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Full-length Article

Skin colour changes during experimentally-induced sickness

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ABSTRACT

Skin colour may be an important cue to detect sickness in humans but how skin colour changes with acute sickness is currently unknown. To determine possible colour changes, 22 healthy Caucasian participants were injected twice, once with lipopolysaccharide (LPS, at a dose of 2 ng/kg body weight) and once with placebo (saline), in a randomised cross-over design study. Skin colour across 3 arm and 3 face locations was recorded spectrophotometrically over a period of 8 h in terms of lightness (L^*), redness (a^*) and yellowness (b^*) in a manner that is consistent with human colour perception. In addition, carotenoid status was assessed as we predicted that a decrease it skin yellowness would reflect a drop in skin carotenoids. We found an early change in skin colouration 1–3 h post LPS injection with facial skin becoming lighter and less red whilst arm skin become darker but also less red and less yellow. The LPS injection also caused a drop in plasma carotenoids from 3 h onwards. However, the timing of the carotenoid changes was not consistent with the skin colour changes suggesting that other mechanisms, such as a reduction of blood perfusion, oxygenation or composition. This is the first experimental study characterising skin colour associated with acute illness, and shows that changes occur early in the development of the sickness response. Colour changes may serve as a cue to health, prompting actions from others in terms of care-giving or disease avoidance. Specific mechanisms underlying these colour changes require further investigation.

1. Introduction

A face flush with colour is attributed with good health and may serve as a cue for mate choice (Stephen et al., 2009b). Conversely, a distinct lack of colour in the face could serve as a cue to ill-health, and promoting actions from others in terms of disease avoidance or care provision. Perceptual studies have shown that faces with slightly raised levels of red and yellow colour are judged as looking healthier (Stephen et al., 2012, 2009b). These findings are likely to be driven by high levels of oxygenated blood (Stephen et al., 2009a) and carotenoids pigments (Whitehead et al., 2012a, 2012b).

These colour associations may be well founded with reference to long-term or general health. Peripheral blood flow is associated positively with physical fitness and negatively with smoking as well as many chronic health conditions (Anton et al., 2006; Carmeliet, 2003). Also, carotenoid colouration of human skin is associated with high levels of fruit and vegetable consumption (Alaluf et al., 2002; Tan et al., 2015; Whitehead et al., 2012b). In many bird species, carotenoid colouration of ornaments (feathers, beaks or skin) are a well-established signal of health, correlating negatively with parasite load (Mougeot et al., 2009), positively with disease resistance (Nolan et al., 1998) and positively with ability to mount a strong immune response (Aguilera and Amat, 2007; Peters et al., 2004). These associations suggest that in evolutionary terms, sensitivity to colour cues may well have provided indirect benefits by allowing individuals to select mates with genes for good health.

There are also potential short-term and direct evolutionary gains to be made if acute illness were characterized by robust changes of colour cues. Observation of sickness in others could induce care-giving behaviour benefiting the sick individual. Alternatively, observers could benefit in terms of avoiding disease. Disease avoidance has attracted much recent attention (Schaller and Park, 2011), but little is known of what signals and cues characterize sick peers. Skin colour changes are readily observable, and

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humans are highly sensitive to subtle colour changes of the skin, i.e. in faces (Lefevre et al., 2013; Tan and Stephen, 2013). Changes in skin colour could therefore provide a sickness cue, but it is at present unknown if and how human skin colour changes in response to an innate immune activation.

Here, we investigated the skin colour changes in 22 healthy Caucasian subjects in response to an intravenous injection of bacterial endotoxin (i.e. lipopolysaccharide, LPS) at a dose of 2 ng/kg body weight and compared with injection of placebo (saline). The model of LPS administration is a well-established model to experimentally induce an acute sickness response in humans (Schedlowski et al., 2014; Suffredini et al., 1999). LPS elicits a transient innate immune response characterised by a production of pro-inflammatory cytokines, and accompanied by flu-like symptoms including fever, headaches, nausea and changes in fatigue, concentration and mood (Benson et al., 2012; Lasselin et al., 2016a; Schedlowski et al., 2014; Suffredini et al., 1999). We set out to investigate skin colour changes during an acute systemic inflammation using spectrophotometric measures and reported change in terms of skin lightness, redness and yellowness. Face (cheeks and forehead) and arm (shoulder, forearm and palm) locations were assessed separately because prior work investigating perceived health has been conducted only in facial stimuli and it is not known whether the arm and face will change the same manner. Consistent with perceptions of health (Stephen et al., 2009b), it was hypothesized that the participants' skin colour will become less yellow, less red and lighter after LPS injection in comparison to saline. Self-reported measures of sickness together with physiological measures (blood pressure, cytokine response and body temperature) were also taken over time in order to investigate how the time-course of colour change mapped onto changes in subjective sickness as well as the body's physiological response. Finally we measured plasma carotenoids to test whether any loss in yellow colour of the skin was consistent with carotenoid loss.

2. Methods

2.1. Participants

Twenty-two Caucasian participants (9 female, mean age = 23.4 years, SD = 3.5 years) were recruited by advertisements on University campuses and high schools in the Stockholm area. Exclusion criteria were related to age (those under 18 or over 50), body mass index (less than 18.5 kg/m^2 or greater than 30 kg/m^2), smoking, excessive use of alcohol and anyone with a diagnosed physiological or psychiatric disease. Participants gave informed consent and all procedures were medically supervised and reviewed by the regional ethical review board in Stockholm, Sweden.

2.2. Procedure

In a double-blinded crossover design, all volunteers participated in two sessions, once receiving an intravenous injection with LPS (*Escherichia coli* endotoxin, Lot H0K354 CAT number 1235503; 2.0 ng/kg body weight) and once with saline (NaCl 0.9%, placebo condition) in a counterbalanced order (see Lasselin et al., 2016b for detailed protocol and also Karshikoff et al., 2015; Sundelin et al., 2015 for similar methods). Throughout the following 7.5 h, measurements of plasma cytokines (IL-6 and TNF-α), body temperature, self-reported sickness, blood pressure, skin colour and plasma carotenoids were performed. The timing of these measures is summarised in table one.

2.3. Cytokines

Plasma concentrations of IL-6 and TNF-α were assessed using multiplexed luminex assays (Human Mag Luminex Performance Assay, LHSCM000, LHSCM206, LHSCM210, RnD Systems, MN, USA) according to the manufacturer's instructions.

2.4. Self-reported sickness (SQ-score)

A 10 item self-report questionnaire of perceived sickness was administered. A translation of the items from Swedish reads: 1) I want to keep still; 2) my body feels sore; 3) I wish to be alone; 4) I don't wish to do anything at all; 5) I feel depressed; 6) I feel drained; 7) I feel nauseous; 8) I feel shaky; 9) I feel tired; 10) I have a headache. Items were rated from 0 to 3 in terms of agreement and summed to provide a sickness score (SQ-score) (Andreasson et al., 2016).

2.5. Skin colour

Skin colour was measured in CIE colour space along three perceptually relevant colour axes: L* represents darkness/lightness; a* represents a scale from green (negative values) to red (positive values); and b* represented a scale from blue (negative values) to yellow (positive values). In the context of skin colour, only positive values are relevant and so a* and b* are hereafter referred to as "redness" and "yellowness" respectively (Stamatas et al., 2004). Measurements were made using a Konica Minolta CM-700d, a Spectrophotometer (d65 illuminant 8° illumination angle, specular component excluded). At each time point (see Table 1), measurements were taken across 3 face locations (left and right cheek, and then forehead) and at 3 arm locations (palm, inner forearm, and shoulder). Each location was measured twice and values for each colour channel (L*, a*, b*) were averaged across the 2 measurement iterations and across constituent locations to obtain values for the face and arm.

Delta e (total perceptual change across all three colour channels) was also calculated for each time point as: $\Delta E^* = \sqrt{((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)}$. This value allows us to consider whether the magnitude of total colour change is likely to be perceivable. A difference greater than 2.2 is often claimed to be the smallest noticeable under optimal lighting (Brainard, 2003; Burriss et al., 2015). However, perceptual studies of facial stimuli have shown that a difference in skin colour associated with a change in blood perfusion of 0.6 ΔE^* is detectable in human skin,

Table 1
Experimental time line summarises when measurements of all variables were collected.

Measured variable	Time post injection (h)									
	0	0.5	1	1.5	2	3	4	5	7	7.5
Cytokines	✓		✓	✓	✓	✓	✓	✓	✓	
Temperature	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Sickness	✓			✓		✓		✓	✓	
Blood Pressure	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Colour	✓		✓		✓	✓	✓	✓		✓
Carotenoids	✓		✓			✓			✓	