Abstract

Aims:

To study bladder sensation during a forced diuresis protocol and to assess differences in sensation perceived by different ethnic groups and after drinking artificially sweetened water.

Methods:

Female Caucasian and south Indian Asian volunteers performed the diuresis protocol drinking water, or water sweetened with saccharine (5 mg/kg body weight). Participants recorded filling sensation every 5 minutes while drinking 250-350 ml/15 minutes. They were asked to record the strongest sensation before voiding as maximum sensation, before voiding. The void was measured and sensation immediately recorded as minimum. The process was repeated. Voided volume, and time required to achieve maximum sensation during cycle 2 were compared by water and sweetener, ethnic group, and age.

Results:

20 Asian and 20 Caucasian volunteers participated. No differences in maximum voided volume or diuresis rate was seen by ethnicity. Median diuresis with sweetener was 16.7 ml/min (8.6-35) compared to 13.2 (7.1-25) with water (p=0.008), a difference accounted for by 16 women with > 5ml/min difference in diuresis rate. These were excluded to leave 24 women with similar diuresis rates with both sweetener and water (14.8 ml/min (8.6-28.0) and 13.2 ml/min (7.1- 25.0). In these women, time to achieve maximum sensation was lower with sweetener than water: 37.5 mins (20-85) vs 50.0 mins (20-80), p =0.002, with no difference in voided volume.

Conclusion:

Water sweetened with saccharine produced an increased diuresis rate in some women. After controlling for this, time to recording maximum sensation was decreased with sweetened water, suggesting saccharin has an effect upon perceived sensation.
Introduction

Lower urinary tract symptoms (LUTS) include storage symptoms such as increased day time frequency, urgency, nocturia, and urinary incontinence, voiding symptoms which include slow stream, intermittency, hesitancy, and post micturition symptoms such as feeling of incomplete emptying. LUTS have significant negative impact on physical, social, psychological, sexual, and occupational aspects of life resulting in impaired high quality of life (1, 2), low self-esteem and social isolation (3).

Conscious bladder control modulated by bladder sensation is central to the pathophysiology of urinary tract dysfunction causing LUTS. This control is a complex interplay of sensory and cognitive processes linked to coordinating reflexes (4, 5). Unconscious processing and integration of afferent noise, including signals emanating from painful stimuli and stretch receptors and those modulated by chemical release from the urothelium (6), and conscious processing and perception of this afferent noise leads to sensation of bladder filling(4). However, the complexities of sensory mechanisms in the lower urinary tract - the generation, transmission, and processing of sensations- which are further influenced by environmental factors (7) and other intrinsic factors such as posture, activity level, bowel status, hydration status and other possible pathology such as chronic pelvic pain syndrome (7) are yet to be fully deciphered.

Bladder sensation can be generated by physiological (sensation of bladder filling, urge or the desire to void, and sensation during micturition), and pathologic (urgency and pain) processes (8). The current established description of three episodic sensations –1.) first sensation of filling, 2.) first desire to void described as a constant sensation gradually becoming more intense, and 3.) strong desire to void, a constant localised sensation to the perineal region or the urethra (9, 10) to void - does not reconcile with the theory that progressive increase in afferent information and constantly growing sensations occurs with progressive bladder filling at a constant rate (5). Further, these symptoms are poor discriminators of physiology versus pathology.

Finding an effective objective way to measure perceived urinary sensation, is subjective and has been of great interest (9). The recently developed bladder sensation tool (5), is a simple approach to identify, record and quantify sensations associated with bladder filling and voiding in an objective and reproducible way. This
approach employs a water loading protocol which achieves a constant high diuresis with rapid, non-invasive bladder filling (11) and does not rely on the use of words to identify and quantify sensation so effectively minimises observer bias which is a factor when performing urodynamics. In this study we have used the bladder sensation tool to investigate differences in bladder sensation in healthy volunteers as a first independent validation study. The potential utility of this tool is that it can evaluate sensory effects in a non-invasive way. It may also be possible to combine the use of this tool with simultaneous urodynamic recording in due course. We have used a cross-over design to allow us to examine two questions within a single study design.

Firstly, it is known that Indian Asian women have a higher incidence of DO compared to Caucasian women and also increased “severity” of detrusor overactivity (DO) (12). There are no data published which examine bladder function in asymptomatic people but data in abstract suggest that Indian Asian women with no symptoms have a higher incidence of detrusor contractions on a urodynamic recording than similar Caucasian women(13). Given that overactive bladder (OAB) and DO are overlapping conditions and that bladder sensation producing urgency is the cardinal symptom of OAB, it is of interest to compare bladder sensation between women of these two ethnic groups.

Secondly, our group has shown artificial sweeteners are associated with enhanced detrusor contractility in vitro due to increased calcium flux (14), via specific urothelial receptors and that the urothelium modulates this response (15). It is currently not known how saccharine, the most potent of the sweeteners tested, influences bladder function in vivo, nor whether there is any effect upon bladder sensation when saccharine is ingested. The bladder sensation tool provides a simple way to explore this.

Thus, in this paper we describe data from the bladder sensation data logging tool to investigate differences of bladder sensation between healthy volunteers of white British and Asian Indian ethnicity, alongside the effect of artificial sweeteners on bladder sensation.

**Methods**

Eligible volunteers were women from White or Asian Indian ethnic background (self-selected) and aged 18 or over. Women of other ethnic background were excluded.
Healthy white and Asian Indian women volunteers contacted the researcher if they were willing to participate after having read the advertisement placed on the weekly University newsletter. Interested women were given an information leaflet and a demographic questionnaire to complete to confirm eligibility for the study. All volunteers provided written consent at the beginning of their first session. All procedures were carried out with Ethical Approval of the Research Governance Office of the University of Leicester, as this was a study of healthy volunteers. Participants were given a small gift voucher at the end of the study.

Following screening and consent, demographic data were collected. Volunteers were also excluded if they have had a stroke, had been diagnosed with multiple sclerosis, spinal injury or other neurological disease or suffer from diabetes. Women who had troublesome urinary symptoms and symptoms of urinary tract infection at the time of the study were also excluded. In addition, volunteers taking anticholinergic drugs within one month of the study were also excluded.

The study aims were to compare sensation experienced during drinking water or sweetened water, and also to compare perceived sensation during each experiment among women from different ethnic groups. Thus, it was conducted as a randomised, single blinded crossover experiment to use each participant as their own control. Randomisation was employed only to determine the sequence of water or sweetened water to be consumed at each visit and was performed immediately prior to administering the first liquid to the volunteers. Jugs were colour coded by researcher (JH). Researcher (EB) was blinded to the contents of the jug and distributed them to the participants on arrival.

Each volunteer attended two 2-hour sessions and received either artificially sweetened flavoured water (saccharine 5 mg/kg) or non-sweetened water. The second session was repeated 28 days later (to control for potential menstrual cycle effects) where the same volunteers were given the type of drink (sweetened or unsweetened) that they did not receive in session one.

Water jugs were colour coded so volunteers did not know what they were drinking, although it was impossible to fully blind participants because of the taste. The researcher (EB) collecting the data forms and analysing them was blind to which liquid volunteers had received.
On arrival for the test, participants were shown into a quiet room and allowed to sit or move around as they pleased. The diuresis protocol was followed as described (accompanying paper). Briefly, volunteers were instructed to begin ‘water loading’ by drinking 250 mls of water every 15 min for the hour preceding arrival. They were given a data-logging sheet (Figure 1) where they recorded their bladder sensations. They continued to drink 250-350 ml water every 15 min and completed the logging sheet as described previously every five minutes. Once the volunteer felt their bladder was full, they were asked to void and this volume was measured (V1). Immediately post void they recorded this as the minimal sensation. Thus the first filing cycle established both the maximum and minimum perceived sensations, and allowed calculation of the diuresis rate for this cycle.

The filling and sensation logging was repeated with either sweetened or unsweetened water and bladder sensation was again recorded every 5 minutes comparing it to their minimum and maximum, which they experienced earlier. At maximum sensation they were asked to void again and this volume was measured (V2). To assess whether the volunteer was in a state of constant diuresis they were asked to continue drinking for a further 30 minutes, still recording sensation. They were asked to void again and the volume measured (V3).

**Sample size and power calculations**
There were no published data available to perform a power calculation. We aimed to recruit 20 women in each ethnic group, and the crossover design achieved group sizes of 40, since each patient acted as her own control. Data from our study of urodynamic parameters (12) showed a mean difference in bladder volume of 69 mls between Caucasian and Asian patients. 36 women per group would be required to confirm a difference of this size (80% power) for either analysis. 40 healthy women volunteers (age 20-50) were recruited of Indian Asian (20 women) and Caucasian (20 women) ethnicity. The mean age of the two groups was comparable. Women recorded their own ethnicity on the demographic data form using standard UK definitions.

**Statistical analysis**
Time to maximum sensation during cycle two was calculated for each woman from the chart, as the time from first void (V1) to immediately before the second void (V2), and reported as median (range). Maximum voided volumes are reported as median
(range). Rate of diuresis was calculated during the second fill (between V1 and V2) by dividing voided volume by time to maximum sensation. To assess whether a fixed diuresis was achieved, the diuresis rate was also calculated based on the same values between V2 and V3.

Voided volume (V2), and diuresis rates (cycle 2 and cycle 3) were compared by water/sweetener and ethnicity using non-parametric analyses (Mann-Whitney U test, Kruskall- Wallis test, or Chi square).

**Results**

20 Asian and 20 Caucasian volunteers participated, with age range 20-50 years. When analysing all women, voided volumes (V2) were the same when comparing Asians to Caucasians (median 600ml (300-1200) for each, p=0.236) and also when comparing volume voided after water to after sweetener (p=0.450). Maximum bladder sensation was achieved sooner with sweetener than water (irrespective of ethnic group) (35 min (20-85) vs. 45 mins (20-80), p <0.001) (Figure 2).

To exclude an increased diuresis rate secondary to drinking sweetened water as the cause, we compared median diuresis rate with water to that with sweetener during the second cycle. Diuresis rate with sweetener was 16.7 ml/min (8.6-35) compared to 13.2 (7.1-25) ml/min with water (p=0.008) for the whole sample. 16 women had more than 5 ml/min differences and excluding these from further analysis rendered the diuresis rate for the remaining 24 women to be the same (14.8 ml/min (8.6-28.0) with sweetener compared to 13.2 ml/min (7.7-24.0) with water, p=0.212).

For these 24 women, time to achieve maximum sensation remained lower with sweetener than with water (37.5 min (20-85) vs. 50.0 min (20-80), p=0.002) (Figure 3). This increased sensation was not due to any difference in bladder volume, since the voided volumes were not significantly different (575 ml (250-1100) for sweetener vs. 600 ml (300-1100) for water, p=0.127). Indeed, there was a trend for the voided volume after sweetener to be lower with sweetener for most women (Figure 4).

Given that this was only the second time this protocol had been used, we examined the diuresis rate during the second and third cycles for both plain water and sweetened water. Interestingly, only four women (three Caucasians and one Asian) demonstrated a less than 10% difference in diuresis rate during second and third cycles for both
water and sweetener. 17 women (eight Caucasian and nine Asian) showed less than 10% difference for one experiment. For these 17 women, the time to achieve maximum sensation remained lower with sweetener than with water: (35.0 min (20-60) vs 40.0 min (20-70), p=0.031). The differences between Asians and Caucasians remained non-significant.

Discussion
To our knowledge this is the first study to investigate differences in bladder sensation between white women and those of south Indian Asian origin and the first controlled study to identify that saccharin had an effect upon perceived sensation. Our data show that bladder sensation during physiological filling was the same between Caucasian and Asian women and there was no difference in bladder capacity since we found no significant difference in voided volumes between the two groups. Most studies looking at the effect of ethnicity on urinary incontinence (UI) have compared black American women with white women and found a higher prevalence of UI in white women (16, 17). There are fewer data available comparing UI between South Asian Indian women and white women. The Nurses’ Health Study (17) showed no difference in the prevalence of UI in white and Asian women, and a study of urodynamic diagnoses in white and Asian women with UI indicated similar rates of USI and DO (18). However, this paper included Asian women from the Far East and IndoChina who are ethnically distinct from Asian woman from India, Pakistan and Bangladesh.

We previously compared urodynamic diagnoses and differences in bladder function between white women and those of Indian Asian origin, and identified a lower incidence of urinary stress incontinence (USI) and a higher incidence of DO and mixed incontinence in Indian Asian patients (12). Similarly, a recent study showed that women of Asian and African origin attending for urodynamics presented with DO more commonly than those of white Caucasian origin (19). We studied the sensory pathway of bladder sensation in the experiments reported here and it is interesting that there were no apparent differences. The aetiology of the higher incidence and more severe urodynamic parameters of DO therefore does not appear to have a sensory component, but the mechanism of this remains obscure.

The sweetened water experiment was the primary focus of this work based upon previous epidemiological (20) and experimental data implicating artificial sweeteners
in the pathology of detrusor overactivity (15, 21). We have established that artificial sweeteners have a stimulatory effect on rat and human detrusor muscle contraction through increased intracellular Ca\(^{2+}\) concentration via extracellular Ca\(^{2+}\) influx through L-type voltage gated Ca\(^{2+}\) channels (21, 22), and that this effect is probably mediated by activation of urothelial sweet taste receptor dimer T1R2/T1R3. The mechanism by which this occurs remains to be elucidated.

This study is the first controlled study exploring the effect of saccharine ingestion on bladder function. We found that water sweetened with saccharine decreased the time to reach maximum bladder sensation despite the voided volumes for these women not being significantly different for sweetener and for water. Thus it appears that saccharine had an effect of increasing perceived sensation in the presence of volumes which are the same (or indeed slightly less) with sweetener, suggesting this is a primary effect upon afferent signals. Of course, it is possible that the increased desire to void was a consequence of induced detrusor overactivity which may be anticipated given the *in vitro* data but we were unable to confirm this. Nevertheless, these data represent the first *in vivo* evidence of an effect of artificial sweeteners upon the lower urinary tract.

The bladder sensation-logging tool used in this study employed a water loading protocol, which achieves a high diuresis with rapid, non-invasive bladder filling (9). It is a simple and non-invasive approach to identifying, recording, and quantifying sensation associated with bladder filling and voiding, based on subjective interpretation without relying on the use of pre-determined terminology from the researcher. We have shown the tool to demonstrate a predictable and reproducible time course of development of sensation in asymptomatic men, illustrating that bladder sensation is a continuous process represented by a sigmoidal curve (accompanying paper) (5). While it can be argued that a water loading protocol is not physiological as it induces a diuresis different from daily life, it is a more physiological filling method than invasive urodynamic investigation (11).

Another limitation associated with the protocol used in our study was the variability in diuresis rate. Because there were no published data on what an expected level of variability was, we analysed the data without modification, but found considerable variation both between filling cycles on the same day, and on different days. By
methodically removing subjects with high variation, we settled on a limit of 5 ml/min in diuresis rate as an appropriate cut-off, but even this would result in a difference in volume of urine produced in 30 minutes of 150 ml (approximately one third bladder volume). There is scope for further work to refine the volume drunk each 15 minutes to achieve a standard diuresis with water or to define what natural variability in diuresis rate might be expected. However it may be partly a function of asking volunteers to drink large volumes of sweetened water, which were difficult to tolerate with some of the volunteers feeling nauseated.

Conclusions

The diuresis chart performed well as a test of bladder sensation. We found no difference in bladder sensation between Asian or Caucasian women, but a more rapid attainment of maximum sensation with saccharin was observed, even after controlling for variations in diuresis rate. These data represent the first in vivo evidence of an effect of saccharine upon bladder sensation but it is not possible to confirm whether this is purely an afferent effect or if it is mediated by detrusor contraction.

2,821 words

References
