not the number of symptomatic episodes. Comparison of the mean IG profiles shown in **Figure 1b** (-60 to 0 mins), using 2-way ANOVA (with repeated measures), indicated that the three symptomatic curves differed over time (P<0.01). There was a trend for mean IG to fall in the 60min preceding neurogenic symptoms (*P= 0.086*). Further analysis of this curve revealed that IG did not change between -60 and -10min (*P=0.229*), but a significant fall in glucose concentration (at a rate equivalent to 1.1mmol/l.hr) occurred between -10 min and t=0 (*P<0.05*). By contrast, a significant change in IG over the preceding 60min prior to autonomic symptoms was observed (*P<0.005*), at a rate of fall over the linear section of the curve (-55min to -10min) equivalent to 1.0 mmol/l.hr. The IG profile prior to 'Mixed' symptoms and controls did not show any change over the preceding 60min (*P=0.457*and *P=0.221* respectively).

***Fig.1a & b here***

When individual data were studied, 6 out of 20 (30%) of events associated with neurogenic symptoms followed a decrease in IG of ≥0.5mmol/l, from a peak, over the previous 30min, compared with 8 out of 14 (57%) for autonomic episodes and 0 out of 7 for episodes with mixed symptoms ($\chi^2$ testing was not valid). In control subjects, this fall in IG of >0.5mmol/l was seen in 2 out of the 20 (10%) postprandial profiles.

After symptoms were reported, both the neurogenic and autonomic groups demonstrated a significant rise in IG over the subsequent 30min (*P<0.05* for neurogenic and *P<0.001* for autonomic events), with no change being observed after t=0 in the 'mixed' group or controls (*P=0.779* and *P=0.490* respectively). However when only those neurogenic

episodes not followed by food intake were analysed, (n=15), there was no significant change in IG over the subsequent 15min (*P=0.253*), whereas autonomic episodes (n=8) were followed by a significant rise, at a mean rate equivalent to 1.2mmol/l.hr (*P<0.05*).

*Dietary & Activity Diaries*

Energy intake, daily activity levels and energy balance were not statistically different between the original test group and controls (*P=0.497, P=0.341, P=0.789* respectively) (Table 1), although there was a trend for a greater energy expenditure (*P=0.078*), in the control group. When the symptomatic group (n=20) were compared with controls, energy intake (*P=0.990*), daily activity levels (*P=0.465*) and energy balance (*P=0.538)*) remained matched and the trend for higher energy expenditure in the control group was no longer observed (*P=0.202)*.

The mean macronutrient composition of the diet during the study period statistically differed only in the percentage of total energy intake derived from fat, with controls consuming a smaller proportion than the symptomatic group (*P<0.05*). The macronutrient composition of the meal preceding symptoms showed a higher proportion of energy derived from total sugars, when compared with the individuals' diet over the study period (*P<0.05*). Moreover, there was a trend for this meal to have a lower proportion of protein (*P=0.083*) and higher proportion of carbohydrate (*P=0.051*) **(Table 4)**.

***Table 4 here***

## Discussion

To our knowledge, the current study is the first to record IG continuously in free-living, non-diabetic subjects who report symptoms that they attribute to hypoglycemia. Most research in this area has focussed on patient groups. However, despite over a third of women in Nottinghamshire UK reporting the experiencing of periodic symptoms (8), there appears to be a low incidence of medical referrals for the condition in the UK (7). As media articles in the UK generally do not present RH as a disease state, but a consequence of a diet high in refined carbohydrate, it seems likely that rather than the UK population being exempt from this phenomenon, symptomatic individuals are similar to those seeking medical help in other countries, but regard their symptoms as benign. Indeed, many studies in the literature investigating RH have used patient groups presenting only with self-reported symptoms that suggest hypoglycemia (6, 24, 29-31). Moreover, a similar non-referral population had previously demonstrated lower capillary blood glucose 3hr after eating, when compared with non-symptomatic controls (32), and a similar incidence of symptomatic BG readings below 3.3mmol/l when compared to other reports in the patient literature (6, 24).

It has long been contentious whether symptoms are actually related to low BG (10, 11), as previous studies using periodic finger prick sampling have not been able to provide information regarding the BG profile preceding the development of symptoms, and biochemical hypoglycemia (defined by symptom thresholds from hyperinsulinemic clamp studies (14)) has not commonly been observed in symptomatic individuals when symptoms were experienced. However, the current data reinforce findings from these other studies, that symptomatic events associated with capillary BG of ≤3.3mmol/l are uncommon (incidence range: 0-17%) (6, 24, 32).

Hyperinsulinemic clamp studies have been employed to investigate BG thresholds at which symptoms of hypoglycemia develop in healthy individuals (14, 25, 33) and these values have been used to define 'hypoglycemia'. However, the prerequisite in the RH literature for BG to be ≤3.3 mmol/l when symptoms are experienced, for diagnosis of hypoglycemia to be confirmed, may be too limited. Brun *et al* reported that after consumption of a high glycemic index breakfast, individuals prone to RH reported symptoms at higher BG (4.0 mmol/l) than traditional threshold values (1). Moreover, volunteers are usually semi-supine during protocols used to investigate symptom thresholds, whereas it has been shown that symptoms and physiological responses to insulin induced hypoglycemia are increased when upright (34, 35). Therefore symptom thresholds, defined by hyperinsulinemic clamp studies, may underestimate hypoglycemia in free-living, ambulatory individuals.

Hyperinsulinemic clamp studies also conclude that rate of fall of BG does not affect whether hypoglycemic symptoms are experienced and that absolute BG determines the initiation of these symptoms (25, 26). However, in the current study, a fall in IG appeared, in some cases, to be associated with initiation of symptoms without an accompanying IG in the hypoglycemic range. Indeed, in just under a third (32%) of all postprandial symptomatic episodes recorded, symptoms were preceded by a fall in IG, but were not associated with a glucose concentration of ≤3.3mmol/l.

Studies of iatrogenic hypoglycemia in diabetes routinely classify symptoms as neurogenic or autonomic, although episodes of hypoglycemia can be accompanied by symptoms from both categories. However, the non-specificity of these symptoms has long been a problem when researching the condition of RH and has led to the condition being dismissed as a non-disease (4). Symptoms classified as 'neurogenic' tend to be vague and could be related to other situations such as poor sleeping habits, caffeine withdrawal, or hypotension. Indeed, in the current study, the majority of neurogenic symptomatic episodes (70%) were

not associated with an IG ≤3.3mmol/l or a prior fall in this variable. Moreover, in those episodes not followed by consumption of food, a rise in IG after symptoms, to suggest counter-regulation by the body, was not observed. It is theoretically possible that dysfunction in glucose transport across the blood-brain barrier may be present, such that neuroglycopenia was occurring despite a subcutaneous IG in the euglycemic range. However, such a dysfunction would not be expected to be intermittent and it is likely that explanations other than those relating to BG regulation are pertinent in these cases. 'Autonomic' symptoms are more specific, but could equally be explained by other situations, unrelated to hypoglycemia, but characterised by an epinephrine mediated response. In these cases, symptoms would not necessarily be related to prior changes in IG, although as a consequence of epinephrine release into the blood, a rise in IG would be expected. Indeed, in those episodes not followed by eating, an increase in IG was observed after symptoms were noted, suggesting that symptoms may have been mediated by epinephrine. Moreover, a prior fall in mean IG was associated with the initiation of autonomic symptoms, and 64% of the individual episodes were either preceded by a decrease in IG of ≥0.5mmol/l over the preceding 30mins, or accompanied by a glucose concentration of ≤3.3mmol/l. However, whether this observation is of clinical significance is unclear, as it was difficult to determine what was unique about these IG profiles (in terms of magnitude or 'rate of fall') to cause a response, when at other times similar IG curves did not induce symptoms. Indeed no differences in mean 24hr IG data could be determined between groups, and the IG profiles around the 2.42hr postprandial time point for both the controls and the symptomatic group on a non-symptomatic occasion did not differ. Therefore it seems reasonable to conclude that the experiencing of these symptoms does not reflect a pathological state with regards to glucose regulation. However, it was interesting to observe that symptomatic IG profiles prior to t=0 were numerically lower than

corresponding IG values in the control group, or in the symptomatic group when asymptomatic. Although it is not ideal to compare symptomatic IG curves with an arbitrary postprandial time period, it suggests that symptomatic events may be associated with small changes in the glucose level around which IG is regulated.

The link between rapidly absorbable sugars and RH long been postulated (13, 36), and is presented as a truism in the media. Previous research and the current study did not observe any differences in the proportion of energy derived from carbohydrate and total sugars in the habitual diet of symptomatic individuals when compared to controls (6, 32). However, a trend for higher total sugars intake (as a proportion of total energy) has previously been reported in symptomatic individuals on days when symptoms were reported (when compared with controls) (32), and in the current study the composition of the meal preceding symptoms showed a higher proportion of energy derived from total sugars, when compared with the individuals' diet over the study period, and that of controls. We recognise the potential problems of comparing the composition of a single meal with that of a complete diet. However, symptoms were recorded at all times of the waking day, so the previous meal represents a variety of different meals in the different subjects. Clearly more work is needed to further investigate the potential role of dietary sugars in RH.

In conclusion, in the current study the majority of symptoms attributed to low BG, by otherwise healthy women, were not associated with hypoglycemia (as assessed by CGMS and defined by counter-regulatory threshold studies). It appears that the experiencing of autonomic symptoms was related to changes in IG, whereas most neurogenic and all 'mixed' symptomatic episodes were unrelated to IG dynamics. It did not appear that the experiencing of symptoms reflected pathology in glucose regulation in these individuals and factors other than BG dynamics *per se* must be involved in the initiation of symptoms, with the consumption of meals high in sugars potentially playing a role.

**Acknowledgements**

Contribution of authors to the work;

EJ Simpson: Study design, collection of data, analysis of data, writing the manuscript

M Holdsworth: Provision of significant advice & consultation regarding study design and interpretation of data, plus review of the manuscript

IA Macdonald: Provision of significant advice & consultation regarding study design and interpretation of data, plus review of the manuscript

None of the authors have any conflict of interests

**References**


1. Brun JF, Fedou C, Mercier J. Postprandial reactive hypoglycemia. Diabetes Metab. 2000 Nov;26(5):337-51.

2. Heller RF, Heller RF. The Carbohydrate Addict's Lifespan Program: Personalized Plan for Becoming Slim, Fit & Healthy in Your 40's 50's 60's and Beyond. Mass Market Paperback ed: Signet Book; 2001.

3. Atkins RC. Dr Atkins New Diet Revolution. Vermillion ed. London: Random House; 2003.

4. Yager J, Young RT. Non-Hypoglycaemia is an epidemic condition. New England Journal of Medicine. 1974;291:907-8.

5. Lefebvre PJ, Andreani D, Marks V, Creutzfeldt W. Statement on post-prandial or reactive hypoglycaemia. Diabet Med. 1988 Mar;5(2):200.

6. Snorgaard O, Binder C. Monitoring of blood glucose concentration in subjects with hypoglycaemic symptoms during everyday life. BMJ. 1990 Jan 6;300(6716):16-8.

7. Simpson E, Holdsworth M, Macdonald I. Prevalence of self reported hypoglycaemic symptoms in the general female population of Nottinghamshire. Int J Behav Med. 2002;9(Supp 1):251.

8. Simpson EJ, Holdsworth M, Macdonald IA. Prevalence of self-reported symptoms attributed to hypoglycaemia within a general female population of the UK. J Psychosom Res. 2006 Apr;60(4):403-6.

9. Marks V. Hypoglycemia--real and imaginary. Horm Metab Res. 1976;Suppl 6:127-8.

10. Gastineau CF. Is reactive hypoglycemia a clinical entity? Mayo Clin Proc. 1983 Aug;58(8):545-9.

11. Service FJ. Hypoglycemia and the postprandial syndrome. N Engl J Med. 1989 Nov 23;321(21):1472-4.

12. Lefebvre PJ. Hypoglycemia or non-hypoglycemia. Acta Clin Belg. 1997;52(4):241-4.

13. Hofeldt FD. Reactive hypoglycemia. Endocrinol Metab Clin North Am. 1989 Mar;18(1):185-201.

14. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, et al. Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. Am J Physiol. 1991 Jan;260(1 Pt 1):E67-74.

15. Snorgaard O, Lassen LH, Rosenfalck AM, Binder C. Glycaemic thresholds for hypoglycaemic symptoms, impairment of cognitive function, and release of counterregulatory hormones in subjects with functional hypoglycaemia. J Intern Med. 1991 Apr;229(4):343-50.

16. Buckingham BA, Kollman C, Beck R, Kalajian A, Fiallo-Scharer R, Tansey MJ, et al. Evaluation of factors affecting CGMS calibration. Diabetes Technol Ther. 2006 Jun;8(3):318-25.

17. Boyne MS, Silver DM, Kaplan J, Saudek CD. Timing of changes in interstitial and venous blood glucose measured with a continuous subcutaneous glucose sensor. Diabetes. 2003 Nov;52(11):2790-4.

18. Steil GM, Rebrin K, Mastrototaro J, Bernaba B, Saad MF. Determination of plasma glucose during rapid glucose excursions with a subcutaneous glucose sensor. Diabetes Technol Ther. 2003;5(1):27-31.

19. Steil GM, Rebrin K, Hariri F, Jinagonda S, Tadros S, Darwin C, et al. Interstitial fluid glucose dynamics during insulin-induced hypoglycaemia. Diabetologia. 2005 Sep;48(9):1833-40.

20. Cutfield WS, Jefferies CA, Jackson WE, Robinson EM, Hofman PL. Evaluation of HOMA and QUICKI as measures of insulin sensitivity in prepubertal children. Pediatr Diabetes. 2003 Sep;4(3):119-25.

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

22. Virally ML, Guillausseau PJ. Hypoglycemia in adults. Diabetes Metab. 1999 Dec;25(6):477-90.

23. Deary IJ, Hepburn DA, MacLeod KM, Frier BM. Partitioning the symptoms of hypoglycaemia using multi-sample confirmatory factor analysis. Diabetologia. 1993 Aug;36(8):771-7.

24. Palardy J, Havrankova J, Lepage R, Matte R, Belanger R, D'Amour P, et al. Blood glucose measurements during symptomatic episodes in patients with suspected postprandial hypoglycemia. N Engl J Med. 1989 Nov 23;321(21):1421-5.

25. Amiel SA, Simonson DC, Tamborlane WV, DeFronzo RA, Sherwin RS. Rate of glucose fall does not affect counterregulatory hormone responses to hypoglycemia in normal and diabetic humans. Diabetes. 1987 Apr;36(4):518-22.

26. Mitrakou A, Mokan M, Ryan C, Veneman T, Cryer P, Gerich J. Influence of plasma glucose rate of decrease on hierarchy of responses to hypoglycemia. J Clin Endocrinol Metab. 1993 Feb;76(2):462-5.

27. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc. 2000 Sep;32(9 Suppl):S498-504.

28. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr. 1985;39 Suppl 1:5-41.

29. Brun JF, Fedou C, Bouix O, Raynaud E, Orsetti A. Evaluation of a standardized hyperglucidic breakfast test in postprandial reactive hypoglycaemia. Diabetologia. 1995 Apr;38(4):494-501.

30. Buss RW, Kansal PC, Roddam RF, Pino J, Boshell BR. Mixed meal tolerance test and reactive hypoglycemia. Horm Metab Res. 1982 Jun;14(6):281-3.

31. Hubinger A, Schimansky F, Gries F. Idiopathic rective hypoglycaemia: The role of glucose counterregulatory hormones during the five hour glucose tolerance test. Diabetes, Nutrition & Metabolism - Clinical & Experimental. 1991;4(3):189-94.

32. Simpson E, Holdsworth M, Macdonald I. Ambulatory blood glucose measurement, dietary composition and physical activity levels in otherwise healthy women reporting symptoms that they attribute to hypoglycaemia. British Journal of Nutrition. 2006;95:1127-33.

33. Schwartz NS, Clutter WE, Shah SD, Cryer PE. Glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. J Clin Invest. 1987 Mar;79(3):777-81.

34. Robinson AM, Parkin HM, Macdonald IA, Tattersall RB. Physiological response to postural change during mild hypoglycaemia in patients with IDDM. Diabetologia. 1994 Dec;37(12):1241-50.

35. Maggs DG, MacDonald IA. Physiological and symptomatic responses to postural change in non-diabetic subjects during hypoglycaemia. Clin Sci (Lond). 1994 Aug;87(2):193-9.

36. Harris S. Hyperinsulinism and Dysinsulinism. JAMA. 1924;83(10):729-33.

Table 1: Characteristics of all subjects (test group who report 'hypoglycemic' symptoms: non-symptomatic control group) and a subset of the test group who experienced postprandial symptoms during the recording period (symptomatic group). Normally distributed data are expressed as means with SD in parentheses, and were compared using an unpaired t-test. Non-parametric data are expressed as the median with range in parentheses, and were compared using the Mann Whitney test. No statistical differences were observed.

| | Control Group (n=20) | Test Group (n=30) | Symptomatic Group (n=20) |
|---|---|---|---|
| Median Age (yrs) | 25 (20-38) | 26 (20-48) | 26 (20-48) |
| Mean BMI ($kg/m^2$) | 22.8 (2.17) | 21.9 (2.40) | 22.2 (2.64) |
| Mean HbA1c (%) | 5.18 (0.23) | 5.16 (0.21) | 5.16 (0.20) |
| Mean Fasting blood glucose (mmol/l) | 4.04(0.37) | 3.98 (0.35) | 3.89 (0.32) |
| Median Fasting Insulin (mIU/l) | 3.35 (2.02-6.70) | 3.12 (1.71-8.24) | 3.13 (1.77-8.24) |
| Mean QUICKI | 0.42 (0.02) | 0.43 (0.03) | 0.43 (0.03) |
| Median HOMA-IR | 0.62 (0.40-1.30) | 0.54 (0.28-1.45) | 0.55 (0.28-1.45) |
| Mean Activity (METS) | 1.64 (0.14) | 1.60 (0.12) | 1.61 (0.87) |
| Mean Energy expenditure (kJ) | 9725 (863) | 9175 (1060) | 9313 (1000) |
| Mean Energy Intake (kJ) | 9751 (1986) | 9357 (1797) | 9743 (2003) |
| Mean Energy balance (kJ) | 26 (1930) | 182 (1862) | 430 (1907) |

Table 2: Comparison of 24hr interstitial glucose concentration (IG) characteristics between the symptomatic group and controls. Data are expressed as means, with SD in parentheses Control data were compared with symptomatic group data using unpaired t-tests and within the symptomatic group, symptomatic days were compared to all days using paired t-test analysis. No statistical differences were observed.

| | Control Group (n=20) | Symptomatic Group (n=20) | Symptomatic days (n=20) |
|---|---|---|---|
| IG over 24hr period, from 12 midnight (mmol/l) | 4.84 (0.49) | 4.86 (0.34) | 4.88 (0.46) |
| Fasting IG (mmol/l) | 4.36 (0.66) | 4.42 (0.49) | 4.43 (0.64) |
| % of time IG was below fasting values | 26.4 (12.3) | 29.7 (11.7) | 31.3 (15.5) |
| % of time IG was above fasting values | 69.3 (13.0) | 65.6 (12.5) | 64.2 (16.2) |
| 'Maximum' IG during recording period (mmol/l) | 6.80 (0.67) | 6.68 (0.90) | 6.69 (1.00)) |
| 'Minimum' IG during recording period (mmol/l) | 3.53 (0.55) | 3.59 (0.49) | 3.61 (0.62) |
| 2hr Postprandial IG – all meals (mmol/l) | 4.88 (0.48) | 4.96 (0.70) | 4.95 (0.79) |
| 3hr Post breakfast IG (mmol/l) | 4.79 (0.62) | 4.73 (0.61) | 4.58 (0.62) |

Table 3: Characteristics of interstitial glucose concentration (IG) when postprandial symptoms were experienced. Mean data are expressed with SD in parentheses. One way ANOVA was used to compared the 3 subgroups defined by symptom type. No statistical differences were observed.

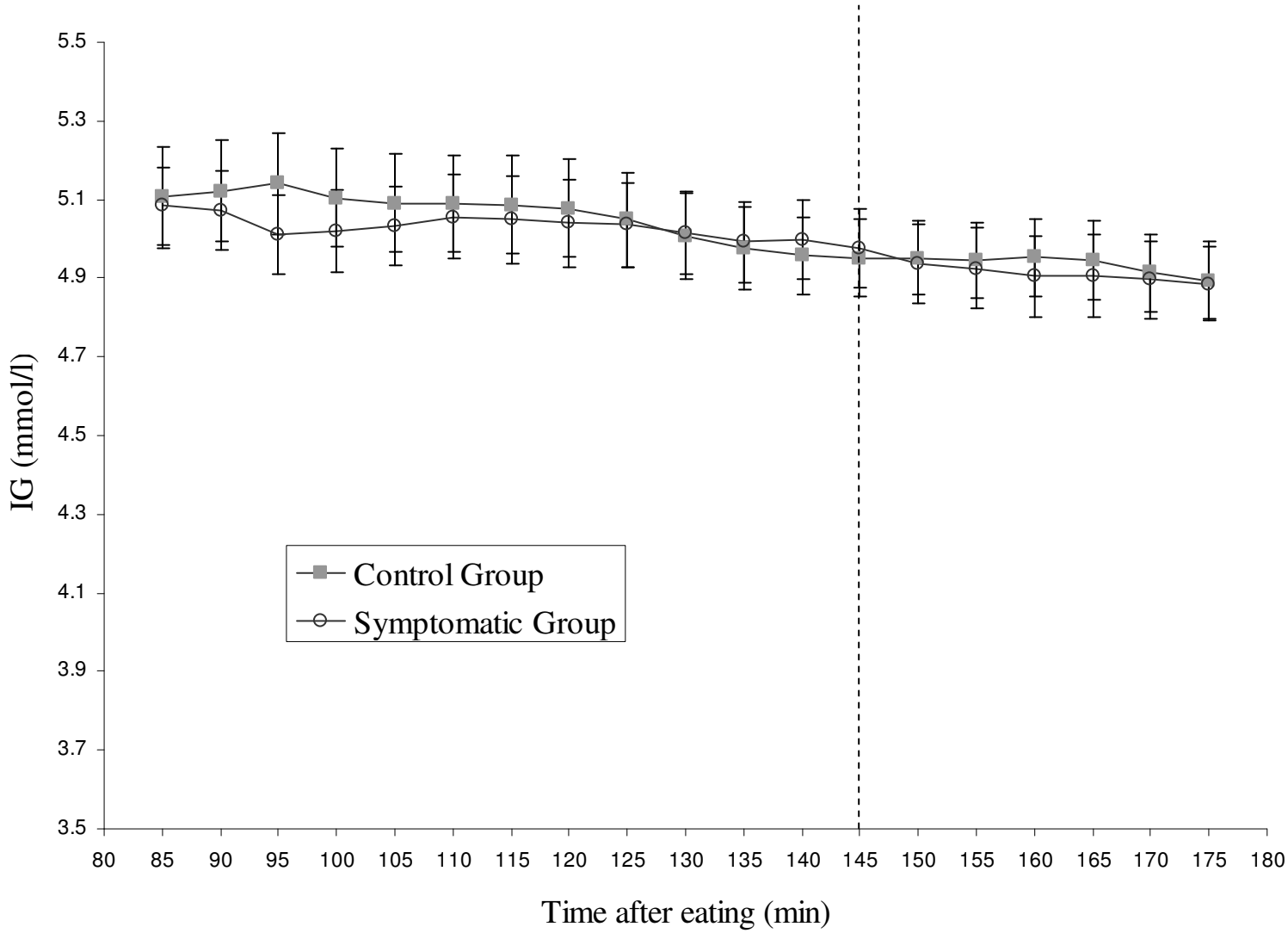
| | All symptoms (n=41) | Autonomic symptoms (n=14) | Neurogenic symptoms (n=20) | Mixed symptoms (n=7) |
|---|---|---|---|---|
| Mean IG (mmol/l) | 4.39 (0.65) | 4.19 (0.65) | 4.46 (0.69) | 4.60 (0.53) |
| Mean time after eating (hrs) | 2.43 (0.97) | 2.41 (0.96) | 2.39 (1.10) | 2.60 (0.67) |
| Number of episodes associated with IG ≤3.3mmol/l | 2 | 1 | 1 | 0 |

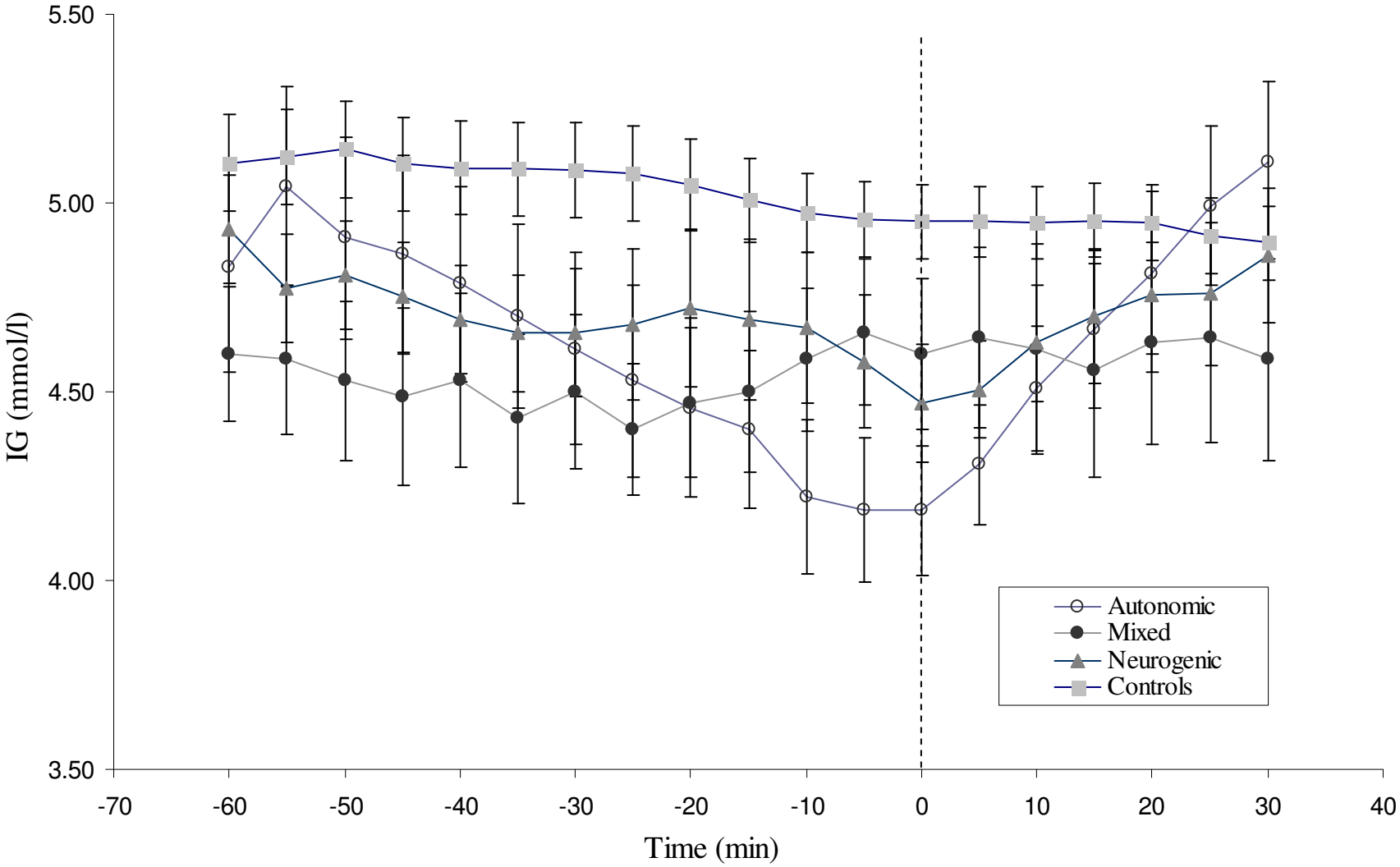
Table 4: Macronutrient composition of the diet in the symptomatic and control groups over the duration of the study period, and the composition of the meal preceding symptoms, expressed as the percentage contribution to total energy. Data are expressed as the mean with SD in parentheses. Controls were compared with the symptomatic group using unpaired t-tests, and within the symptomatic group the meal preceding symptoms was compared to the diet on all days using paired t-test analysis. *P<0.05 compared to mean diet composition of the symptomatic group.

| | Mean diet composition during study | | Meal composition |
|---|---|---|---|
| | Control Group (n=20) | Symptomatic group (n=20) | preceding symptoms (n=20) |
| Protein (%) | 13.5 (2.3) | 13.5 (2.2) | 11.2 (4.6) |
| Total fat (%) | 31.7 (5.1)* | 35.2 (5.0) | 31.2 (14.7) |
| Carbohydrate (%) | 47.2 (6.1) | 45.2 (5.2) | 54.9 (19.0) |
| Total sugars (%) | 21.2 (5.4) | 20.1 (5.2) | 35.0 (27.6)* |
| Starch (%) | 24.6 (3.9) | 23.6 (4.6) | 18.4 (14.1) |

Figure 1a: Mean interstitial glucose concentration (IG) profiles around the 2hr 25min (2.42hr) postprandial time point in the control group (n=20: ■ ) and the symptomatic group when asymptomatic (n=20: ○ ). Error bars display SEM. Change in the profiles over time was investigated using one-way ANOVA and the 2 profiles were compared using two-way ANOVA with repeated measures. No statistical differences were observed.

Figure 1b: Mean interstitial glucose concentration (IG) profiles associated with autonomic (14 symptomatic events in 10 subjects: ⎔ ), neurogenic (20 symptomatic events in 13 subjects: △) and 'mixed' symptoms (7 symptomatic events in 6 subjects: ⬢ ). T= 0 indicates time when symptoms were reported in symptomatic individuals and the 2hr 25min (2.42hr) postprandial time point in controls (n = 20: ■ ). Error bars display SEM calculated from the number of subjects in each group. The three IG profiles preceding symptoms differed over time ($P<0.01$ using two-way ANOVA with repeated measures), with a significant fall in IG observed prior to autonomic symptoms ($P<0.005$ using one-way ANOVA).


5.5
5.3
5.1
4.9
4.7
4.5
4.3
4.1
3.9
3.7
3.5
IG (mmol/l)
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
Time after eating (min)
Control Group
Symptomatic Group


5.50
5.00
4.50
4.00
3.50
IG (mmol/l)
-70
-60
-50
-40
-30
-20
-10
0
10
20
30
40
Time (min)
Autonomic
Mixed
Neurogenic
Controls