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Cutaneous Microdialysis as a Novel Means of Continuously Stimulating Eccrine Sweat Glands *In Vivo*

Caroline J. Morgan[1], Peter S. Friedmann[1], Martin K. Church[1] and Geraldine F. Clough[1]

Previous studies of the pharmacological regulation of sweat gland function in humans have administered agonists or antagonists systemically, by local intradermal injection or by iontophoresis. This has not allowed prolonged or steady-state activation of sweat glands to be examined. In this study, we used the technique of dermal microdialysis to administer pharmacological agents singly and in combination for up to 5 hours. Muscarinic stimulation with pilocarpine nitrate (50 μg ml^{-1} to 1.66 mg ml^{-1}) produced a sigmoid dose response curve, with maximal sweating (measured as transepidermal water loss) (mean 70 g m^{-2} hour^{-1}) after 15 minutes. This was sustained at steady-state levels (55 g m^{-2} hour^{-1}) until perfusion stopped. Perfusion with atropine (0.003 mg ml^{-1}) reduced sweating below baseline and blocked pilocarpine-induced sweating completely. Noradrenaline (0.005 mg ml^{-1}) induced much lower sweat rates than pilocarpine (56.8$\pm$1.62 g m^{-2} hour^{-1} vs 8.2$\pm$1.2 g m^{-2} hour^{-1}, respectively, $P<0.001$) and this was unaffected by co-administration of atropine. This method has made it possible to show that sweat glands are capable of sustaining near maximal activity for at least 5 hours. The method has future application in investigation of conditions with disordered sweat gland activity.

Journal of Investigative Dermatology (2006) **126,** 1220–1225. doi:10.1038/sj.jid.5700197; published online 9 February 2006

INTRODUCTION

Human eccrine sweat glands have two major functions; first, they are critically involved in thermoregulation, producing sweat, the evaporation of which allows body cooling, and second, they improve the grip of the palms and soles at times of activity. Sweat production is under the control of the sympathetic nervous system, but unusually, the post-ganglionic sudomotor fibers release acetylcholine as their main neurotransmitter, acting on muscarinic receptors situated on the secretory coil (Kurzen *et al.*, 2004). The secretory coil also has alpha and beta-adrenergic receptors. However, adrenergic receptor-induced sweat is only about 10% as copious as that resulting from activation of cholinergic receptors (Sato, 1983).

Clinically abnormal sweat production can be due to an alteration in the number, distribution or structure of sweat glands, or to a problem with their innervation. The pathophysiology of altered sweating in many disorders is not fully understood. Although idiopathic hyperhidrosis can be reduced by chemodenervation using botulinum A toxin, the effect is temporary and the pathophysiology of excessive sweating is uncertain. Similarly the understanding of idiopathic generalized anhydrosis is poor.

Studies of the pharmacological control of sweat glands *in vivo* have reported the introduction of agonists or antagonists into the dermis by iontophoresis (Kato *et al.*, 1999; DiPasquale *et al.*, 2003), skin prick, or intradermal injection (Wolf and Maibach, 1974; Szabadi *et al.*, 1980; Sato and Sato, 1983) as single or repeated doses. Pharmacological agents have also been given orally (Foster *et al.*, 1970) systemically or via intravenous or intra-arterial injection. The disadvantage of all of these techniques is that they do not allow continuous delivery of agonist or antagonist to the sweat gland. Hence, the extended effects of steady-state drug concentrations on sweat gland activity have been difficult to explore.

Cutaneous microdialysis has been used previously for the continuous local delivery of pharmacological agents under minimally traumatic conditions to investigate cutaneous vascular and neurogenic function (Schmelz *et al.*, 1997; Clough, 1999; Minson *et al.*, 2001; Shibasaki and Crandall, 2001). The present study set out to re-examine some of the previously defined aspects of sweat gland physiology using microdialysis to deliver a muscarinic agonist (pilocarpine nitrate) and antagonist (atropine sulfate) directly to the skin of healthy volunteers and to investigate whether pharmacological stimulation to sweat glands *in vivo* in this way can provide a greater understanding of sweat gland activity.

[1]*IIR Research Division, School of Medicine, University of Southampton, Southampton General Hospital, Southampton, UK*

Correspondence: Dr Geraldine F. Clough, IIR Research Division, School of Medicine, University of Southampton, Level F South Block, MP 825, Southampton General Hospital, Southampton SO16 6YD, UK.
E-mail: gfc1@soton.ac.uk

Abbreviation: TEWL, transepidermal water loss

Received 10 September 2005; revised 18 November 2005; accepted 1 December 2005; published online 9 February 2006

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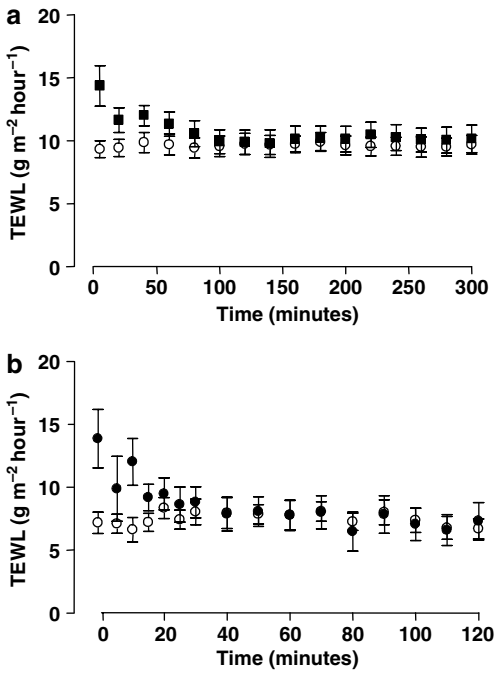

Figure 1. TEWL measurements. (**a**) TEWL measured at 20 minutes intervals in the skin directly above a microdialysis probe during 5 hours continuous perfusion with Ringer's solution at a rate of 5 μl minute^{-1} (closed squares) and at a control uninstrumented skin site on the contralateral forearm (open circles). (**b**) TEWL measured at 10 minutes intervals in uninstrumented skin at control sites (open circles) and at sites treated with EMLA cream for 1.5 hours before measurement of TEWL (closed circles) Data are mean ± SEM, $n = 8$ and $n = 5$, respectively.

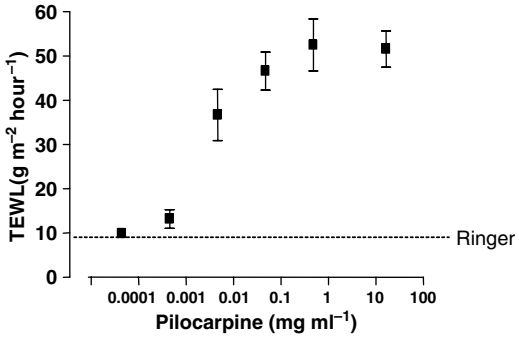

Figure 2. Concentration-dependent effects on steady-state TEWL in response to pilocarpine nitrate (5×10^{-5} to 5×10^{-1} mg ml^{-1}) in Ringer's solution. TEWL was measured directly above each probe at 20 minutes intervals. Data are mean ± SEM for $n = 8$ healthy volunteers. Also shown on the graph is the steady-state TEWL in response to pilocarpine nitrate (1.66 mg ml^{-1}) from a further six volunteers from Study 2. The values for TEWL measured above Ringer perfused probes in the steady state are represented as a dotted line.

RESULTS

Transepidermal water loss (TEWL) measured at 20 minute intervals during the 5 hours study did not differ significantly at instrumented Ringer-perfused sites from that measured in control skin at un-instrumented sites on the contralateral forearm ($P = 0.065$). TEWL showed an initial small increase during the first 20 minutes of Ringer perfusion, but then fell rapidly to reach a steady-state value of 10.2 ± 1.1 g m^{-2} hour^{-1}, similar to that seen at control sites (9.7 ± 0.8 g m^{-2} hour^{-1}) (Figure 1a). As the instrumented sites used in this initial validation study had been exposed to topical local anesthetic cream (EMLA) for 1.5 hours before probe insertion, a second series of measurements were made to compare sweating rates at EMLA-treated and EMLA-free uninstrumented skin sites. A similar, but not significant, increase in TEWL was seen in uninstrumented skin treated with EMLA cream compared to that at instrumented sites over the first 20–40 minutes of measurement (Figure 1b).

Pilocarpine induced sweating

Blood pressure and resting pulse rate were unaffected by the concentrations of pilocarpine used, nor was there evidence of excessive salivation, tear secretion, or other systemic cholinergic-mediated effects in any of the volunteers. An area of erythema was visible in all volunteers along the length of the pilocarpine-perfused probe at the higher concentrations of pilocarpine used. There was no evidence of a wider spreading neurogenic flare at any concentration.

Pilocarpine administered via the probe caused a concentration-dependent increase in TEWL (Figure 2). At all concentrations, the maximal effects of pilocarpine on TEWL were seen within the first 20 minutes of perfusion. At the three higher concentrations of pilocarpine, this initial increase was followed by a decline in TEWL to reach a steady state of approximately four times baseline, 90–120 minutes after the start of perfusion (Figure 3). The steady-state values for TEWL measured in the eight individuals in response to pilocarpine are shown in Figure 2 together with the response of a further six individuals to the higher concentration of pilocarpine (1.66 mg ml^{-1}) used in study 2.

Effect of atropine on sweating induced by pilocarpine or noradrenaline

Addition of atropine to the probe perfusate caused a rapid reduction in pilocarpine-stimulated sweating towards baseline levels ($P < 0.01$). (Figure 3). Pilocarpine had no effect on noradrenaline-induced sweating (data not shown). However, atropine alone did cause a small but significant reduction in steady-state TEWL to below baseline levels (−4.2 ± 0.8 g m^{-2} hour^{-1}, $P < 0.01$). Both the maximal and total sweating induced by noradrenaline was significantly lower than that induced by pilocarpine (max TEWL pilocarpine 56.8 ± 1.62 g m^{-2} hour^{-1}; noradrenaline 8.2 ± 1.2 g m^{-2} hour^{-1}, $P < 0.001$) (Figure 4). Addition of noradrenaline to the pilocarpine probe perfusate had little additional effect on maximum sweating (51.3 ± 2.39 g m^{-2} hour^{-1}) (Figure 4).

DISCUSSION

Much of the previous work on sweat gland physiology and pharmacology has involved the use of *in vitro* preparations of isolated animal or human sweat glands (reviewed in K. Sato, 1983). Where *in vivo* studies have been performed, they have generally investigated sweating responses to short-term

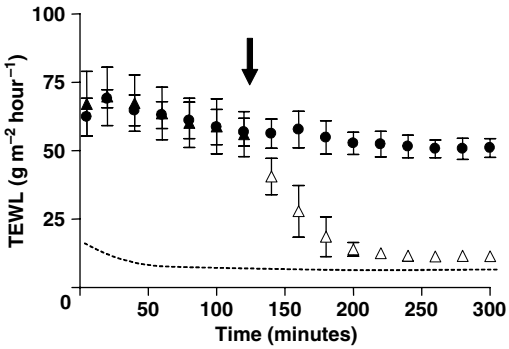

Figure 3. Effect of muscarinic blockade on cholinergic stimulation of sweating. Two microdialysis probes were initially perfused with pilocarpine (1.66 mg ml^{-1}) and TEWL readings taken every 20 minutes for 2 hours (closed symbols). The perfusate of one of the probes was then switched to one containing pilocarpine (1.66 mg ml^{-1}) and atropine (0.003 mg ml^{-1}) (open symbols) and TEWL readings continued at 20 minutes intervals for a further 3 hours. Atropine caused a significant reduction in TEWL ($P<0.01$, analysis of variance). Data are mean ± SEM for $n=3$ healthy volunteers. Dotted line represents unstimulated TEWL measured over Ringer-perfused microdialysis probes (see Figure 1).

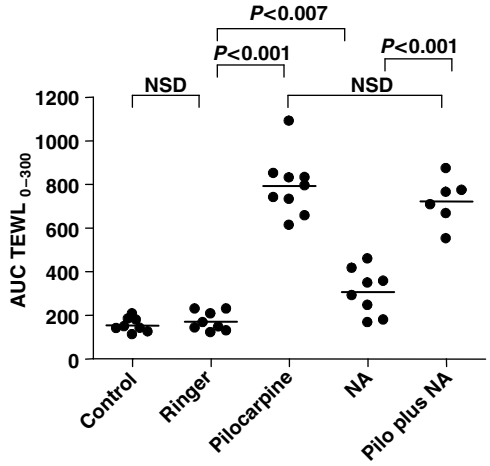

Figure 4. Total sweating response. Total sweating response calculated from the area under the TEWL curve $AUC_{0-300\,min}$ during perfusion of the microdialysis probes with either Ringer's solution, pilocarpine (1.66 mg ml^{-1}), noradrenaline (0.005 mg ml^{-1}), or pilocarpine and noradrenaline. Control measurements were taken from the contralateral uninstrumented forearm. Data are from eight healthy volunteers. Bar represents median value. NSD, no significant difference.

delivery of pharmacological agonists and antagonists by iontophoresis or intradermal injection. In the present study, we have used intradermal microdialysis to co-deliver agonists and antagonists to the sweat glands over extended (5 hours) periods in order to investigate sustained cholinergic and adrenergic sweating in healthy human skin.

Pilocarpine delivered continuously to the skin via a microdialysis probe caused a sustained, concentration-dependent increase in sweating. The increase in TEWL was maximal during the first two hours of the study for all concentrations of pilocarpine. At the higher concentrations of pilocarpine, this maximal increase in TEWL was followed by a small but significant reduction in sweat production suggesting a decline in gland sensitivity following extended periods of agonist exposure. A reduction in sweat gland activity was seen following repeated intradermal injection of high doses of pilocarpine in human (Thaysen and Schwartz, 1955) and murine skin (Vilches *et al.*, 1995). However, it is difficult to follow the time course of eccrine sweating using bolus injection or iontophoresis (Benarroch and Low, 1991; Namer *et al.*, 2004). Where agonists have been delivered continuously, only maximal sweating rates have been reported or sudomotor function has been followed for only relatively short (30 minutes) periods of time (Shastry *et al.*, 2000; Bickel *et al.*, 2002, 2004; Namer *et al.*, 2004). Thus, our data provide a novel insight into the effects of sustained delivery and its consequences on sweat gland function. They also reveal new information about the stamina of sweat glands and their ability to sustain sweat production for many hours.

Pilocarpine-induced sweating was significantly attenuated by addition of the muscarinic blocker atropine to the probe perfusate. This is consistent with other studies in which it has been demonstrated that local modulation of cholinergic activity can inhibit thermally induced sweat rate in humans (Shibasaki and Crandall, 2001). Interestingly, the continuous delivery of atropine sulfate alone to sweat glands also reduced sweat gland activity below basal levels. This suggests that under resting conditions, there is continuous muscarinic sweat gland stimulation.

The role of noradrenaline in sweat production is unclear. In the forearm, cholinergic sweating was much greater than that induced by adrenergic stimulation. At steady state, noradrenaline increased sweating by only 20% of that induced by pilocarpine. This figure is similar to that previously reported for adrenergic stimulation of sweat glands (Foster *et al.*, 1970; Szabadi *et al.*, 1980; Sato and Sato, 1983) following a single injection of agonist. It is interesting to note that co-delivery of pilocarpine and noradrenaline caused less sweating than pilocarpine alone. One possible explanation for this is that sweat gland function is compromised by noradrenaline-induced vasoconstriction resulting in a reduced supply of nutrients and water. If vasoconstriction was limiting the delivery of essential nutrients and water to the glands, it might be expected that the shape of the sweating curves following stimulation by pilocarpine and pilocarpine with noradrenaline would differ. We found no such differences. Therefore, if vasoconstriction is affecting sweat gland nutrient supply, it does not appear to be an absolute rate-limiting step in sweat production. An alternative explanation for the reduced noradrenaline-induced sweating is enhanced sweat reabsorption in the sweat duct through beta-adrenergic stimulation, this may lead to an imbalance in primary sweat production and reabsorption (Sato, 1983).

Delivery and recovery of substances by microdialysis have been extensively used to investigate tissue metabolism, vascular and neurogenic function in a wide range of physiological and pathophysiological conditions (Muller, 2000; Schmelz and Petersen, 2001; Clough and Church,

2002). In this study, it proved an effective method by which known concentrations of agonists and antagonists could be delivered relatively a-traumatically to the skin. The effective concentration of these molecules reaching the tissue will depend on the dialysis efficiency (E_d) of the microdialysis membranes (Clough, 2005). However, whereas no estimates of E_d of atropine, pilocarpine, or noradrenaline have been made, previous *ex vivo* studies demonstrate that delivery of molecules between 150 and 300 Da molecular weight is greater than 40% using the 5 kDA membrane perfused at 3–5 μl minute^{-1} (Clough, 2005).

Measurements of sweating rate at baseline over the microdialysis probes, and at control and EMLA-treated uninstrumented sites, confirm that neither pretreatment with topical local anesthetic cream nor probe insertion significantly affects basal TEWL (Namer *et al.*, 2004). These data also suggest that the transient increase in TEWL seen over the first 20–30 minutes of probe perfusion is most probably a result of a reduction in the barrier properties of the skin following application of the EMLA cream used for probe insertion rather than an increased sweating consequent to the start of perfusion of the probes. The data from the Ringer-perfused probes further show that, following the initial transient effect on TEWL as perfusion commenced, there was little change in basal sweating during the course of the study. All sites were equilibrated for approximately 15 minutes before the start of perfusion. This is shorter than we have previously allowed for recovery from the initial trauma of probe insertion and thus it might be expected that blood flow in the vicinity of the probe would remain raised for some time after the start of perfusion (Clough, 1999). However, the values of TEWL at instrumented sites that we report are similar to those we measured in uninstrumented skin and to those previously reported by Bickel *et al.* (2002, 2004) in healthy volunteers and in patients with familial dysautonomia in whom microdialysis probes had been inserted without local anesthesia. These observations suggest that any insertion induced increase in superficial blood flux did not impact significantly on the chemically induced sweating that we subsequently went on to investigate. Further, all of our studies were carried out in a thermoneutral environment as it has been shown that sweat glands show maximal cholinergic sensitivity at normal skin temperature (Shastry *et al.*, 2000; DiPasquale *et al.*, 2003) and to minimized changes in local blood flow in the skin.

In summary, we have shown that continuous delivery of the muscarinic agonist pilocarpine to the skin results in a directly mediated and sustained sweating that was sensitive to atropine blockade. This study has, for the first time, provided information about sweat gland activity with prolonged stimulation within the same individual using both cholinergic and adrenergic agonists. We have further shown that combined delivery of agonists and antagonists via the microdialysis probe provides an important tool with which to address the underlying pathologies of sweat gland dysfunction. In time this technique may be used to investigate and clarify the complex irregularities that underlie sweat gland dysfunction.

MATERIALS AND METHODS

Study group

Twenty-one healthy volunteers (11 females and 10 males) were recruited (age range 23–48; median 28 years). All volunteers gave written informed consent. The study was approved by the South and West Research Ethics Committee (ref 265/98) and was conducted according to the Declaration of Helsinki Principles. All volunteers were requested to refrain from exercise and caffeine-containing drinks on the day of study. None were taking any medication nor had applied moisturizing creams or other topical agents to either arm for at least 24 hours before the study.

Drugs

Noradrenaline (Levophed, Sanofi Winthrop, UK), pilocarpine nitrate (Mandeville Medicines, Stoke Mandeville Hospital, UK), lidocaine/prilocaine (5% EMLA cream, Astra Pharmaceuticals Ltd, Macctesfield, UK), Ringer's intravenous solution (Baxter Healthcare Ltd, Newbury, UK), atropine sulfate (Aurum Pharmaceuticals, Brentwood, UK), were obtained from the hospital pharmacy.

Microdialysis probes

Drugs were delivered using linear microdialysis membranes of 2 kDa molecular mass cutoff, 216 μm outer diameter, 8 μm wall thickness (Gambro model GFE 18, Gambro Dialysaten AG, Hechingen, Germany). The membranes were glued into a short length of Portex tubing (0.28 mm internal diameter, 0.61 mm outer diameter) and a 0.1 mm diameter, 5 cm long stainless-steel wire (Goodfellow Cambridge Ltd, Cambridge, UK) inserted into each fiber to strengthen the membrane. Probes were sterilized with ethylene oxide before use. Once inserted (see below) the probes were connected via flexible tubing (length 200 cm, volume 1.5 ml; Alaris Medical Systems, Basingstoke, UK) to 10 ml syringes (Terumo Europe N.V, 3001 Leuven, Belgium) and perfused at a rate of 5 μl minute^{-1} using a syringe pump (P3000 IVAC Medical Systems, Hants, UK).

Study protocol

All studies were performed in a clinical setting at an ambient temperature of 22°C (±2°C), with the volunteer lying supine throughout. Up to six sites on the volar surface of the non-dominant forearm were anesthetized with EMLA cream under occlusion for 90 minutes. Following removal of the EMLA, a microdialysis probe was introduced into the dermis at each site to run parallel to the epidermal surface for a length of 25 mm using a 23-gauge guide needle (0.6 × 30 mm, Terumo Europe N.V, 3001 Leuven, Belgium). Probes were separated by at least 20 mm, avoiding the more distal part of the forearm. On removal of the guide needles, the probes were connected to the infusion pumps and perfused at a rate of 5 μl minute^{-1} using a syringe pump (P3000 IVAC Medical Systems, Hants, UK). The outflow from each probe was collected to avoid wetting the skin, which might affect TEWL readings. As sweating has been shown to be higher at the wrist (Panisset *et al.*, 1992), probe perfusion was randomized to site in all studies.

Sweating was assessed directly above each microdialysis probe and at a control area of skin at the same site on the contralateral arm using a TEWL meter (Tewameter TM 210, Courage & Khazaka, Koln, Germany). Measurements of TEWL were made for up to 5.5 hours at 20 minutes intervals, commencing 5 minutes after the start of

perfusion. A stable value of TEWL was achieved within 90 seconds. A second study to investigate the effects of application of the EMLA cream used as an anesthetic for probe insertion were also performed in a subset of five volunteers in whom values of TEWL were measured at 10 minutes intervals for up to 2 hours at EMLA-exposed and control skin.

At the end of the study, probe depth was measured in a subset of eight volunteers using high-frequency (20 MHz) ultrasound (Dermascan C, Cortex Technology, Hadsund, Denmark). Using the 2D B mode, the wire insert was followed along its length to ensure that its depth within the dermis was uniform. Three measurements of fiber depth were taken using the A mode and the average calculated. Mean probe depth was 1.02 ± 0.1 mm (SEM), placing them in close proximity to the secretory portion of the dermally located eccrine sweat glands.

Study 1: dose–response of pilocarpine-induced sweating

Six microdialysis probes were inserted into the forearm of eight volunteers (four males and four females). Five of the probes were perfused, each with a randomized concentration of pilocarpine nitrate (5×10^{-5} to 0.5 mg ml^{-1}) in Ringer's solution; the remaining probe was perfused with Ringer's solution alone. TEWL was measured every 20 minutes for the 5 hours perfusion period directly above each probe and from control sites on the contralateral arm. Arterial blood pressure and pulse were monitored hourly to ensure that there were no significant systemic effects.

Study 2: effect of atropine on sweating induced by pilocarpine or noradrenaline

To investigate the differential effects of cholinergic and adrenergic stimulation on sweating, TEWL was measured in six volunteers (three males and three females) in response to pilocarpine (1.66 mg ml^{-1}), noradrenaline (0.005 mg ml^{-1}), or a combination of pilocarpine (1.66 mg ml^{-1}) and noradrenaline (0.005 mg ml^{-1}). Six probes were inserted per volunteer with randomization of perfusate (two for each agonist) to probe site. A high concentration of both pilocarpine nitrate and noradrenaline were used to ensure maximal effects on sweat gland activity. As before, TEWL measurements were taken every 20 minutes for 5 hours above each probe and from sites on the contralateral arm as control.

In three further volunteers (one male and two females), the effect of muscarinic blockade on cholinergic and adrenergic stimulation on sweating was investigated using pairs of probes perfused initially with either pilocarpine (1.66 mg ml^{-1}) or noradrenaline (0.005 mg ml^{-1}). TEWL readings were taken every 20 minutes for 2 hours. The perfusate was then switched to one to which atropine (0.003 mg ml^{-1}) had been added, and TEWL readings continued at 20 minutes intervals for a further 3 hours.

Data handling

Data are presented as mean $\pm$ SEM for either peak or steady-state TEWL. Total sweating was estimated as the $AUC_{0\text{–}300\ minutes}$ of the TEWL response over the 5 hours probe perfusion. Where two probes were perfused with the same solution, the mean of the two values was taken for any individual. All volunteers acted as their own controls. Data are compared using non-parametric Mann–Whitney *U*-test or Wilcoxon's signed-rank tests or by analysis of variance using a repeated-measures analysis and Bonferroni's *post hoc* test as appropriate. Differences were regarded as significant at $P < 0.05$.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was funded by GlaxoSmithKline Consumer Healthcare. G.F.C. was supported by the Wellcome Trust (Grant ref 057474/99). Carolyn Gill is acknowledged for her technical support.

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