Local action of exogenous nitric oxide (NO) on the skin blood flow of rock pigeons (*Columba livia*) is affected by acclimation and skin site

Liisa M. Peltonen[1],* and Ahti Pyörnilä[2]

[1]*Department of Basic Veterinary Sciences, Physiology, 00014 University of Helsinki, PO Box 66, FIN 00014 Helsinki, Finland* and [2]*Department of Biology, University of Oulu, PO Box 3000, FIN 90014 Oulu, Finland*

*Author for correspondence (e-mail: liisa.m.peltonen@helsinki.fi)

Summary

We studied the blood flow over dorsal and abdominal, non-brooding patch skin of two groups of pigeons: one group was thermally acclimated to cold (winter-acclimatized, WAC) while the other group was acclimated to a mesic environment (thermally non-challenged, NOC). Two bilateral patches at the measurement sites were treated simultaneously with a gel containing sodium nitrate and ascorbic acid, to generate nitric oxide (NO), and a vehicle gel. Blood flow was measured by laser Doppler velocimetry. Changes induced by these treatments were calculated against basic blood flow values for the corresponding patch. The results showed that the basic blood flow over the abdominal skin patches at room temperature was higher than over the dorsal skin in both acclimation states, but comparison revealed a sustainably higher level of basic skin blood flow in the WAC pigeons. The local response to exogenous NO was non-uniform over the two skin areas measured, and a specific vasodilatory action on the abdominal microvessels was recorded in the NOC pigeons. Abdominal vasodilatation in the WAC pigeons seemed to involve other mechanisms as well as local NO-dependent ones, among which the role of cold-induced vasodilatation (CIVD) is discussed here. Interestingly, the dorsal skin seemed to be less responsive to the action of NO, irrespective of the acclimation state.

Our results show that acclimation state and skin site affect the action of exogenous NO on local skin blood flow, and we suggest that the differences reflect acclimation-induced changes in the vascularity of the skin and in its sensitivity to thermal stimuli and that the roles of the abdominal and dorsal skin are different with respect to environmental changes.

Key words: skin blood flow, nitric oxide, temperature, acclimation, cold-induced vasodilatation, laser Doppler velocimetry, pigeon, *Columba livia*.

Introduction

Cutaneous blood vessels have two major functions: the exchange of nutrients, metabolic products or blood gases with tissue fluids, which is carried out mostly by the capillaries (Ryan, 1973), and thermoregulation, which is carried out mostly by the arteriovenous anastomoses (AVA). In birds, AVAs have been found in the feet, the thoracic skin anterior to the brooding patch, abundantly in the brooding patch itself and in the naked skin of the head (Jones and Johansen, 1972; Lucas and Stettenheim, 1972; Molyneux and Harmon, 1982; Midtgård, 1984, 1986; Midtgård et al., 1985). The dorsal skin is covered with feathers and is thus not considered to be a favourable site for heat loss. This holds true only for non-evaporative heat loss, however, since the dorsal skin has a crucial role in cutaneous water evaporation (CWE) heat loss in desert birds (Marder, 1983; Marder and Ben-Asher, 1983; Marder and Gavrieli-Levin, 1987; Arieli et al., 2002). The increased skin blood flow (SBF) in heat-acclimated pigeons during heat-induced CWE greatly enhances evaporative heat loss but it is not a prerequisite for CWE, as has recently been shown by Ophir et al. (2002). The maintenance of heat balance in extreme climates seems to involve dynamic changes in both the arterial:venous pressure gradient and the hydrostatic pressure in the capillary network (Arieli et al., 2002; Ophir et al., 2002) and also functional and structural modifications to the epidermis (Peltonen et al., 1998, 2000).

Cutaneous blood flow (BF) is regulated by the vasomotoric tone of the different structural components of the cutaneous vascular tree. The sympathetic nerves release neuronal factors that act on the small arterioles supplying the skin capillaries, on the post-capillary venules draining blood from the skin and on the AVAs. Neuronal regulation is mediated in mammals by an adrenergic vasoconstrictor system and an active vasodilator system that involves cholinergic cotransmission (Kellogg et al., 1995), while the resting vascular tone seems to be regulated by both vasoconstrictor fibres and nitric oxide (NO; Kellogg et al., 1999; Pergola et al., 1993). Capillary blood flow *per se* is thought to be primarily regulated by local factors, of which NO seems to be the most powerful (Hales et al., 1978; Pergola et al., 1993; Kellogg et al., 1999).

The corresponding BF control mechanisms in birds are still

unclear, although recent studies on the effects of various adrenergic stimuli on the SBF in pigeons have revealed that the vasoconstrictory tone is maintained by the adrenergic sympathetic nerves. Thermal vasodilatation takes place, at least in part, passively through the release of the vasoconstrictory tone mediated by β- and α-adrenergic receptors (ARs; Arieli, 1998; Ophir et al., 2000). It is important to note that there seems to be a clear difference in the functional role of these receptors between birds and mammals. Passive vasodilatation, which is mediated by α_2-ARs in mammals, seems also to be mediated by the β-adrenergic pathway in birds, at least in the pigeon (Marder and Raber, 1989; Ophir et al., 2000). Even though the β_2 subtype predominates in the central nervous system (CNS) of the pigeon (Fernandez-Lopez et al., 1997), the β_1 subtype seems to be more responsive to changes in acclimation state, thus contributing to the fine-tuning of responses to thermal stimuli (Ophir et al., 2000). Recent results suggest that the fundamental difference between birds and mammals in the haemodynamic control exercised by the sympathetic nervous system could be partly explained by differences in the distribution, number and affinity of α- and β-ARs (Arieli, 1998) and, furthermore, by the hierarchical relationship between the receptor subtypes in the CNS and periphery (Ophir et al., 2000).

Even though no evidence so far exists for an active vasodilatory mechanism in birds, speculations that an NO-dependent local mechanism may exist have been put forward by Arieli et al. (2002).

The aim of the present work was to elucidate the role of NO in the local control of BF by measuring the action of non-invasively administered NO on the cutaneous BF in different acclimation states and at different skin sites.

Materials and methods

All the experiments conformed to the guidelines for proper animal care and use and had been authorized by the local ethical committee.

Animals

Winter-acclimatized (WAC; $N=5$) and thermally non-challenged (NOC; $N=6$) pigeons (*Columba livia* Gmelin 1789) were used. The WAC pigeons were exposed to cold in an outdoor aviary in Finland during the winter, with an air temperature (T_a) ranging from +7°C to –29°C, while the NOC birds were acclimated to room temperature (21–23°C) for at least three weeks. All the birds had free access to water and food.

Laser Doppler velocimetry

Skin blood flow was measured by laser Doppler velocimetry, which uses monochromatic laser light and the frequency shift of back-scattered light from the tissues to assess the number and velocity of the blood cells (Shepherd and Öberg, 1990). The equipment used was an Oxford Array

flowmeter (Oxford Optronix, Oxford, UK). The signals were recorded as blood perfusion units (BPU), which is a relative unit scale defined by reference to a controlled motility standard. Two fibre-optic button probes (diameter 1.5 cm) were attached to the patches over the dorsal and abdominal skin. To avoid erroneous readings resulting from movement of the probes, the birds were anaesthetized (see below) and the buttons kept in steady contact with the skin. The baseline flow in each intact skin area was measured for 10–15 min, or until stabilized, after which the probes were detached and the blood flow was stimulated locally as described below. The probes were reattached on the treated patch, and the stabilized flow was again measured for 10–15 min. The changes in blood flow in each skin patch were determined relative to the baseline flow (zero line). Means were calculated from the stabilized 10-min flow recordings for use in the baseline, control and 'treatment' flow assessments. All measurements were performed at room temperature in a dim light and at a relative humidity of 30–35%. To prevent possible interference from external illumination, the probes were covered with a piece of cloth.

Anaesthesia and experimental set-up

In order to ensure the immobility required for laser Doppler velocimetry, the pigeons were anaesthetized with intraperitoneal ketamine in combination with xylazine, at doses of 25 mg kg^{-1} (Ketalar®; Pfizer, Espoo, Finland) and 15 mg kg^{-1} (Rompun®; Bayer, Leverkusen, Germany), respectively. This combination, which is frequently used for avian anaesthesia, results in good muscle relaxation (xylazine), analgesia and calm recovery. Xylazine, a specific α_2-agonist, occupies the presynaptic receptors, leading to reduced release of endogenous catecholamines from the sympathetic nerve endings (Adams, 1995). At the central level, this leads to reduced adrenergic tone and sedation. On the other hand, ketamine induces a dissociative anaesthesia in which cardiac function and circulation are stimulated *via* central sympathetic activation (Adams, 1995). We therefore hypothesized that the stimulatory effect of ketamine on the vasomotoric tone would be able to balance the depressant effects of xylazine, leaving the central factors affecting the skin blood flow relatively unaltered.

When measuring blood flow in the dorsal skin, the pigeons were placed in a Styrofoam frame, which minimized heat loss and allowed free movement of the thoracic cage. During the abdominal measurements, they were placed on their backs on a Styrofoam frame without any additional attachments, thus allowing appropriate free orientation.

For blood flow stimulation, a vasoactive agent and a vehicle gel were applied bilaterally to the patches on the dorsal and abdominal skin. The patches on the dorsal skin were at the level of the scapulae on both sides of the spinal column, and those on the abdominal skin were on both sides of the mid-sternum.

Local stimulation of skin blood flow by nitric oxide

A gel generating nitric oxide was prepared as described in detail by Tucker et al. (1998). Briefly, NO can be generated in
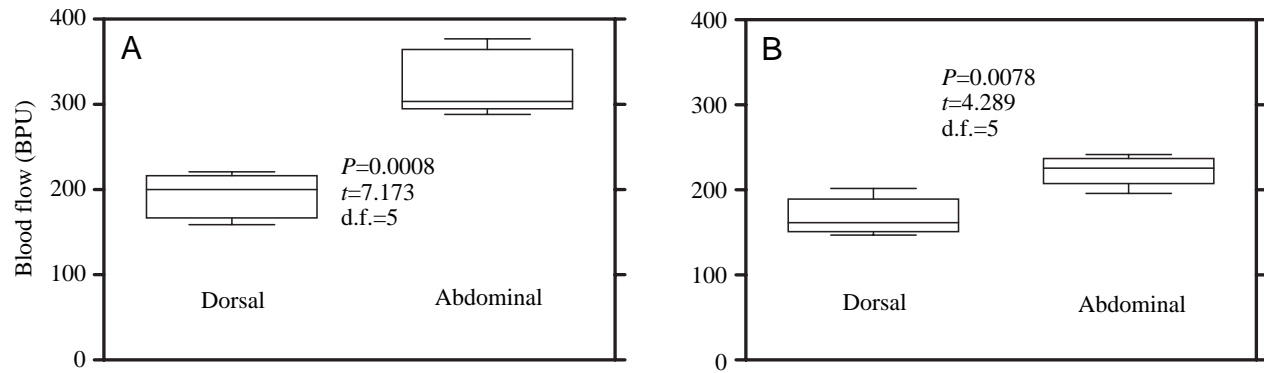
Fig. 1. Baseline blood flow over the dorsal and abdominal skin. (A) Winter-acclimatized (WAC) pigeons (95% confidence interval –171.9 to –81.17). (B) Thermally non-challenged (NOC) pigeons (95% confidence interval –89.69 to –22.47). BPU, blood perfusion unit. The box extends from the 25th to the 75th percentile, with a line at the median (50th percentile). The 'whiskers' extend above and below the box to show the highest and lowest values.

Table 1. *Mean basic blood flow velocities (mean BPU ± S.E.M.) over the dorsal and abdominal skin of winter-acclimatized (WAC) and thermally non-challenged (NOC) pigeons*

	Dorsal skin			Abdominal skin		
	WAC	NOC	$P*$	WAC	NOC	$P^\dagger$
Baseline	194.4±9.84 (N=10)	167.1±8.05 (N=12)	0.0371	320.9±14.35 (N=10)	223.2±6.32 (N=12)	0.0008

N, number of measurements (one measurement on two adjacent patches in each bird); BPU, blood perfusion unit; baseline, stabilized pre-treatment values for the two adjacent patches on each bird; *P*-value in the unpaired *t*-test, assuming equal variances; †*P*-value in the unpaired *t*-test with Welch's correction, assuming unequal variances.

large amounts through the rapid reduction of nitrite (A) by ascorbic acid (B). Substance A contained sodium nitrite (5% w/v) in a sterile lubricant gel (KY-Jelly; Johnson & Johnson, Maidenhead, UK), and it was added to substance B (5% w/v) by gently mixing equal volumes (0.02 ml) on the skin with a cotton bud. The reaction was allowed to proceed for 1 min, after which it was stopped by cleansing the skin with a soft tissue.

Statistics

Paired and unpaired *t*-tests and one-way and two-way analyses of variance (ANOVA) were used for the statistical analyses. If the variances of the two populations were revealed as 'unequal' by Bartlett's test, the Welch correction was used in obtaining the *P*-value in the unpaired *t*-test. To confirm the validity of the probabilities obtained from statistical tests, we randomised the order of the skin site (dorsal/abdominal) and patch (right/left) chosen for stimulation by tossing a coin.

Results
Baseline blood flow (BBF)

The results show the BBF over the abdominal skin to be higher than that over the dorsal skin in both acclimation states (Fig. 1; Table 1), although the difference (abdominal *vs* dorsal) was more than twice as great in the WAC birds (Fig. 1A). A general comparison of the BBF values showed consistently higher velocities in the WAC birds (Table 1).

Effect of the vehicle gel on blood flow

The measurements indicated that even the vehicle gel, as such, was not totally inert but affected the SBF, the response being variable and depending on the acclimation state and skin site (Table 2). The difference between the dorsal and abdominal skin (Fig. 1) disappeared in the NOC birds, since application of the vehicle gel to the abdominal skin led to a reduction in flow suggestive of vasoconstriction of the superficial vessels (Table 2). By contrast, the mean abdominal SBF in the WAC pigeons was significantly higher than the corresponding baseline (*P*=0.0016), indicating vasodilatation of the vessels over the measurement area (Table 2; Fig. 2C).

The dorsal skin seemed to be unresponsive in the WAC pigeons (Fig. 2A), while displaying higher flow velocities in the NOC birds (Table 2). Analysis with two-way ANOVA did indeed indicate that the differences between the skin sites caused by the vehicle gel were not consistent for the two acclimation states (interaction, *P*=0.017; skin site, *P*=0.292; acclimation state, *P*=0.038).

Effect of the NO-generating gel on blood flow

Topical application of the NO gel induced a significant increase in BF velocity over the abdominal skin in both acclimation states (Table 3; Figs 2D, 3D), and a moderate stimulation of the BF was also seen over the dorsal skin of the WAC pigeons, although that of the NOC birds was less responsive (Table 3). The changes induced by the vehicle and
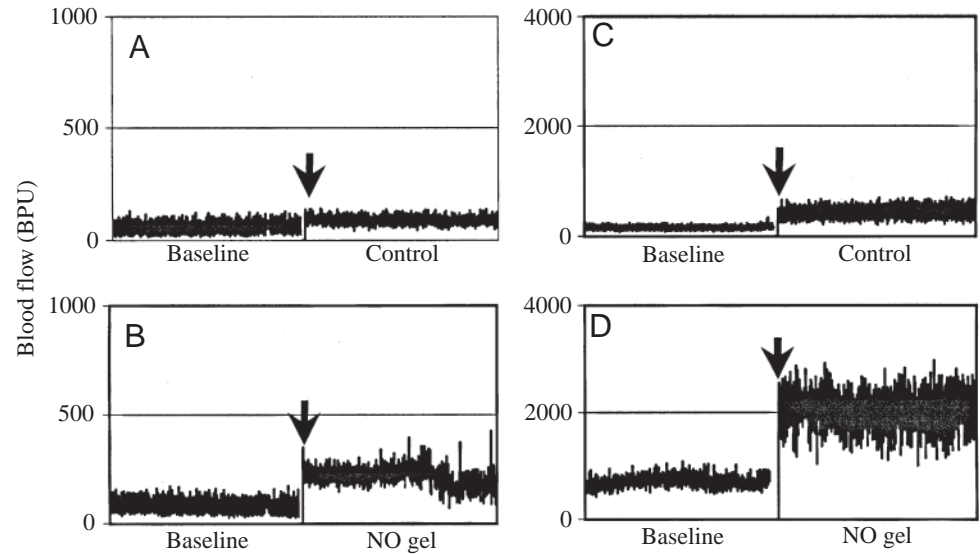
Fig. 2. Blood flow over the (A,B) dorsal and (C,D) abdominal skin of one winter-acclimatized pigeon (*Columba livia*). (A) Control. (B) Nitric oxide (NO) gel treatment. (C) Control. (D) NO gel treatment. BPU, blood perfusion unit. The arrow denotes the time of gel application. Stabilized blood flow over a 10-min period is presented as charts based on raw numerical flow data.

Table 2. *Mean blood flow velocities (mean BPU ± s.e.m.) over dorsal and abdominal 'control' skin patches on winter-acclimatized (WAC) and thermally non-challenged (NOC) pigeons*

	Dorsal skin			Abdominal skin		
	WAC	NOC	P^*	WAC	NOC	P^*
Baseline	146.9±8.92 (N=5)	112.4±9.69 (N=6)	0.0009	226.4±16.01 (N=5)	231.2±8.06 (N=6)	0.7912
	0.6048†	0.0003		0.0016†	0.0091	
Control	140.1±6.61 (N=5)	182.5±4.71 (N=6)	0.0004	473.4±35.73 (N=5)	156.5±11.74 (N=6)	0.0002

BPU, blood perfusion unit; baseline, calculated from stabilized pre-treatment values for the control skin patch; control, calculated from stabilized values for the control skin patch after vehicle gel application; *P-value in horizontal comparison between acclimation states (unpaired t-test); $^†P$-value in vertical comparison between baseline and control (paired t-test).

Table 3. *Mean blood flow velocities (mean BPU ± s.e.m.) over dorsal and abdominal 'treatment' skin patches on winter-acclimatized (WAC) and thermally non-challenged (NOC) pigeons*

	Dorsal skin			Abdominal skin		
	WAC	NOC	P^*	WAC	NOC	P^*
Baseline	245.1±15.56 (N=5)	215.7±19.04 (N=6)	0.2587	415.4±15.02 (N=5)	215.2±5.84 (N=6)	$P<0.0001$
	0.0252†	0.3693		0.0014†	$P<0.0001$	
Treatment	301.2±9.45 (N=5)	232.2±10.58 (N=6)	0.0007	1084±96.48 (N=5)	680.6±35.63 (N=6)	0.0078

BPU, blood perfusion unit; baseline, calculated from stabilized pre-treatment values for the treatment skin patch; treatment, calculated from stabilized values for the treatment skin patch after NO gel application; *P-value in horizontal comparison between acclimation states (unpaired t-test); $^†P$-value in vertical comparison between treatments (paired t-test).

NO gel in two adjacent patches on the dorsal and abdominal skin are summarized in Figs 4, 5, which show that a specific response to topically administered NO is present in the abdominal vascular network of the NOC pigeons (Table 4; Fig. 5), while in the WAC pigeons NO seems to amplify the vasodilator action of the vehicle gel (Figs 2C,D, 4). The mean BPU values for the vehicle and the NO treatment differ significantly (P=0.0015), being 473.4±35.73 and 1084±96.48, respectively (Tables 2, 3).

The superficial vessels on the dorsal skin of the WAC pigeons seem to respond only moderately to the vasodilator action of exogenous NO (Table 3), while the dorsal skin of the NOC pigeons seems to be practically unresponsive to NO action.

Two-way ANOVA analysis of the mean changes caused by application of the NO gel strongly points to the skin site as the major source of the variation (interaction, P=0.1333, not

Fig. 3. Blood flow over the (A,B) dorsal and (C,D) abdominal skin of one thermally non-challenged pigeon (*Columba livia*). (A) Control. (B) Nitric oxide (NO) gel treatment. (C) Control. (D) NO gel treatment. BPU, blood perfusion unit. The arrow denotes the time of gel application. Stabilized blood flow over a 10-min period is presented as charts based on raw numerical flow data.
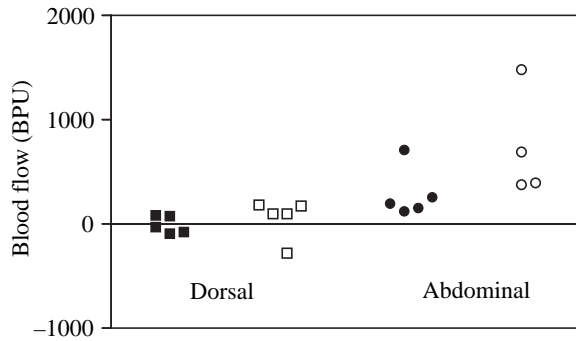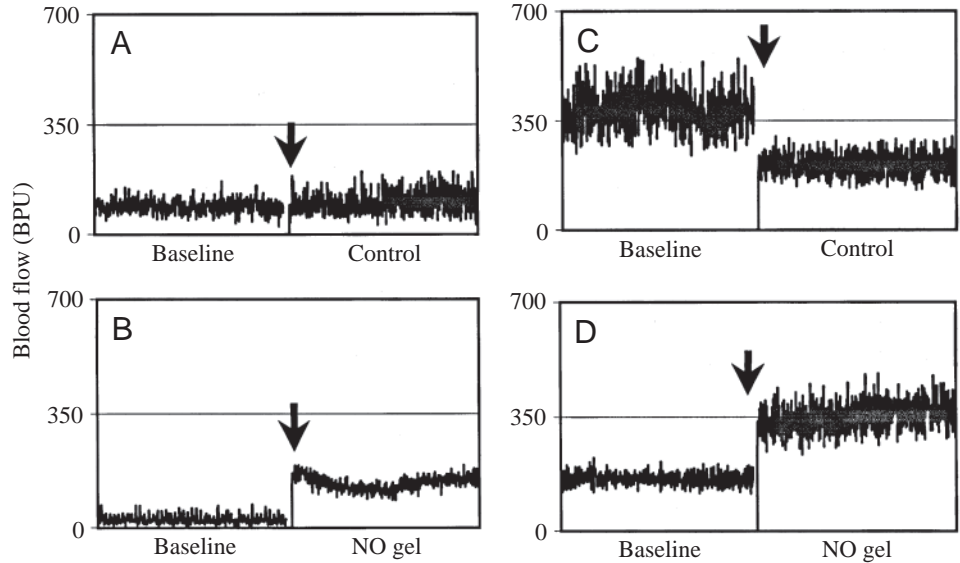

Fig. 4. Changes in blood flow over the dorsal and abdominal skin after topical application of the vehicle (controls ■ and ●, respectively) and NO-generating gel (treatments □ and ○, respectively) in five winter-acclimatized pigeons (*Columba livia*). Zero denotes the baseline flow. BPU, blood perfusion unit.
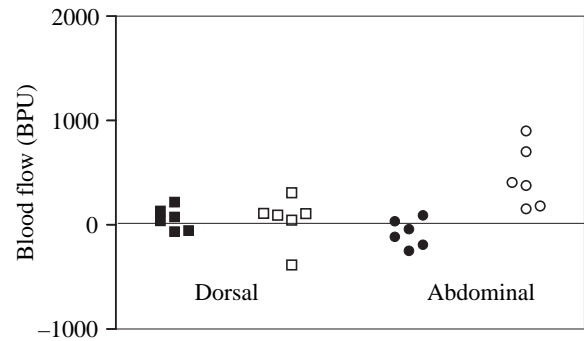

Fig. 5. Changes in blood flow over the dorsal and abdominal skin after topical application of the vehicle (controls ■ and ●, respectively) and NO-generating gel (treatments □ and ○, respectively) in six thermally non-challenged pigeons (*Columba livia*). Zero denotes the baseline flow. BPU, blood perfusion unit.

significant; skin site, $P<0.0001$; acclimation state, $P=0.0554$, not significant).

Discussion

We studied the blood flow over the dorsal and the abdominal skin of pigeons acclimated to either a cold or a mesic environment by treating two bilateral patches of diameter 1.5 cm simultaneously with an aqueous gel containing sodium nitrate and ascorbic acid, to generate NO, and a vehicle gel. The changes induced by these treatments were calculated against the BBF values of the corresponding patch. The results show that the local response of the skin to exogenous NO at room temperature is not uniform and that a specific NO-induced vasodilatation is clearly seen in the abdominal microvessels of NOC pigeons. In the WAC pigeons, mechanisms other than NO-dependent vasodilatation seem to be involved in the response, among which cold-induced vasodilatation (CIVD) will be discussed below. Interestingly,

the dorsal skin seems to be less responsive to the action of NO irrespective of the acclimation state.

Aspects of basic skin blood flow

The results suggest that acclimation to cold is associated with a higher basic SBF over the dorsal and abdominal skin areas than is found in thermally non-challenged birds. These findings are in line with the few *in vivo* studies of the effects of acclimation on BF. Vogel and Sturkie (1963) found that the peripheral resistance of the vascular system is lower in winter-acclimatized chickens than in summer-acclimatized ones. Data on humans suggest that the basic peripheral blood flow in arctic regions is higher in cold-acclimatized than in non-acclimatized subjects (Adams and Smith, 1962; Naidu and Sachdeva, 1993).

Why was the BBF velocity higher in the WAC pigeons? It is well known that a higher flow velocity may be caused by either release of the tonic vasoconstriction induced by the sympathetic adrenergic nerves or by stimulation of the activity of a vasodilator mechanism. These mechanisms are known to

Table 4. *Mean changes in blood flow (ΔBF) relative to the baseline flow velocity over the dorsal and abdominal skin of winter-acclimatized (WAC) and thermally non-challenged (NOC) pigeons*

	Dorsal skin			Abdominal skin		
	WAC	NOC	P*	WAC	NOC	P
Control	−7.1±37.0 $P^{\dagger}$>0.05	57.9±44.5 P>0.05	0.3034	287.9±108.2 $P^{\dagger}$>0.05	−78.8±53.6 0.0156	0.0107
NO gel	55.1±85.4	47.5±93.7	0.9542	735.4±259.0	453.5±120.2	0.2991

The differences in the blood flow between the 'baseline' and 'control' values and between the 'baseline' and 'treatment' values were assessed by collecting ΔBF values every 2 min over a 10-min period of stabilized BF. Mean values were calculated for every 10-min period in each individual. *P*-values in horizontal and vertical comparisons are presented as in Tables 2, 3.

cooperate in the presence of thermal stress, for example, so that the thermal vasodilatation observed in humans is far greater than that caused by blockade of the noradrenergic vasoconstrictor nerves alone (Kellogg et al., 1999; Wilson et al., 2002). Ophir and coworkers showed recently that release of the vasoconstrictor tone by the non-specific β-adrenergic antagonist propranolol does not solely account for the maximal vasodilatory response induced by heat in heat-acclimated pigeons (Ophir et al., 2002). Contrary to mammals, in which α-ARs primarily mediate vasoconstriction in the peripheral vessels (Kellogg et al., 1999), β-ARs seem to be involved in the pigeon. Depending on the location and function within the vascular tree, different receptors seem to be employed. A recent hypothesis on the interplay between receptor types in the pigeon's cutaneous vasculature suggests that, while the β-ARs (especially β_2) primarily act on precapillary sites, the α-ARs (especially α_2) act on postcapillary sites (Ophir et al., 2002). Nevertheless, in order to produce appropriate physiological effects, the process of acclimation to a changing external environment seems to be crucial in modulating the function of these receptors.

The reasons for the higher SBF over the abdominal skin than over the dorsal skin may be both physiological and anatomical. The skin, as a site for dry heat flux, is functionally heterogeneous. The areas covered with feathers do not offer as effective a route for dry heat loss as those that are without feathers. On the other hand, the apteria do not offer as good insulation against heat loss as do the feathered skin areas. The constantly higher BBF over the abdominal skin documented here in both acclimation states can be seen as reflecting the crucial role of the abdominal apteria in dry heat flux, since the brooding patch and associated thoracic areas (and their heat transfer) are important for successful incubation and offspring survival. On the ventral side of the bird, the high vascular density and the active vascular responses seem to correspond to the sparse feather layer (Lucas and Stettenheim, 1972). The fivefold response of the total SBF to total body warming over the skin of the ventral side relative to that on the back is thought to be associated with the presence of AVAs in these areas (Wolfenson et al., 1981). In general, AVAs have been found in areas that are important for temperature regulation (Jones and Johansen, 1972; Lucas and Stettenheim, 1972; Molyneux

and Harmon, 1982; Midtgård, 1984, 1986; Midtgård et al., 1985). The present findings suggest indirectly that AVAs may be present in the anterior areas of the featherless abdominal skin of pigeons as well. We found that topical application of the vehicle gel, which had a cooling effect at the site of application, resulted in a specific dilatory response over the abdominal skin that was similar to the AVA-mediated CIVD reaction to cold observed in birds (Johansen and Millard, 1974; Midtgård et al., 1985).

Cold-induced vasodilatation over the non-brood patch abdominal skin

The results regarding the WAC pigeons suggest the existence of CIVD over the non-brood patch areas of the apterial ventral skin. Moreover, the CIVD response seems to be augmented by NO. The increased blood flow in the WAC pigeons therefore seems to be due to at least two separate mechanisms. The initial reaction to local cooling seems to be an acclimation-dependent axon reflex. There is strong evidence for the fact that the main targets of neuronal control are the richly innervated AVAs, while the capillary flow is under local control (Kellogg et al., 1995). The local mechanisms, on the other hand, are thought to be largely, if not completely, independent of neurogenic control (Roberts et al., 2002). This independence has also been demonstrated in birds. Artificial cooling of hens' eggs, for instance, induced purely local vasodilatation, which was unaffected by nerve blockade (Midtgård et al., 1985).

If CIVD is indeed controlled by nerves, functional cold receptors are mandatory. We suggest that, despite the analgesia, the epidermal cold receptors may have been stimulated under ketamine anaesthesia. Ketamine tends to increase adrenergic tone in the CNS, so that animals remain sensitive to sound, for example (Plumb, 2002). Thus, the sensory input from the epidermal cold receptors may have induced CIVD, possibly by intervening in the nerve traffic controlling the thermoregulatory AVA flow.

In addition to a neural mechanism, a CIVD response may have been induced locally by the temperature around the vessel. Temperature seems to be able to modulate the vessel's response to neuronal transmitters. Local cooling has been shown to change the sensitivity of ARs within these structures

by reducing the amount of noradrenaline (NA) released from the nerves (Vanhoutte and Verbeuren, 1976), by reducing the enzymatic degradation of NA within the vessel wall (Roberts et al., 1986) and by altering the binding affinity of NA for α_1- and α_2-ARs (Janssens and Vanhoutte, 1978; Roberts et al., 1986). A 1-min application of the vehicle gel alone resulted in a $1.22\pm0.67°C$ ($N=11$) drop in the abdominal skin temperature (L.M.P., unpublished information), but it is uncertain whether the temperature around the superficial vessels is reduced as well. A direct action of cooling on the vascular nerves would nevertheless be able to cause a temporary release of the vasoconstrictor tone and thereby an increase in the SBF.

The CIVD reaction is a protective response of the cutaneous vessels to cold injury. This was first reported in humans after immersion of an extremity to cold water (Lewis, 1930). Cold stimulus leads to a rapid decrease in skin temperature, accompanied by pronounced vasoconstriction of the cutaneous vessels. After a few minutes, the skin temperature starts to increase as a consequence of vasodilatation and an increase in the superficial BF. Depending on the species, acclimation state and skin site, several mechanisms may lie behind CIVD. It has been suggested that cholinergic, β-adrenergic and purinergic nerves may be involved in the control of CIVD in birds (Johansen and Millard, 1974; Murrish and Guard, 1977; Hillman et al., 1982), and Midtgård (1988) has suggested that the VIP-immunoreactive fibres may be mediators of CIVD, in view of their abundance in the AVAs of the brood patch in the chicken. The proposed mechanisms in humans are axon reflex *via* peripheral pain fibres and the release of vasodilator substances (Lewis, 1930; Daanen and Ducharme, 2000), CNS involvement (Werner, 1977; Kunimoto, 1987) and sympathetic activation to modulate the dilatory response (Sendowski et al., 2000). The most common explanation, though, may be the interruption of adrenergic neurotransmission by cold (Johnson et al., 1986; Daanen and Ducharme, 2000). Just as in humans (LeBlanc, 1975), cold acclimation also seems to enhance the CIVD reaction in the pigeon. Acclimation to mesic environmental conditions seems to abolish the vasodilatory response, indicating that it further modulates the systems that control cutaneous blood flow.

Aspects of the local effects of NO on skin blood flow

Nitric oxide, due to its short half-life of only a few seconds (Feelisch and Stamler, 1996), is considered to be a strictly local regulating factor. Various cells that may be associated with the control of SBF have the capacity to produce NO, notably the endothelial cells, perivascular nervous cells and keratinocytes, which can all produce it either by a constitutive or an inducible mechanism (reviewed in Bruch-Gerharz et al., 1998). In order to find out the effects and possible targets of NO action in the case of the pigeon skin, we studied the effects of exogenous NO on the SBF in small areas on the dorsal and abdominal skin. Methodologically, the topical applications used here can be considered to represent a non-invasive procedure, and we can therefore exclude any stimulatory effects of tissue trauma on BF. NO most probably acted directly on the contractile

elements around the vessel walls of the small skin patches, as it diffuses freely through cell membranes and acts on smooth muscle cells, which may be present adjacent to the precapillary arterioles or postcapillary venules draining from the skin, or on the AVA, if present. In smooth muscle cells, NO binds to soluble guanylyl cyclase, the source of second-messenger cGMP (Feelisch and Stamler, 1996).

The present results show that the dorsal vasculature is far less responsive to exogenous NO than the ventral vasculature. This is an unexpected result, since NO is a very potent vasodilator and the present NO-generating system could be expected to yield local concentrations high enough to cause vasodilatation (Tucker et al., 1998). Since the structural characteristics of the dorsal and abdominal epidermis are similar (Peltonen et al., 1998), the barrier to penetration by NO would be similar in these areas. Hence, a reduction in the number of vessels with contractile perivascular structures seems to be the most probable reason for the moderate haemodynamic changes. We have previously shown that acclimation to different thermal environments may lead to local changes in the vascular tree and in the number of superficial microvessels (Peltonen et al., 1998). The capillary bed over the dorsal skin tends to become more dense when pigeons are acclimated to heat. The WAC pigeons appeared to be devoid of microvessels in an area reaching from epidermal basal lamina to the depth of at least twice the thickness of the viable epidermis, whereas the NOC pigeons possessed an intermediate number of superficial vessels, and heat-acclimated pigeons (HAC) are known to have the greatest abundance of microvessels adjacent to the epidermal basement membrane. The abdominal skin, on the other hand, seems to maintain its superficial vascularity in WAC and NOC states, while its tendency for a dense vascularity in the HAC pigeons is similar to that in the dorsal skin (Peltonen et al., 1998).

The role of NO in the SBF of birds is still unclear, but studies made in mammals on its interactions with the catabolism of cAMP, the second messenger system of the adrenergic pathway, suggest that the second messenger of the NO pathway, cGMP, is able to inhibit phosphodiesterase III, which catalyses the transformation of cAMP to AMP (Vanhoutte, 2001). The net effect would thus be an increase in the cAMP concentration and an enhancement of the biological effects of adrenergic stimuli. Acclimation to cold probably modulates the sensitivity of various receptors and thereby adjusts the character of the response to thermal stimuli.

To summarize, the response of the skin to exogenous NO is affected by the skin site. The abdominal skin of the NOC pigeon shows a specific response to NO, whereas in the WAC pigeon NO seems to amplify the dilatory action of the vehicle, which exercises a cooling effect. We suggest that this may be due to different vascular arrangements in the dorsal and abdominal skin, reflecting their functional differences in dry and wet heat loss, and that the moderate responsiveness of the dorsal skin may be due to lack of AVAs in the measurement area. The consistently higher flow velocity over the dorsal and abdominal skin that is associated with cold acclimation is

thought to be caused by an acclimation-dependent reduction in the tonic vasomotor tone. The acclimation process seems to affect the local CIVD reaction by modifying the sensitivity of the skin to thermal stimuli. CIVD seems to be enhanced by cold acclimation and amplified by NO. Acclimation to a mesic environment, on the other hand, seems to abolish the local response. These functional differences between acclimation states are probably associated with the acclimation-dependent modulation of the sensitivity of the local BF for thermal protection of the cutaneous tissue or the offspring.

References

Adams, T. and Smith, R. E. (1962). Effect of chronic cold exposure on finger temperature responses. *J. Appl. Physiol.* **17**, 317-322.

Adams, H. R. (ed.) (1995). *Veterinary Pharmacology and Therapeutics.* Ames: Iowa State University Press.

Arieli, Y. (1998). Heat acclimation physiology, the role of cells, organelles, and membranous receptors in the cutaneous water evaporation cooling mechanism in the rock pigeon (*Columba livia*). PhD Thesis, The Hebrew University of Jerusalem, Israel.

Arieli, Y., Peltonen, L. and Ophir, E. (2002). Cooling by cutaneous water evaporation in the heat-acclimated rock pigeon (*Columba livia*). *Comp. Biochem. Physiol. A* **131**, 497-504.

Bruch-Gerharz, D., Ruzicka, T. and Kolb-Bachofen, V. (1998). Nitric oxide in human skin, current status and future prospects. *J. Invest. Dermatol.* **110**, 1-7.

Daanen, H. A. M. and Ducharme, M. B. (2000). Axon reflexes in human cold exposed fingers. *Eur. J. Appl. Physiol.* **81**, 240-244.

Feelisch, M. and Stamler, J. S. (1996). *Methods in Nitric Oxide Research.* Chichester: Wiley.

Fernandez-Lopez, A., Revilla, V., Candelas, M. A., Gonzales-Gil, J., Diaz, A. and Pazos, A. (1997). A comparative study of α_2- and β-adrenoceptors distribution in pigeon and chick brain. *Eur. J. Neurosci.* **9**, 871-883.

Hales, J. R. S., Iriki, M., Tsuchiya, K. and Kozawa, E. (1978). Thermally-induced cutaneous sympathetic activity related to blood flow through capillaries and arteriovenous anastomoses. *Pflügers Arch.* **375**, 17-24.

Hillman, P. E., Scott, N. R. and van Tienhoven, A. (1982). Vasomotion in chicken foot, dual innervation of arteriovenous anastomoses. *Am. J. Physiol.* **242**, R582-R590.

Janssens, W. J. and Vanhoutte, P. M. (1978). Instantaneous changes of alpha-adrenoceptor affinity caused by moderate cooling in canine cutaneous veins. *Am. J. Physiol.* **234**, H330-H337.

Johansen, K. and Millard, R. W. (1974). Cold-induced neurogenic vasodilatation in skin of the giant fulmar *Macronectes giganteus*. *Am. J. Physiol.* **227**, 1232-1235.

Johnson, J. M., Brengelmann, G. L., Hales, J. R. S., Vanhoutte, P. M. and Wenger, C. B. (1986). Regulation of the cutaneous circulation. *Fed. Proc.* **45**, 2841-2850.

Jones, D. R. and Johansen, K. (1972). The blood vascular system of birds. In *Avian Biology* (ed. D. S. Farner and J. R. King), pp. 157-285. New York: Academic Press.

Kellogg, D. L., Pergola, P. E., Piest, K. L., Kosiba, W. A., Crandall, C. G., Grossman, M. and Johnson, J. M. (1995). Cutaneous active vasodilatation in humans is mediated by cholinergic nerve cotransmission. *Circ. Res.* **77**, 1222-1228.

Kellogg, D. L., Liu, Y., Kosiba, I. F. and O'Donnell, D. (1999). Role of nitric oxide in the vascular effects of local warming of the skin in humans. *J. Appl. Physiol.* **86**, 1185-1190.

Kunimoto, M. (1987). Where is the most useful point in the skin temperature curve of the fingertip immersed in the cold water to evaluate the function of the skin sympathetic function? *Biomed. Thermogr.* **7**, 254-268.

LeBlanc, J. (1975). *Man in the Cold.* Springfield, IL: American Lecture Series.

Lewis, T. (1930). Observations upon the reactions of the vessels of the human skin to cold. *Heart* **15**, 177-208.

Lucas, A. M. and Stettenheim, P. R. (1972). *Avian Anatomy. Agriculture Handbook 362*. Washington: US Government Printing Office.

Marder, J. (1983). Cutaneous water evaporation II. Survival of birds under extreme thermal stress. *Comp. Biochem. Physiol. A* **75**, 433-439.

Marder, J. and Ben-Asher, J. (1983). Cutaneous water evaporation I. Its significance in heat-stressed birds. *Comp. Biochem. Physiol. A* **75**, 425-431.

Marder, J. and Gavrieli-Levin, I. (1987). Heat-acclimated pigeon, an ideal physiological model for a desert bird. *J. Appl. Physiol.* **62**, 952-958.

Marder, J. and Raber, P. (1989). Beta-adrenergic control of trans-cutaneous evaporation cooling mechanisms in birds. *J. Comp. Physiol. B* **159**, 97-103.

Midtgård, U. (1984). Density of arteriovenous anastomoses in some skin areas of the domestic fowl (*Gallus domesticus*). *Anat. Rec.* **209**, 455-459.

Midtgård, U. (1986). The blood vascular system in the head of the herring gull (*Larus argentatus*). *J. Morphol.* **179**, 135-152.

Midtgård, U. (1988). Innervation of arteriovenous anastomoses in the brood patch of the domestic fowl. *Cell Tissue Res.* **252**, 207-210.

Midtgård, U., Sejrsen, P. and Johansen, K. (1985). Blood flow in the brood patch of Bantam hens, evidence of cold vasodilatation. *J. Comp. Physiol. B* **155**, 703-709.

Molyneux, G. S. and Harmon, B. (1982). Innervation of arteriovenous anastomoses in the web of the foot of the domestic duck, *Anas platyrhyncos*. Structural evidence for the presence of non-adrenergic non-cholinergic nerves. *J. Anat.* **135**, 119-128.

Murrish, D. E. and Guard, C. L. (1977). Cardiovascular adaptations of the giant petrel *Macronectes giganteus*, to the Antarctic environment. In *Adaptations within Antarctic Ecosystems* (ed. G. A. Llano), pp. 511-530. Washington, DC: Smithsonian Institute.

Naidu, M. and Sachdeva, U. (1993). Effect of local cooling on skin temperature and blood flow of men in Antarctica. *Int. J. Biometeorol.* **37**, 218-221.

Ophir, E., Arieli, Y., Raber, P. and Marder, J. (2000). The role of β-adrenergic receptors in the cutaneous water evaporation mechanism in the heat-acclimated pigeon (*Columba livia*). *Comp. Biochem. Physiol. A* **125**, 63-74.

Ophir, E., Arieli, Y., Marder, J. and Horowitz, M. (2002). Cutaneous blood flow in the pigeon *Columba livia*, its possible relevance to cutaneous water evaporation. *J. Exp. Biol.* **205**, 2627-2636.

Peltonen, L., Arieli, Y., Pyörnilä, A. and Marder, J. (1998). Adaptive changes in the epidermal structure of the heat-acclimated Rock pigeon (*Columba livia*), a comparative electron microscopy study. *J. Morphol.* **235**, 17-29.

Peltonen, L., Arieli, Y., Harjula, R., Pyörnilä, A. and Marder, J. (2000). Local cutaneous water barrier in cold- and heat-acclimated pigeons (*Columba livia*) in relation to cutaneous water evaporation. *J. Morphol.* **246**, 118-130.

Pergola, P. E., Kellogg, D. L., Johnson, J. M., Kosiba, W. A. and Solomon, D. E. (1993). Role of sympathetic nerves in the vascular effects of local temperature in human forearm skin. *Am. J. Physiol.* **265**, H785-H792.

Plumb, D. C. (ed.) (2002). *Veterinary Drug Handbook*. Ames: Iowa State University Press.

Roberts, M., Chilgren, J. and Huang, M. (1986). Effect of temperature on degradation of norepinephrine in rabbit ear artery. *Physiologist* **29**, 181.

Roberts, M., Rivers, T., Oliveria, S., Texeira, P. and Raman, E. (2002). Adrenoceptor and local modulator control of cutaneous blood flow in thermal stress. *Comp. Biochem. Physiol. A* **131**, 485-496.

Ryan, T. J. (1973). Structure, pattern and shape of the blood vessels of the skin. In *The Physiology and Pathophysiology of the Skin* (ed. A. Jarret), pp. 577-653. London: Academic Press.

Sendowski, I., Savourey, G., Launay, J.-C., Besnard, Y., Cottet-Emard, J.-M., Pequignot, J.-M. and Bittel, J. (2000). Sympathetic stimulation induced by hand cooling alters cold-induced vasodilatation in humans. *Eur. J. Physiol.* **81**, 303-309.

Shepherd, A. P. and Öberg, P. Å. (ed.) (1990). *Laser-Doppler Blood Flowmetry*. Boston: Kluwer Academic Publishers.

Tucker, A. T., Pearson, R. M., Cooke, E. D. and Benjamin, N. (1998). Effect of nitric-oxide-generating system on microcirculatory blood flow in skin of patients with severe Raynaud's syndrome, a randomised trial. *Lancet* **354**, 1670-1675.

Vanhoutte, P. M. (2001). Endothelial adrenoceptors. *J. Cardiovasc. Pharmacol.* **38**, 796-808.

Vanhoutte, P. M. and Verbeuren, T. J. (1976). Depression by local cooling

of ^{3}H-norepinephrine release evoked by nerve stimulation in cutaneous veins. *Blood Vessels* **13**, 92-99.

Vogel, J. A. and Sturkie, P. D. (1963). Cardiovascular responses on chicken to seasonal and induced temperature changes. *Science* **140**, 1404.

Werner, J. (1977). Influences of local and global temperature stimuli on the Lewis-reaction. *Pflügers Arch.* **367**, 292-294.

Wilson, T. E., Cui, J. and Crandall, C. G. (2002). Effect of whole-body and local heating on cutaneous vasoconstrictor responses in humans. *Auton. Neurosci.* **31**, 122-128.

Wolfenson, D., Frei, Y. F., Snapir, N. and Berman, A. (1981). Heat stress effects on capillary blood flow and its redistribution in the laying hen. *Pflügers Arch.* **390**, 86-93.