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Characterization of Temperature Profiles in Skin and Transdermal Delivery System When Exposed to Temperature Gradients *In vivo* and *In Vitro*

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Abstract

Purpose—Performance of a transdermal delivery system (TDS) can be affected by exposure to elevated temperature, which can lead to unintended safety issues. This study investigated TDS and skin temperatures and their relationship *in vivo*, characterized the effective thermal resistance of skin, and identified the *in vitro* diffusion cell conditions that would correlate with *in vivo* observations.

Methods—Experiments were performed in humans and in Franz diffusion cells with human cadaver skin to record skin and TDS temperatures at room temperature and with exposure to a heat flux. Skin temperatures were regulated with two methods: a heating lamp *in vivo* and *in vitro*, or thermostatic control of the receiver chamber *in vitro*.

Results—*In vivo* basal skin temperatures beneath TDS at different anatomical sites were not statistically different. The maximum tolerable skin surface temperature was approximately 42–43°C *in vivo*. The temperature difference between skin surface and TDS surface increased with increasing temperature, or with increasing TDS thermal resistance *in vivo* and *in vitro*.

Conclusions—Based on the effective thermal resistance of skin *in vivo* and *in vitro*, the heating lamp method is an adequate *in vitro* method. However, the *in vitro-in vivo* correlation of temperature could be affected by the thermal boundary layer in the receiver chamber.

Keywords

heat effect; *in vitro* permeation test (IVPT); temperature; transdermal; transdermal delivery system (TDS)

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INTRODUCTION

Many transdermal delivery systems (TDS) exhibit altered performance such as enhanced drug delivery under conditions of elevated temperature (1–10). Due to the potential effects of elevated temperature on transdermal delivery and the clinical efficacy and safety of TDS, a number of TDS products have warning labels against exposure to elevated temperature during drug administration. Furthermore, some TDS product formulations and designs may be relatively more susceptible to the effect of elevated temperature (11). An understanding of the effects of heat transfer on the performance of TDS can assist pharmaceutical scientists in anticipating the potential adverse heat effects of different TDS designs and formulations. An understanding of the heat transfer processes across skin can also be beneficial for the development of local thermal therapies and use of thermo-responsive materials. To facilitate the evaluation of drug delivery from TDS under elevated temperature conditions relevant to actual product use, a bio-relevant *in vitro* test method that correlates with *in vivo* results is needed. This test method can be potentially used in the assessment of the comparative safety of generic TDS to reference listed drug (RLD) under elevated temperature conditions.

Currently, there is no standard *in vivo* or *in vitro* test method to evaluate and compare the performance of TDS under the influence of elevated temperature. *in vivo* studies exploring heat effects on TDS have utilized differing conditions where durations of elevated temperature exposure have ranged from 5 min to 10 h, test temperatures have ranged from 39°C in a hot tub to air temperature of 90°C in a sauna. The heat sources themselves have also represented fundamentally different scenarios ranging from an internal heat source induced by strenuous exercise to various external sources of heat (e.g., heating lamps, electric heating pads or blankets, heated water baths, saunas) (12). A variety of different protocol parameters have also been utilized for *in vitro* studies with different durations and frequencies of elevated temperature exposure, elevated temperature ranges, and methods of exposure. In addition, the apparatus employed in these *in vitro* studies have not been consistent. The lack of consistency in study designs and protocol parameters for the existing body of *in vivo* and *in vitro* work may be the consequence of two issues. First, there is no right or wrong scenario for the exposure of a TDS to elevated temperature during the course of patient use because this can potentially arise in different ways—from sources of heat either internal or external to the body, across a range of different temperatures, and for any duration or frequency during the period of wear. Second, while certain test conditions may be more representative and/or discriminating, how specific differences in heat transfer protocol parameters (e.g., temperature, duration, frequency, method of exposure, and directionality of the temperature gradient) alter the manner in which elevated temperature affects the fundamental characteristics of the skin and TDS, and whether *in vitro* observations correlate with *in vivo* effects, have not been well characterized.

There are also fundamental differences between the *in vivo* and *in vitro* scenarios, which can impact potential *in vitro-in vivo* correlations (IVIVC) of the temperatures at the skin and TDS surfaces. Notably, such potential temperature differences *in vitro* and *in vivo* may affect the IVIVC of bioavailability (a different IVIVC) under certain conditions. This work focuses on the fundamental heat transfer, and the IVIVC between temperatures and temperature gradients produced *in vitro* and *in vivo* under different scenarios, and discusses the

theoretical implications of these effects on the ability of an *in vitro* system to predict heat effects on transdermal bioavailability *in vivo*. In a conventional *in vitro* permeation test (IVPT) using excised human skin mounted on a Franz diffusion cell, the skin is maintained at a physiological state of hydration and at a normal skin surface temperature of approximately 32°C when exposed to ambient (room temperature) conditions. This IVPT model has demonstrated IVIVC (13,14). However, in humans, skin temperature is modulated by thermoregulation (15–17). Under conditions of external heat transfer, as the environmental temperature rises substantially above the core body temperature of 37°C, the body counteracts the rise in skin temperature in an effort to maintain homeostasis. Thus, the effect of elevated temperature on skin temperature during TDS application *in vitro* could be different from that *in vivo*. Even when the temperature at the skin surface is maintained the same *in vitro* and *in vivo*, the temperature at the TDS surface could be different due to the heat transfer *in vitro* and *in vivo*. This lack of temperature IVIVC could affect the outcomes of heat effect evaluations when heat influences the rate of drug transport not only in the skin, but also in the TDS. To develop an *in vitro* test method for the evaluation of heat effects on TDS that could appropriately model *in vivo* conditions therefore requires understanding the relationship between skin and TDS temperatures and the influence of TDS thermal resistance on this relationship *in vitro* and *in vivo*.

The overall goals of this work were to characterize, *in vivo* and *in vitro*, the fundamental transdermal heat transfer—the response of skin and TDS temperatures to the influence of elevated temperature under different conditions—in order to support the development of appropriate, representative *in vitro* conditions for evaluating the effects of elevated temperature on drug delivery from TDS. The objectives of this work were to (a) identify appropriate skin temperatures for evaluating heat effects in IVPT via the characterization of normal skin temperature at different anatomical locations and conditions and skin temperature under exposure to a radiant heat flux *in vivo*, (b) explore how differences in heat transfer through TDS of potentially differing thicknesses and thermal resistance impact the TDS and skin temperatures *in vivo* and *in vitro*, (c) characterize the influence of temperature gradient direction from either internal or external heat sources on factors like the temperature gradient across the TDS and skin *in vitro* based on heat transfer theory, and (d) ultimately compare the temperature gradients *in vivo* and *in vitro* in a manner that can characterize how specific *in vitro* study design parameters may support or limit the relevance of the *in vitro* results to different *in vivo* scenarios.

MATERIALS AND METHODS

Materials

Phosphate buffered saline (PBS) of pH 7.4 (consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride) was prepared by dissolving PBS tablets (MP Biomedicals, Solon, OH) in deionized water. Sodium azide (Acros Organics, Morris Plains, NJ) was added to PBS at 0.02% as bacteriostat. Sodium chloride was purchased from Fisher Scientific (Pittsburgh, PA).

Ultrafine IT-Series flexible microprobes IT-21 (0.016" diameter, 0.08 s time constant) and IT-24P (0.009" diameter and 0.004 s time constant) were purchased from Physitemp

Instruments (Clifton, NJ). The temperature probes were calibrated by the manufacturer and verified in the present study by immersion into a water bath at temperatures ranging from 32–46°C. The probes were found to be within $\pm 0.2^\circ\text{C}$ accuracy compared to an NIST traceable thermometer and within $\pm 0.1^\circ\text{C}$ precision and accuracy among the probes themselves.

The model TDS were prepared using a nicotine transdermal system (Equate Clear Nicotine Transdermal System, 21 mg/day; Walmart, Bentonville, AR) manufactured by Alza Corp (Mountain View, California). Two model TDS were used in the present study: a 1-layer and a 5-layer model TDS (hereafter referred to as TDS-1 and TDS-5, respectively). The term “TDS” is used to refer collectively to both TDS-1 and/or TDS-5 in the present study. The TDS-1 was a single nicotine TDS. TDS-5 was a simple stacked assembly of five nicotine TDS. TDS-5 was used to mimic a thick transdermal system of high thermal resistance to study the influence of TDS thickness (i.e., thermal resistance). Since the analysis in the present study was based on TDS thermal resistance and not the specific TDS product used or the properties of the nicotine TDS, such as materials, dimensions, or the existence of multiple backing layers in the 5-layer system, these specifics were not expected to affect the conclusion of this study. TDS-1 had a thickness of 0.027 cm and TDS-5 was 0.128 cm thick (measured using a micrometer; Mitutoyo, Kanagawa, Japan).

Prior to using the nicotine TDS in the human studies, the nicotine in the TDS was extracted using saline (0.9% sodium chloride in deionized water) over an extended period of time to ensure that human exposure to nicotine would be minimal in the studies. Briefly, the nicotine TDS was immersed in saline in a 1-L beaker under stirring at room temperature for up to 40 days, and the solution in the beaker was replaced with fresh saline solution on days 1, 2, 3, 9, 17, 28, and 40. Before each solution replacement, a 1-mL aliquot was withdrawn from the beaker to check for drug content using a high performance liquid chromatography (HPLC) assay.

Human Subjects and Human Skin

For the *in vivo* studies, healthy male and female subjects between the ages of 25 and 60 years were recruited by referral and personal contact at the Academic Health Center at the University of Cincinnati. Informed consent was obtained from the subjects. No vulnerable subjects were involved and subjects with skin sensitivity to heat and light were excluded from the study. Further details about the study design and methodologies utilized for *in vivo* studies are described in subsequent sections. For the *in vitro* studies, excised split-thickness human cadaver skin dermatomed from the posterior torso of males and females between the ages of 47 and 70 years was obtained from the New York Firefighters Skin Bank (New York, NY). The thickness of the split-thickness skin from the skin bank was measured by a micrometer (Mitutoyo, Kanagawa, Japan) and found to be 0.025 ± 0.014 cm (mean $\pm$ SD; range ~ 0.01– 0.06 cm; four skin donors with measurements at six different positions each). The cadaver skin was stored at -80°C before use and was thawed by immersing the tissue in PBS in a 600-mL beaker. The skin was then patted dry with a Kimwipe and cut into appropriate sizes. Unused skin was wrapped in aluminum foil and stored in a freezer at -20°C for later use. The use of human subjects and tissues was approved by the Institutional

Review Board (IRB) at the University of Cincinnati and the Research Involving Human Subjects Committee (RIHSC) at the U.S. Food and Drug Administration (FDA).

***In Vivo* Human Studies**

Three anatomical locations were studied *in vivo*: the forearm, the upper arm and the abdomen. The experiments on the forearm and upper arm were performed without the cover of clothing. The experiments on the abdominal area were covered with clothing (a shirt and/or sweater). Sites on the extensor surface of the forearm or upper arm or on the abdominal area of the subject were randomly selected for the study. The TDS (after nicotine extraction) was cut to a size of $\sim 1.5 \text{ cm} \times 1.8 \text{ cm}$. A temperature probe (Ultrafine IT-Series Flexible Microprobe, IT-24P) was connected to the bottom adhesive surface of the TDS. Another temperature probe (IT-24P) was placed on the top surface of the TDS and was secured by a small piece of adhesive tape (3M Scotch tape). The TDS was placed on the selected site of the subject and was secured at the edges with adhesive tape (3M Scotch tape) when necessary. The ambient temperature of the room for the experiments was $23.6 \pm 1.5^\circ\text{C}$ (mean $\pm$ SD). Temperature measurements were recorded by data logger and computer software (Thermes USB-Temperature Data Acquisition System and DasyLab-Lite Software; Physitemp Instruments). Parallel experiments were conducted with TDS-1 and TDS-5, and the experimental procedure for the multi-layer TDS-5 was the same as that described for the single layer TDS-1. The use of TDS-5 allowed examination of the effect of thermal resistance differences in TDS upon skin temperature. The basal temperatures of skin and TDS surfaces were measured by the temperature probes at the interface between the skin surface and the TDS, and at the external surface of the TDS, respectively.

Before exposure to a heat flux, an insulating cover ($\sim 30 \text{ cm} \times 30 \text{ cm}$ and $\sim 0.8 \text{ mm}$ thick; neoprene rubber, Rubber-Cal, Santa Ana, CA) was used to protect the skin around the testing site. The protective cover included an opening ($\sim 2.5 \text{ cm} \times 2.5 \text{ cm}$) in the middle for placing the TDS on the skin and exposure to the heating lamp. The protective cover was used to prevent heat-induced skin damage around the TDS application site. Two types of protective covers were used: a black neoprene cover (normal cover) and a neoprene cover with a reflective surface of aluminum foil (reflective cover). The normal cover was used initially in the first set of experiments, and upon receiving feedback from the subjects about their heat sensation, the normal cover was modified with the addition of a reflective surface (i.e., the attachment of an aluminum foil to its surface) to reduce human subjects' discomfort in all other experiments.

To evaluate the heat transfer through the skin and TDS, probe temperatures were monitored to provide a baseline reference before the exposure to a heat flux. Heat was then supplied to the external surface of the TDS using a heating lamp (250 W, HL-1, Physitemp Instruments) placed approximately 15–20 cm from the TDS surface. The heating lamp was controlled by a proportional-integral-derivative (PID) controller (TCAT-2, Physitemp Instruments) to maintain the temperature of the skin surface, and the following skin temperature protocol was used. Heat was supplied for 10 min to maintain a TDS surface temperature of 40°C , with the skin surface and the TDS external surface monitored throughout the duration. Within approximately 3 min, once the temperatures at the surfaces of the skin and TDS had

stabilized, the temperature was increased to 42°C for at least 3 min or until a steady-state skin surface temperature was achieved for 1 min. This procedure was repeated to measure skin and TDS temperatures at 1°C increments up to 45°C. Skin surface temperature generally reached a steady state within 1 min in these subsequent temperature stages. Heat sensation was monitored by surveying each subject during the study. Sensations of heat and (dis)comfort were evaluated using a rating scale from 0–6: 0 = no feeling; 1 = warm comfortable; 2 = hot comfortable; 3 = hot not comfortable, but fine; 4 = hot uncomfortable, but sustainable; 5 = hot unsustainable; 6 = hot that requires immediate termination.

***In Vitro* Franz Diffusion Cell Studies for IVPT**

Franz Diffusion Cell General Setup—Temperatures at the skin surface (interfacing with the bottom surface of the TDS) and at the external surface of the TDS applied upon the skin were measured in Franz diffusion cells *in vitro* as shown in Fig. 1a using a methodology similar to the *in vivo* studies. The dimensions of the receiver chamber in the diffusion cell were approximately 1 cm in diameter × 6 cm high, and the effective diffusional area was approximately 0.6 cm². Prior to the IVPT experiments, skin samples were cut into the desired sizes (~ 1.5 cm × 1.5 cm) and mounted on Franz diffusion cells. The receiver chamber was filled with approximately 6 mL PBS, and the donor chamber was exposed to the room environment. When the temperature of the receiver solution was maintained constant, the influence of the receiver solution volume on the temperature gradient in the TDS and skin was not expected to be significant because of the large heat capacity of the receiver solution compared to that of the skin. The TDS was cut into a small circular piece with a diameter of 0.86 cm and connected to two temperature probes (IT-24P) at its top and bottom surfaces as previously described for the *in vivo* experiments. The TDS was then placed on the external surface of the skin in the diffusion cell.

Uniquely for the *in vitro* studies, in addition to the temperature probes described above, another temperature probe (IT-24P) was placed beneath the skin near the skin/receiver interface (underside of the skin interfacing the receiver solution). The temperature of the receiver solution was controlled by a water jacket surrounding the receiver chamber of the Franz diffusion cell, and the water jacket was connected to a circulating water bath. The temperature in the receiver chamber was monitored by a temperature probe (Ultrafine IT-Series Flexible Microprobe, IT-21). To maintain temperature uniformity in the receiver chamber, the receiver solution was continuously stirred with a magnetic stir bar (Spinbar 8 mm × 3 mm, Bel-Art Products, Wayne, NJ) impelled by a stirring plate (Fisher Scientific Isotemp Basic Magnetic Stirrer, 7 in × 7 in. Ceramic, Pittsburgh, PA) operating just beneath the Franz diffusion cell. Temperature uniformity in the receiver solution was verified by moving the probe randomly to different locations within the receiver chamber for temperature measurements. The temperature of the receiver solution was first adjusted to a temperature (typically ~34°C) that provided a skin surface temperature of 32°C (under the TDS). The skin surface, TDS surface, and skin/receiver interface baseline temperatures were measured. The heating protocols were similar to those described in the *in vivo* human studies. Heat was supplied to the Franz diffusion cells to increase skin surface temperature stepwise from 32–46°C (in stages, from reference Stage 0 to Stages 1–4) using one of the two methods as described below. The temperatures at the skin surface, TDS surface, and

skin/receiver interface were monitored and recorded for at least 5 min within which steady state temperatures were routinely achieved.

Heating Lamp Method—In the heating lamp method, heat was provided by a heating lamp from above the external surface of the TDS to model an external source of heat *in vivo*. The diffusion cells were heated by a lamp placed approximately 15–20 cm from the surface of the TDS, with the diffusion cells arranged in a concentric configuration under the lamp (Fig. 1b). This distance allowed for an even distribution of the heat energy from the heating lamp to the multiple diffusion cells. The temperature of the skin surface (under the TDS) was monitored in one of these diffusion cells by an extra temperature probe that was connected to a PID controller. The PID controller regulated the heating lamp to modulate the heat flux and maintain the skin surface temperature at the desired level. Only 6 of 8 potential positions in this concentric configuration (Fig. 1b) were used, however, due to formation of a relative cold spot corresponding to a discontinuity in the heating filament in the lamp bulb (a “horse-shoe” shape filament) (Fig. 1c).

Water Bath Heating Method—When the water bath was used to modulate the heat flux, the temperature of the skin in the diffusion cells was controlled by the heat flux from the circulating water bath via the receiver chamber of the diffusion cell. The water bath circulated the water through the water jacket surrounding the receiver chamber and provided heat from beneath the skin via the receiver solution. The temperature of the skin surface in one of the diffusion cells was regulated by the circulating water bath, and all diffusion cells were heated from the same circulating water bath. The water bath method can be viewed as a model for an internal source of heat *in vivo*. It also represents a convenient method that requires only Franz diffusion cells without additional equipment.

TDS Thermal Resistance

The thermal conductivity and thermal resistance of the TDS were measured with ThermTest TPS 2500 S Thermal Constants Analyzer by ThermTest, Inc. (Fredericton, Canada). Prior to setting the TDS in the equipment for testing, the release liners were removed and the thickness of the samples was measured with digital calipers. The thermal properties of the TDS were determined using the TPS Thin Film Analysis Module at 40°C. The thermal resistance data were reported as the thermal resistance normalized by the surface area (in m²K/W).

Statistical Analysis

All data are presented as means and standard deviations (mean ± SD). Statistical differences were determined using Student's *t*-tests and considered to be significant at a level of $p < 0.05$. For the comparison of the slopes in the regression analyses, the least squares regression slopes were examined using both SigmaPlot (Systat Software, San Jose, CA) and Student's *t*-tests. Variances were examined using the F-test and considered to be significantly unequal when the F value > F Critical one-tail. Outliers in the *in vitro* experiments were identified using the Q-test and were excluded from the analyses. Linear least-squares regression analyses were performed using Microsoft Excel (Redmond, WA).

Heat Transfer Theory and Model

Figure 2 illustrates the temperature gradients across the human skin when heat is externally supplied to TDS *in vivo* and in Franz diffusion cells *in vitro* in a 1-dimensional (1-D) model. A more complex 2-D or 3-D model was not used because it is reasonable to assume that heat transfer at the edges of the TDS and skin layers is small. In the model of Fig. 2, similar heat transfer processes are expected *in vivo* and *in vitro*: the thermally controlled, “well stirred” receiver solution *in vitro* is analogous to the blood circulation in the dermis *in vivo*, which regulates the skin temperature by thermoregulation. In both cases, at elevated external temperatures (e.g., 42°C), a temperature gradient would exist between the surface of the skin and the deeper tissue in the skin. A temperature transition/boundary layer is likely to exist in the skin at the depth near the circulating blood vasculature in the dermis (*in vivo*) or in the receiver chamber at a similar depth in contact with the “well stirred” receiver solution (*in vitro*). This thermal boundary layer is conceptually analogous to the aqueous boundary layer that has been described for the transport of hydrophobic compounds (18). At steady state, the heat flux across the TDS (q_{TDS}) is theoretically proportional to the difference in temperature across the TDS and inversely related to TDS thermal resistance (e.g., higher temperature difference or lower membrane thermal resistance leads to higher heat flux) under the assumption that the thermal resistance is independent of temperature with small temperature variation (19,20):

$$q_{TDS} = \frac{T_{TDS} - T_{TDS/skin}}{R_{TDS}} \quad (1)$$

where T is temperature and subscripts TDS and $TDS/skin$ represent the TDS surface and skin surface under the TDS, respectively. R_{TDS} is the thermal resistance of the TDS (in m²K/W) and can be expressed as (20):

$$R_{TDS} = \frac{h_{TDS}}{k_{TDS} A} \quad (2)$$

where h_{TDS} is the TDS thickness, A is the area of the TDS, and k_{TDS} is the TDS thermal conductivity (the reciprocal of TDS thermal resistivity). The heat flux across the skin (q_{skin}) is theoretically equal to that across the TDS at steady state and can be described as:

$$q_{skin} = \frac{T_{TDS/skin} - T_{derm}}{R_s} \quad (3)$$

where T_{derm} is the temperature of the tissue around blood circulation under the skin and R_s is the thermal resistance of the skin. T_{derm} can be assumed to be close to human body temperature of 37°C. Combining Eqs. 1 and 3 yields:

$$T_{\text{TDS/skin}} = \frac{R_s}{R_{\text{TDS}} + R_s} T_{\text{TDS}} + \frac{R_{\text{TDS}}}{R_{\text{TDS}} + R_s} T_{\text{derm}} \quad (4)$$

and

$$T_{\text{TDS}} - T_{\text{TDS/skin}} = \frac{R_{\text{TDS}}}{R_{\text{TDS}} + R_s} (T_{\text{TDS}} - T_{\text{derm}}) \quad (5)$$

In the *in vivo* study, R_s is defined as the effective thermal resistance of the skin (i.e., comprising the epidermis and thermal boundary layer discussed earlier). R_s can be determined using the slope of the $T_{\text{TDS}} - T_{\text{TDS/skin}}$ versus T_{TDS} plot. Since R_s is related to the temperature gradients across the TDS and skin, this parameter can be used to predict skin surface temperature using Eq. 4, when R_{TDS} is known.

In the Franz diffusion cell study *in vitro*, measuring the temperature at the skin/receiver interface allows Eq. 5 to be rewritten as:

$$T_{\text{TDS}} - T_{\text{TDS/skin}} = \frac{R_{\text{TDS}}}{R_{\text{TDS}} + R_{\text{sts}}} (T_{\text{TDS}} - T_{\text{skin/receiver}}) \quad (6)$$

where $T_{\text{skin/receiver}}$ is the temperature at the skin/receiver interface and R_{sts} is the thermal resistances of the split-thickness skin. This modification of Eq. 5 allows determination of the split-thickness skin thermal resistance. Similar to the *in vivo* study, a plot of $T_{\text{TDS}} - T_{\text{TDS/skin}}$ versus $T_{\text{TDS}} - T_{\text{skin/receiver}}$ can be used to determine the R_{sts} .

To evaluate the effect of the thermal boundary layer on heat transfer within the *in vitro* diffusion cell, Eq. 6 could be modified:

$$T_{\text{TDS}} - T_{\text{TDS/skin}} = \frac{R_{\text{TDS}}}{R_{\text{TDS}} + R_{\text{sts}} + R_{\text{ABL}}} (T_{\text{TDS}} - T_{\text{receiver}}) \quad (7)$$

where T_{receiver} is the temperature of the receiver solution ("well stirred" solution in the receiver chamber), and R_{ABL} is the thermal resistance of the aqueous boundary layer in the diffusion cell. In Eq. 7, the combined thermal resistance of the skin and thermal boundary layer is collectively considered to be the effective thermal resistance of the skin in the diffusion cell. The contribution of the thermal boundary layer to the thermal resistance of the diffusion cell system can be evaluated when $T_{\text{TDS}} - T_{\text{TDS/skin}}$ is plotted against $T_{\text{TDS}} - T_{\text{receiver}}$.

RESULTS AND DISCUSSION

***In Vivo* Basal Skin Temperature beneath TDS (without Exposure to a Heat Flux)**

The first objective of this study was to characterize normal skin temperature at different anatomical locations and conditions (e.g., uncovered or covered by clothing) and to evaluate how an external heat source influenced these temperatures. Table 1 presents the skin temperature under TDS-1 and TDS-5 at the forearm, upper arm, and abdominal area of the human subjects without exposure to a heat flux. The TDS-5 was developed to provide a model TDS of different thermal barrier thickness (i.e., thermal resistance) with which to investigate the effect of TDS thermal resistance on the relationship between skin and TDS temperatures. The average skin surface temperatures under the TDS-1 and TDS-5 were 32.2–32.8°C at the application sites. This temperature range is consistent with the reported skin temperatures under fentanyl TDS in a previous study (8). This temperature is also essentially the same or slightly higher than the skin surface temperature recommended for *in vitro* percutaneous penetration studies (21). The resistance of the thermal barrier (and the TDS thickness) did not meaningfully affect the skin surface temperature in this study; the skin surface temperatures under the TDS-1 and TDS-5 systems were not statistically different ($p > 0.05$). In contrast, the temperature difference between the skin surface and the external surface of the TDS-5 (a difference of 0.5–0.6°C) was significantly greater than that observed with the TDS-1 (a difference of 0.04–0.2°C) ($p < 0.05$, Student's *t*-tests). A TDS with higher thermal resistance (i.e., a relatively thicker barrier) appeared to better insulate the surface of the TDS from the warmth emanating from the underlying skin, leading to a larger temperature difference between the skin surface and the TDS surface. Yet, the insulating effect of the thicker TDS had minimal influence on the temperature at the skin surface under the TDS without exposure to a heat flux, possibly due to human thermoregulation that controls skin temperature and heat loss from the body.

***In Vivo* Elevated Skin and TDS Temperatures and Tolerable Skin Temperature**

The effect of elevated temperature applied by an external heat source (a heating lamp) was studied *in vivo*. While a relatively high temperature may be likely to exacerbate heat effects on TDS and better represent a worst-case scenario, patients would likely limit their exposure to levels of heat greater than they can tolerate. To determine this maximum tolerable skin temperature, the intensity of heat sensation and perceptions of tolerability were assessed by the subjects. The presence of the reflective cover reduced the area of collateral exposure and substantially lessened the intensity of the heat sensation. Without the reflective protective cover around the heating site, the maximum tolerable skin surface temperature for the human subjects was approximately 42–43°C under the TDS using the heating lamp. This temperature may represent an upper limit for the skin surface temperature appropriate to study heat effects on drug delivery from TDS because patients may experience intolerable distress at this temperature and either avoid the heat or modulate the heat source to a level that is tolerable. While there are conceivable situations in which patients may tolerate (or acclimate to) higher temperatures for extended periods, this maximum tolerated temperature of 42–43°C may appropriately represent the worst-case scenario for TDS exposure to an elevated temperature. This maximum tolerable temperature value is also supported by observations reported in the literature (9,22,23). Together, the results in the present study

suggest a baseline skin temperature range of 32–33°C and an upper skin temperature range of 42–43°C to evaluate heat effects on transdermal delivery in Franz diffusion cell experiments *in vitro*.

Effective Skin Thermal Resistance *In Vivo*

Figure 3 presents the *in vivo* temperature data and the analysis using the heat transfer model described in Eq. 5. The figure shows the temperature difference between skin and TDS surfaces as a function of the temperature at the TDS surface with the linear regression slope related to the skin and TDS thermal resistances. Assuming that the thermal resistances of the TDS remain constant in the temperature range of 38–45°C, the linear relationship in the figure suggests a constant effective thermal resistance of the skin beneath the TDS under the heat conditions studied. In addition, the x-intercept values for the TDS-1 and TDS-5 in Fig. 3 are consistent with the temperature of the tissue under the skin being close to 37°C, as expected.

Using the temperature data in Fig. 3, TDS-1 thermal resistance, and Eq. 5, the effective skin thermal resistance *in vivo* was calculated. For the nicotine TDS (TDS-1), the thermal resistance normalized by the surface area was $0.00091 \pm 0.00001 \text{ m}^2 \text{ K/W}$ (mean $\pm$ SD, $n = 5$). From the slopes of the linear regressions of the TDS-1 and TDS-5 in the figure (0.16 and 0.34, respectively), the average effective thermal resistance of the skin *in vivo* was $0.007 \text{ m}^2 \text{ K/W}$, which was approximately 7.6 times the thermal resistance of a single nicotine TDS. This effective skin thermal resistance value was in the same order of magnitude as the thermal resistance of skin ($\sim 0.002\text{--}0.005 \text{ m}^2 \text{ K/W}$) calculated using the thermal conductivity of skin ($0.2\text{--}0.6 \text{ W/m/K}$) reported previously (24,25) when skin thickness of 1 mm was assumed. This thickness value was more than 10 times larger than the epidermis thickness (0.08 mm, (26)), suggesting that the dermis likely contributed to the effective thermal resistance of skin under the TDS during exposure to a heat flux *in vivo*. The effective skin thermal resistance value obtained here can also be used to predict the temperature at the TDS surface at a given skin surface temperatures using Eq. 5 and the thermal resistance of TDS. In general, the temperature difference between the TDS and skin surface is small for a thin TDS such as the nicotine TDS with low thermal resistance ($\sim 0.0009 \text{ m}^2 \text{ K/W}$) *in vivo*. When the TDS thermal resistance increased (e.g., with thicker TDS), the difference between the surface temperatures of the skin and TDS increased.

Skin and TDS Temperature in Franz Diffusion Cell and Skin Thermal Resistance *In Vitro*

The relationships between the TDS surface temperature, skin surface temperature under the TDS, and thermal resistances of the skin and TDS were investigated in two experimental designs: (a) a heating lamp to control the skin surface temperature, where the receiver temperature was kept constant with the water bath at the temperature that initially provided 32°C at the skin surface before exposure to a radiant heat flux (heating lamp method), and (b) a water bath to control the skin surface temperature, in which the diffusion cell receiver fluid was heated but the TDS was not directly heated (water bath method). Figure 4 shows the relationship of the temperature difference between the exterior surfaces of the TDS and skin (i.e., $T_{\text{TDS}} - T_{\text{TDS/skin}}$) versus the temperature difference between the TDS surface and skin/receiver interface (i.e., $T_{\text{TDS}} - T_{\text{skin/receiver}}$). Because the temperature gradient for the

water bath method (providing upward heat transfer from the receiver solution toward the TDS) is opposite to that of the heating lamp method (providing heat transfer down from the TDS toward the receiver solution), the water bath method values in Fig. 4 are negative. Whether evaluated based upon the results obtained with the heating lamp method or the water bath method, the thermal resistances of the skin *in vitro* were essentially the same within the precision of the data (illustrated by the similarity in the slopes of the regression lines from the two methods in the figure). The thermal resistance of the skin *in vitro* calculated using the slope in Fig. 4 and Eq. 6 was smaller than that *in vivo* ($\sim 0.002 \text{ m}^2\text{K/W}$ versus $\sim 0.007 \text{ m}^2\text{K/W}$, respectively). This may be partly due to the dermis being a relatively good thermal barrier and *in vitro* dermatomed split-thickness skin being thinner than whole skin *in vivo*. It should be noted that although the dermis provides a relatively good thermal barrier to heat transfer, the dermis is not a significant diffusion barrier for most compounds for drug delivery (27) (e.g., hydrophilic and moderately lipophilic drugs), a major difference between the characteristics of heat and mass transfer in the skin.

Effect of a Thermal Boundary Layer in Franz Diffusion Cell

Figure 5 shows the difference between surface temperatures of the skin and TDS as a function of the temperature difference between the exterior surface of the TDS and the receiver solution (i.e., the temperature gradient collectively across the TDS, skin, and thermal boundary layer in the receiver) in Franz diffusion cells *in vitro* (see Eq. 7). The figure is similar to Fig. 4 except that the contribution of the thermal resistance of the aqueous boundary layer in the diffusion cells was included in the temperature analysis; the difference between Fig. 4 and Fig. 5 is related to the boundary layer between the skin/receiver interface and receiver solution. The effective thermal resistance of the skin and the boundary layer together *in vitro* was calculated using the slope in Fig. 5 according to Eq. 7. The effective thermal resistance of this combined skin and boundary layer in the heating lamp method ($\sim 0.02\text{--}0.03 \text{ m}^2\text{K/W}$, corresponding to $\sim 20\text{--}30$ times the thermal resistance of the nicotine TDS) was larger than that of the water bath method ($\sim 0.003 \text{ m}^2\text{K/W}$). This difference could be attributed to the presence of a greater thermal boundary layer at the skin/receiver interface in the heating lamp method compared to that in the water bath method, due to relatively diminished fluid convection at the skin/receiver interface in the heating lamp method. In contrast, the heated receiver fluid in the water bath method had the propensity to rise and improve convective mixing at the skin/receiver interface and hence lower resistance for heat transfer.

It should be pointed out that the resistance of drug diffusion in the aqueous boundary layer is several orders of magnitudes smaller than that of the stratum corneum (18,28,29), and is therefore considered negligible for drug transport under normal circumstances. However, the thermal resistance of the aqueous boundary layer is significant; the specific thermal resistance of water is in the same order of magnitude as that of the skin. Without the aqueous boundary layer, the effective thermal resistance of the skin in the diffusion cells is significantly smaller ($\sim 0.002 \text{ m}^2\text{K/W}$) and more consistent with the values in the literature (25). The effective thermal resistance of the skin in the heating lamp method is significantly larger than that of the skin *in vivo* ($\sim 0.02\text{--}0.03 \text{ m}^2\text{K/W}$ versus $\sim 0.007 \text{ m}^2\text{K/W}$, respectively) due to the significant contribution of the thermal resistance of the aqueous boundary layer,

leading to a difference between the temperature gradients across the TDS *in vitro* compared to *in vivo*. The impact of this difference will be discussed in the next section. The large thermal resistance of the aqueous boundary layer in the heating lamp method suggests that the thermal resistance of dermis may not be an important factor affecting the temperature gradient in IVPT studies. Since the thermal resistance of the dermis is a function of its thickness, this also suggests that the effect of variability in the thickness of the dermis across the different skin samples used for IVPT (e.g., from dermatomed split-thickness skin versus non-dermatomed skin) may be masked by the effect of the thermal boundary layer in the heating lamp method.

Comparison of TDS Temperature *In Vivo* and *In Vitro* under Exposure to a Heat Flux and Effect of TDS Thermal Resistance

To evaluate the influence of TDS thermal resistance on the IVIVC of skin and TDS temperatures with different *in vitro* study designs, the heat transfer *in vivo* and *in vitro* was compared. Figure 6 presents the predicted *in vitro* versus *in vivo* relationship between the external surface temperature of the TDS and the thermal resistance of the TDS from Eq. 7 when the skin surface temperature is maintained at 42°C with the water bath and heating lamp methods. This analysis mimics the temperature conditions of IVPT studies that control skin surface temperature but have different TDS temperatures as a result of the two IVPT heating methods versus the *in vivo* conditions of external heat supply. In the figure, the dashed line represents the temperature required at the external surface of the TDS to achieve 42°C at the skin surface (i.e., the TDS and skin interface) using the heating lamp method. The solid line in the figure is the temperature at the external surface of the TDS resulting from the skin surface temperature of 42°C using the water bath method. A comparison of the results from the Franz diffusion cells (dashed and solid lines) and those calculated using the *in vivo* parameters (dash-dotted line) suggests that a TDS would be exposed to a lower temperature *in vitro* compared to that *in vivo* when using either the heating lamp or water bath method to control temperature *in vitro*. The TDS temperature difference between the *in vivo* and *in vitro* conditions was generally small, between 1–2°C, with the heating lamp method (the difference between the dash-dotted line and dashed line), but could be considerable with the water bath method (the difference between the dash-dotted line and solid line) depending upon the thermal resistance of the TDS. For example, for a thin TDS with a thermal resistance of 0.001 m²K/W, when the skin surface temperature is 42°C, the temperature at the top of a TDS would be approximately 42.4°C *in vitro* using the heating lamp method compared to 42.8°C *in vivo*. At the same skin temperature and thermal resistance of the TDS, the surface temperature of the TDS would be 41.1°C using the water bath method *in vitro*. When the TDS thermal resistance increased to 0.003 m²K/W, a larger difference between surface temperatures of the TDS *in vivo* and *in vitro* would be expected. Following the same example, the temperatures at the external surface of the TDS would be 44.3°C, 43.0°C, and 39.5°C for the *in vivo* condition, heating lamp method *in vitro*, and water bath method *in vitro*, respectively.

This analysis illustrates that the difference of the effective skin thermal resistance *in vivo* and *in vitro* could lead to temperature-related systematic differences in the evaluation of external heat effects on transdermal drug delivery using the Franz diffusion cell *in vitro*

under certain conditions, when (a) the TDS possesses sufficient thermal resistance that its surface has a substantially different temperature than the surface of the skin and (b) the TDS temperature influences the rate at which it delivers drug across the skin. In both the heating lamp and water bath methods, the temperature of the TDS is lower than that encountered *in vivo* when the skin surface temperature is controlled by the heating lamp, and the magnitude of this difference is related to TDS thermal resistance. With a decrease in the effective thermal resistance of the combined skin and thermal boundary layer, which may result from more effective stirring, the temperature gradient across the TDS in the heating lamp method *in vitro* would approach that *in vivo* for external heat supply (i.e., an improvement of IVIVC when the dashed line approaches the dash-dotted line in Fig. 6). The opposite would be true in the water bath method, in which a slower stirring rate may diminish the temperature difference between the *in vivo* and *in vitro* conditions (i.e., the solid line approaches the x-axis in Fig. 6) by increasing the thermal resistance of the combined skin and thermal boundary layer and reducing the temperature gradient across the TDS; while the temperatures at the TDS would not be the same in the *in vivo* and *in vitro* settings with receiver water bath heating (except when the TDS thermal resistance approaches zero), this difference could be reduced. In summary, the modulation of the thermal boundary layer (e.g., by controlling the stirring rate or modifying the dimensions of the diffusion cell receiver chamber) could affect the relative temperatures of the skin and TDS and improve the precision with which the temperatures of both the skin and TDS surfaces are matched *in vivo* and *in vitro*. Further studies are warranted to explore the potential effect of stirring and other apparatus or methodological parameters on the heat transfer in diffusion cells using the heating lamp and water bath methods *in vitro*.

Significance of Temperature Effect

To examine the significance of the temperature differences between the heating lamp and water bath heating methods in the present Franz diffusion cell setup *in vitro* and the situation encountered *in vivo*, and to contextualize this temperature analysis from the perspective of transdermal drug delivery, the mechanism of skin transport should be considered. First, using the Arrhenius equation and activation energy of 20–25 kcal/mol for skin transport across the lipoidal pathway (30–32), an increase of 1°C, 2°C, and 3°C would translate into approximately 10–13%, 23–30%, and 40–50% increase in flux, respectively. The variability caused by an error of 1–2°C is therefore within the skin-to-skin variability normally observed in skin permeation experiments. The effect of temperature on free aqueous diffusion or skin transport across the polar pathway is expected to be smaller due to lower activation energy involved (32): for example, an increase of 2°C would increase the flux by approximately 5–10% under this condition. Second, it is well-accepted that the stratum corneum is the major barrier for transdermal permeation of most drugs. The stratum corneum is the outmost layer of skin and is ~15 µm thick. This barrier as a whole is therefore likely to have temperature close to the skin surface temperature. In general, unless the thermal resistance of TDS is large (e.g., > 0.002 m²K/W), the temperatures of the TDS and the stratum corneum under both diffusion cell *in vitro* and human *in vivo* conditions should not differ by more than 1–2°C according to the analyses in the present study. The impact on TDS drug release from such a temperature difference between the TDS *in vivo* and *in vitro* is expected to be small unless the transition temperatures of the materials in the

TDS are in the same temperature range (e.g., 40–45°C range). Hence, the temperature errors identified in the present study will not be a major concern when using the Franz diffusion cell to mimic skin temperature conditions *in vivo* for the evaluation of transdermal delivery *in vitro* in cases where the TDS thermal resistance is small (e.g., $< 0.002 \text{ m}^2\text{K/W}$). In terms of heating methods *in vitro*, when TDS thermal resistance is small relative to that of the skin, the use of the bottom-to-top water bath method to heat the system would likely provide similar transdermal flux results as the top-to-bottom heating lamp method in evaluating drug delivery from a TDS because the effects from the temperature difference would likely be masked by skin-to-skin variability. For TDS with higher thermal resistance, the water bath method may not be appropriate to model external heat conditions and the heating lamp method is preferred. Future studies are required to investigate the potential impact of temperature differences between the *in vivo* and *in vitro* settings on drug permeation across skin in Franz diffusion cell testing using the heating lamp and water bath methods *in vitro*.

CONCLUSION

In order to support the development of a bio-relevant *in vitro* test system (e.g., IVPT) for the evaluation of differential TDS performance under elevated temperature, an understanding of heat transfer in the skin and TDS *in vivo* and *in vitro* is required. The present study investigated the influence of TDS thermal resistance on the temperatures of the skin and TDS *in vivo*. Without exposure to a heat flux, the skin temperature was not affected by the thermal barrier created by the TDS covering the skin at different anatomical sites and remained at 32–33°C under the conditions investigated. With exposure to a heat flux, it was found that the maximum tolerable skin surface temperature in humans was approximately 42–43°C. Together, this established the skin temperature range of 32–33°C to 42–43°C as the likely range representative of temperature normally encountered during the application of TDS. Therefore, this temperature range can be used for the evaluation of heat effects on transdermal drug delivery *in vitro* using a Franz diffusion cell.

In both *in vivo* and *in vitro* settings, it is difficult to control the skin and TDS temperatures independently because modulating the temperature of one can affect the temperature of the other. For example, depending upon the heat transfer dynamics of the *in vitro* test configuration, even when the skin surface temperature is controlled *in vitro* at a temperature matched to an *in vivo* condition (e.g., 42°C), the temperature of the TDS *in vitro* could be different than it is *in vivo*, under certain conditions. This can be important in situations when transdermal drug delivery is controlled not only by the skin but also by the TDS. Therefore, the comparison of the temperature gradients *in vitro* (IVPT) and *in vivo* was important. The present study evaluated two *in vitro* methodologies (heating lamp and water bath methods) and compared the influence of TDS thermal resistance on the temperatures of the skin and TDS under the *in vitro* and *in vivo* conditions. The results suggest that the heating lamp method *in vitro* can have advantages as a model for external heat *in vivo* because it can provide better IVIVC of skin and TDS temperatures than the water bath method, particularly for TDS with high thermal resistance (e.g., $> 0.002 \text{ m}^2\text{K/W}$). That said, for TDS with low thermal resistance (e.g., $< 0.001 \text{ m}^2\text{K/W}$), when the skin surface temperature is matched *in vivo* and *in vitro* at 42°C, there is less than 1°C and 2°C difference between the *in vivo* and *in vitro* temperatures at the TDS surface with the heating lamp and water bath methods,

respectively. Another important finding in the present study was the substantial contribution of the thermal resistance of the aqueous boundary layer in the receiver chamber to the temperature gradients across the TDS and the skin in the diffusion cells in the heating lamp method, which was not the case in the water bath method. This finding suggests that IVIVC of temperature gradients in the diffusion cell could be affected by this thermal boundary layer in the receiver chamber. It also implies that the thickness of the skin sample (e.g., dermatomed split-thickness skin versus non-dermatomed skin) in IVPT may not significantly impact this IVIVC when the thermal resistance of the aqueous boundary layer is relatively large compared to that of the skin sample. However, any discrepancy *in vitro* and *in vivo* for temperature gradients beneath the dermis (in an aqueous thermal boundary layer) may not necessarily be meaningful, since the drug delivery rate is predominantly controlled by the TDS and/or the stratum corneum.

In summary, the Franz diffusion cell heating lamp method, or a similar method with an external heat source, can provide adequate IVIVC of TDS and skin temperatures to evaluate heat effects on transdermal drug delivery from the external heat source. In other *in vivo* situations not examined in the present study, e.g., temperature gradients due to increases in core body temperature rather than due to an exposure to external heat supply, the corresponding heat transfer should be appropriately modeled *in vivo* based upon the considerations elucidated in this work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

FDA	U.S. Food and Drug Administration
HPLC	High performance liquid chromatography
IVIVC	<i>In vitro-in vivo</i> correlation
IVPT	<i>In vitro</i> permeation test
PBS	Phosphate buffered saline
PID	Proportional integral derivative

q_{skin}	Heat flux across the skin
q_{TDS}	Heat flux across the TDS
R_{ABL}	Aqueous boundary layer thermal resistance
R_s	Skin thermal resistance <i>in vivo</i>
R_{sts}	Split-thickness skin thermal resistances <i>in vitro</i>
R_{TDS}	TDS thermal resistance
RLD	Reference listed drug
T_{derm}	Temperature of the tissue around blood circulation under the skin
$T_{receiver}$	Receiver solution temperature in diffusion cell
$T_{skin/receiver}$	Temperature at the skin/receiver interface
T_{TDS}	TDS surface temperature
$T_{TDS/skin}$	Skin surface temperature under TDS
TDS	Transdermal delivery systems
TDS-1	1-layer model TDS
TDS-5	5-layer model TDS

References

1. Ashburn MA, Ogden LL, Zhang J, Love G, Basta SV. The pharmacokinetics of transdermal fentanyl delivered with and without controlled heat. *J Pain*. 2003; 4(6):291–7. [PubMed: 14622685]
2. Barkve TF, Langseth-Manrique K, Bredesen JE, Gjesdal K. Increased uptake of transdermal glyceryl trinitrate during physical exercise and during high ambient temperature. *Am Heart J*. 1986; 112(3):537–41. [PubMed: 3092610]
3. Bur A, Joukhadar C, Klein N, Herkner, Mitulovic G, Schmid R, Agneter E, Muller M, Brunner M. Effect of exercise on transdermal nicotine release in healthy habitual smokers. *Int J Clin Pharmacol Ther*. 2005; 43(5):239–43. [PubMed: 15906589]
4. Fujimura A, Sasaki M, Harada K, Kumagai Y, Ohashi K, Ebihara A. Influences of bathing and hot weather on the pharmacokinetics of a new transdermal clonidine, M-5041T. *J Clin Pharmacol*. 1996; 36(10):892–6. [PubMed: 8930775]
5. Gjesdal K, Klemsdal TO, Rykke EO, Bredesen JE. Transdermal nitrate therapy: bioavailability during exercise increases transiently after the daily change of patch. *Br J Clin Pharmacol*. 1991; 31(5):560–2. [PubMed: 1909540]
6. Klemsdal TO, Gjesdal K, Bredesen JE. Heating and cooling of the nitroglycerin patch application area modify the plasma level of nitroglycerin. *Eur J Clin Pharmacol*. 1992; 43(6):625–8. [PubMed: 1493844]
7. Klemsdal TO, Gjesdal K, Zahlsten K. Physical exercise increases plasma concentrations of nicotine during treatment with a nicotine patch. *Br J Clin Pharmacol*. 1995; 39(6):677–9. [PubMed: 7654487]

8. Moore KT, Sathyan G, Richarz U, Natarajan J, Vandenbossche J. Randomized 5-treatment crossover study to assess the effects of external heat on serum fentanyl concentrations during treatment with transdermal fentanyl systems. *J Clin Pharmacol.* 2012; 52(8):1174–85. [PubMed: 21878578]
9. Petersen KK, Rousing ML, Jensen C, Arendt-Nielsen L, Gazerani P. Effect of local controlled heat on transdermal delivery of nicotine. *Int J Physiol Pathophysiol Pharmacol.* 2011; 3(3):236–42. [PubMed: 21941614]
10. Shomaker TS, Zhang J, Ashburn MA. A pilot study assessing the impact of heat on the transdermal delivery of testosterone. *J Clin Pharmacol.* 2001; 41(6):677–82. [PubMed: 11402637]
11. Prodduturi S, Sadrieh N, Wokovich AM, Doub WH, Westenberger BJ, Buhse L. Transdermal delivery of fentanyl from matrix and reservoir systems: effect of heat and compromised skin. *J Pharm Sci.* 2010; 99(5):2357–66. [PubMed: 19967778]
12. Hao J, Ghosh P, Li SK, Newman B, Kasting GB, Raney SG. Heat effects on drug delivery across human skin. *Expert Opin Drug Deliv.* 2016; 13(5):755–68. [PubMed: 26808472]
13. Franz TJ, Lehman PA, Raney SG. Use of excised human skin to assess the bioequivalence of topical products. *Skin Pharmacol Physiol.* 2009; 22(5):276–86. [PubMed: 19707043]
14. Lehman PA, Raney SG, Franz TJ. Percutaneous absorption in man: *in vitro-in vivo* correlation. *Skin Pharmacol Physiol.* 2011; 24(4):224–30. [PubMed: 21455015]
15. Gazerani P, Arendt-Nielsen L. Cutaneous vasomotor reactions in response to controlled heat applied on various body regions of healthy humans: evaluation of time course and application parameters. *Int J Physiol Pathophysiol Pharmacol.* 2011; 3(3):202–9. [PubMed: 21941611]
16. Kellogg DL Jr, Liu Y, Kosiba IF, O'Donnell D. Role of nitric oxide in the vascular effects of local warming of the skin in humans. *J Appl Physiol* (1985). 1999; 86(4):1185–90. [PubMed: 10194201]
17. Pergola PE, Kellogg DL Jr, Johnson JM, Kosiba WA, Solomon DE. Role of sympathetic nerves in the vascular effects of local temperature in human forearm skin. *Am J Phys.* 1993; 265(3 Pt 2):H785–92.
18. Miller MA, Kasting G. A measurement of the unstirred aqueous boundary layer in a Franz diffusion cell. *Pharm Dev Technol.* 2012; 17(6):705–11. [PubMed: 21554190]
19. Bird, RB., Stewart, WE., Lightfoot, EN. Transport phenomena. New York: Wiley; 1960.
20. McCabe, WL., Smith, JC. Unit operations of chemical engineering. New York: McGraw Hill; 1976.
21. Skelly JP, Shah VP, Maibach HI, Guy RH, Wester RC, Flynn G, Yacobi A. FDA and AAPS report of the workshop on principles and practices of *in vitro* percutaneous penetration studies: relevance to bioavailability and bioequivalence. *Pharm Res.* 1987; 4(3):265–7.
22. Aliouat Bellia S, Saidane A, Hamou A, Benzohra M, Saiter JM. Transmission line matrix modelling of thermal injuries to skin. *Burns.* 2008; 34:688–97. [PubMed: 18321649]
23. Becker S, Kuznetsov A. Thermal damage reduction associated with *in vivo* skin electroporation: a numerical investigation justifying aggressive pre-cooling. *Int J Heat Mass Transf.* 2007; 50:105–16.
24. Agache, P., Humbert, P. Measuring the skin. New York: Springer; 2004.
25. Cohen ML. Measurement of the thermal properties of human skin. A review. *J Invest Dermatol.* 1977; 69(3):333–8. [PubMed: 894075]
26. Sandby-Moller J, Poulsen T, Wulf HC. Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type, and smoking habits. *Acta Derm Venereol.* 2003; 83:410–13. [PubMed: 14690333]
27. Flynn, GL. Physicochemical determinants of skin absorption. In: Gerrity, TR., Henry, CJ., editors. Principles of route-to-route extrapolation for risk assessment. New York: Elsevier; 1990. p. 93–127.
28. Li SK, Suh W, Parikh HH, Ghanem A-H, Mehta SC, Peck KD, Higuchi WI. Lag time data for characterizing the pore pathway of intact and chemically pretreated human epidermal membrane. *Int J Pharm.* 1998; 170(1):93–108.
29. Ibrahim R, Kasting GB. Improved method for determining partition and diffusion coefficients in human dermis. *J Pharm Sci.* 2010; 99(12):4928–39. [PubMed: 20533434]

30. Mitragotri S. Temperature dependence of skin permeability to hydrophilic and hydrophobic solutes. *J Pharm Sci.* 2007; 96(7):1832–9. [PubMed: 17094128]
31. Scheuplein RJ. Analysis of permeability data for the case of parallel diffusion pathways. *Biophys J.* 1966; 6(1):1–17. [PubMed: 5903151]
32. Peck KD, Ghanem AH, Higuchi WI. The effect of temperature upon the permeation of polar and ionic solutes through human epidermal membrane. *J Pharm Sci.* 1995; 84(8):975–82. [PubMed: 7500283]

Fig. 1.

(a) Top Picture: Franz diffusion cell (side view); bottom picture: diffusion cell donor chamber with TDS (translucent), skin, and two temperature probes at their respective locations (top view). (b) Schematic diagram showing an example of the concentric configuration (dotted circle) of the diffusion cells (six small open circles) located under the heating lamp placed above the center of the concentric configuration in the Franz diffusion cell experiments *in vitro*. The Franz diffusion cells at one end are missing due to a cold spot in the configuration as a result of the “horse-shoe” shape filament in the heating lamp, depicted in yellow in the center of (c) thermal image of the heating lamp.

Fig. 2.

Schematic diagram comparing heat transfer dynamics *in vivo* and *in vitro*. *In vivo*: heat transfers across the TDS, skin surface, mid-dermis tissue and into the circulating blood (and the dermis beneath). By comparison, *in vitro*: heat transfers across TDS, skin surface, mid-dermis, and into the circulating receiver compartment. The effective thermal resistance of the aqueous boundary layer where the receiver solution interfaces with the underside of the skin is depicted as R_{ABL} and is only relevant *in vitro*. Circulating blood and receiver compartments were assumed to be at a constant temperature *in vivo* and *in vitro*, respectively. The temperature, thickness (span) of each layer, and temperature gradients in the diagram are not to scale.

Fig. 3.

Analysis of heat transfer *in vivo* using Eq. 5: plots of temperature difference between TDS and skin surfaces as a function of temperature at the TDS surface under the heating lamp.

From Eq. 5, slope = $R_{TDS}/(R_{TDS} + R_s)$ and x-intercept = T_{derm} . Symbols: TDS-1 (squares) and TDS-5 (triangles). Mean $\pm$ SD ($n = 6$ subjects). The slopes of TDS-1 and TDS-5 are statistically different (Student's t -tests, $p < 0.05$).

Fig. 4.

Analysis of heat transfer *in vitro* using Eq. 6: plots of temperature difference between TDS and skin surfaces as a function of the temperature difference between TDS surface and skin/receiver interface in Franz diffusion cells. From Eq. 6, slope = $R_{TDS}/(R_{TDS} + R_{sts})$. *In vivo* data from Fig. 3 are also shown here for comparison (dotted lines and dashed lines for TDS-1 and TDS-5, respectively). Symbols: TDS-1 (closed symbols) and TDS-5 (open symbols); heating lamp (triangles) and water bath methods (circles). Data are presented as mean $\pm$ SD from eight diffusion cells, with one outlier data set excluded from the analysis. The slopes of TDS-1 and TDS-5 are statistically different (Student's *t*-tests, $p < 0.05$). Average values of T_{TDS} in Stages 1–4 of heating lamp methods: 37.7°C, 43.1°C, 45.2°C, and 47.2°C for TDS-1 and 38.1°C, 43.8°C, 45.9°C, and 48.7°C for TDS-5, respectively. Average values of T_{TDS} in Stages 0–4 of water bath methods: 31.7°C, 36.5°C, 41.7°C, 43.7°C, and 45.5°C for TDS-1 and 31.6°C, 35.9°C, 40.4°C, 42.0°C, and 43.9°C for TDS-5, respectively. Stage 0 is the reference without exposure to a heat flux.

Fig. 5.

Analysis of heat transfer *in vitro* using Eq. 7: plots of temperature difference between TDS and skin surfaces as a function of the temperature difference between TDS surface and receiver in Franz diffusion cells. From Eq. 7, slope = $R_{TDS}/(R_{TDS} + R_{sts} + R_{ABL})$. *In vivo* data from Fig. 3 are also shown here for comparison (dotted lines and dashed lines for TDS-1 and TDS-5, respectively). Symbols: TDS-1 (closed symbols) and TDS-5 (open symbols); heating lamp (triangles) and water bath methods (circles). Data are presented as mean $\pm$ SD from eight diffusion cells, with one outlier data set excluded from the analysis. The slopes of TDS-1 and TDS-5 are statistically different (Student's *t*-tests, $p < 0.05$).

Fig. 6.

TDS surface temperatures predicted using Eq. 7 *versus* TDS thermal resistance when the skin surface temperature is 42°C in Franz diffusion cells with the heating lamp method (dashed line) and water bath method (solid line) *in vitro*. The same analysis of TDS surface temperatures was performed using the *in vivo* parameters of the same condition for comparison (dash-dotted line). When TDS thermal resistance decreases, the TDS surface temperature approaches that of the skin surface temperature.

Table 1Basal skin temperature under TDS (without exposure to a heat flux) *in vivo*

	Temperature at skin surface under TDS ^a	Temperature at TDS surface ^a	Temperature difference between skin and TDS ^{a, b}
TDS-1			
Forearm	32.8 ± 1.2°C	32.5 ± 1.1°C	0.25 ± 0.13°C
Upper arm	32.2 ± 0.7°C	32.0 ± 0.7°C	0.18 ± 0.05°C
Abdomen	32.3 ± 1.6°C	32.2 ± 1.6°C	0.04 ± 0.08°C
TDS-5			
Forearm	32.4 ± 0.6°C	31.8 ± 0.6°C	0.62 ± 0.19°C
Upper arm	32.3 ± 1.0°C	31.8 ± 1.0°C	0.51 ± 0.12°C
Abdomen	32.8 ± 1.5°C	32.3 ± 1.5°C	0.59 ± 0.13°C

^a mean ± SD (*n* = 6 subjects)^b $T_{\text{TDS/skin}}$ minus T_{TDS} calculated directly from the data on individual subjects