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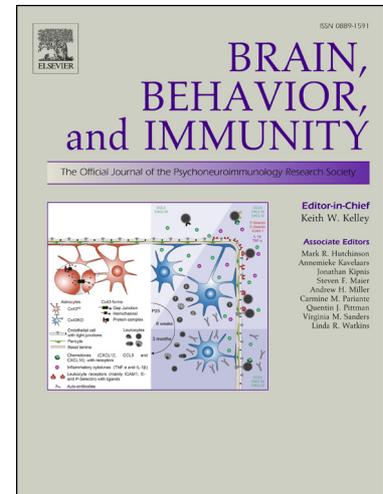
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Skin colour changes during experimentally-induced sickness

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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 hours 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 hours 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 hours 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.

Keywords: skin colour, inflammation, lipopolysaccharide, sickness response, spectrophotometry, carotenoids, blood

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 2011), but little is known of what signals and cues characterize sick peers. Skin colour changes are readily observable, and 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., 2016; 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 (seeLasselin et al 2016 for detailed protocol and also Karshikoff et al., 2015; Sundelin et al., 2015 for similar methods). Throughout the following 7.5 hours,

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.

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

Measured Variable	Time Post Injection (hours)									
	0	0.5	1	1.5	2	3	4	5	7	7.5
Cytokines	✓		✓	✓	✓	✓	✓	✓	✓	
Temperature	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Sickness	✓			✓		✓		✓	✓	
Blood Pressure	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Colour	✓		✓		✓	✓	✓	✓		✓
Carotenoids	✓		✓			✓			✓	

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, and a difference as small as 1.4 ΔE^* is enough to influence judgements of health (Re et al., 2011).

2.6. Plasma Carotenoids

Plasma samples were analysed by Craft Technologies (City, State, USA) by high performance liquid chromatography (HPLC). Plasma concentrations of lutein, zeaxanthin, lycopene and beta-carotene were summed to give a value of total carotenoids for each participant at each sampled time point (T0, T1, T3, T7.5) in both conditions.

2.7. Data Preparation

For three participants, arm data was based on two locations rather than 3 since their tattoos did not allow for proper measures of colour of the inner forearm and or shoulder.

Of remaining participants and locations, 18 (0.8%) data points were missing in the LPS condition (all locations from 1 participant at T4 plus shoulder for 1 participant at T7.5). Fifteen (0.7%) data points were missing in placebo condition (all locations from 1 participant T4). These missing values were replaced with values extrapolated by averaging colour values from the relevant individual and location for the time point immediately prior and subsequent (for T4); or continued solely from the prior time (for T7.5).

Cytokine data were log transformed. There were 32 cases of missing data (9.1%) but no imputations were made because values were missing at critical time points around peaks, where missing values could be increasing or decreasing.

2.8. Statistical Analysis

2-way (condition x time) repeated ANOVAs were employed to test for treatment effects and interactions with time with reference to cytokine (IL-6 & TNF-α) concentrations, temperature,

self-reported sickness, blood pressure (systolic and diastolic), skin colour (L* a* and b* for arm and face locations) and plasma carotenoid content.

To aid interpretation of interactions, all measured values were plotted overtime in response to LPS and Placebo administration with 95% confidence intervals of the paired difference (Pfister and Janczyk, 2013). These error bars are equivalent to a paired sample t-test between conditions (LPS and Placebo) at each sample time point and were used as post-hoc analyses. Where these error bars were not sufficient to explain a significant interaction, planned contrasts were employed to test whether the *change from baseline* differed significantly by condition at any given time point.

3. Results

3.1. Physiological and self-reported sickness change in response to LPS administration

The ANOVAs for cytokine concentrations, body temperature, self-reported sickness and blood pressure showed significant interactions between condition and time (all ps< .001, all ηp^2>.633). Graphs showing effects of condition (Figure 1) demonstrate that the administration of LPS, as compared to placebo, was associated with an increased production of pro-inflammatory cytokines IL-6 and TNF-α, an increase in body temperature and higher levels of self-reported sickness. Systolic and diastolic blood pressure also showed significant interactions between condition and time, showing that blood pressure changes differently over time in response to the LPS and placebo injections (systolic: $F(8,152) = 3.55$, $p=.001$, $\eta p^2 =.158$; diastolic: $F(8,152) = 5.03$, $p<.001$, $\eta p^2 =.209$). However, they did not follow the same pattern of change as other physiological variables. As indicated in Figure 1, systolic blood pressure was significantly higher

after LPS relative to placebo 1.5 hours post injection and significantly lower at 5-7 hours post injection whilst diastolic blood pressure was significantly lower at 4 and 7 (but not 5) hours post LPS injection.
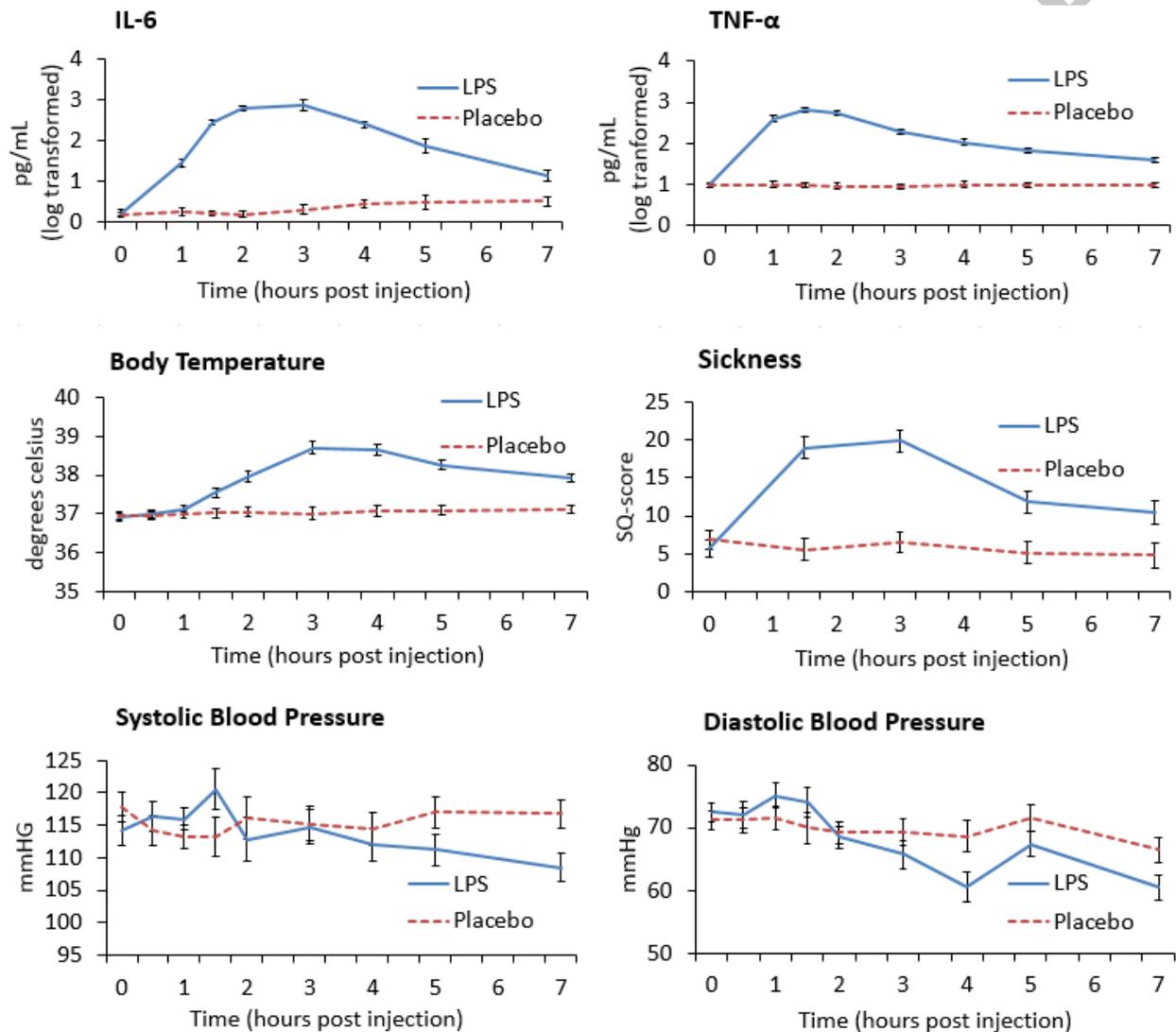

Figure 1. Mean plasma concentrations of cytokines (IL-6, TNF-α), body temperature, self-reported sickness and blood pressure over time in response to injection of bacterial endotoxin

(LPS) (solid lines) or saline placebo (dashed lines). Error bars represent 95% confidence intervals for the paired difference between two means at each time point.

3.2. Colour changes in response to LPS administration

3.2.1. Face Colour

Both redness and lightness showed a significant interaction (condition x time) suggesting that these colour channels changed differently over time in response to LPS and saline administration (redness: $F(3.36,70.58)=8.85$, $p<.001$ $\eta p^2=.296$; lightness: $F(3.64,76.45)=2.95$, $p=.029$ $\eta p^2=.123$). Graphs detailing these interactions can be viewed in Figure 2 and show a clear a drop in skin redness 1-3 hours after the LPS injection followed by a recovery; this is coupled with a smaller increase in skin lightness at 1-2 hours post LPS administration when redness loss was highest. Skin yellowness at the face showed no significant interaction and no main effect of time or condition (all ps$< .305$). The largest change in overall face colour occurred 2 hours post injection with a magnitude of 1.74 ΔE^* (see Table 1).

3.2.2. Arm Colour

All three colour channels showed a significant interaction (condition x time) suggesting that skin colour changed differently over time in response to LPS and saline administration in terms of redness ($F(3.40,71.30)=3.67$, $p=.013$ $\eta p^2=.149$), yellowness ($F(3.72,78.18)=5.45$, $p=.001$ $\eta p^2=.206$) and lightness ($F(4.06, 85.18)=4.20$, $p=.004$ $\eta p^2=.167$). Graphs detailing these interactions can be viewed in Figure 2 and show an early drop in redness, yellowness and lightness. Colour had recovered 4 hours post injection. The largest change in overall arm colour occurred at T1 with a magnitude of 1.29 ΔE^* (see Table 1).
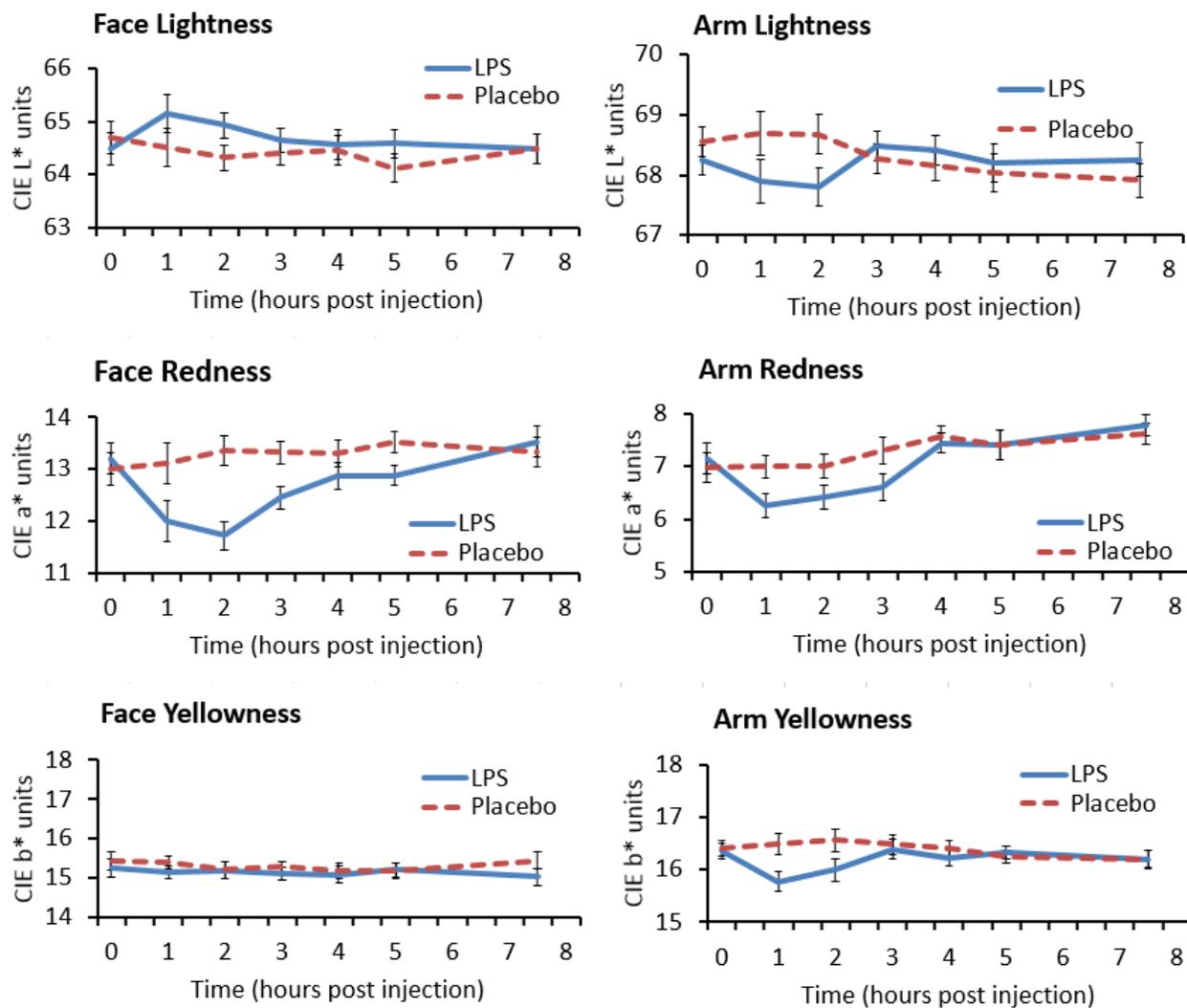
Figure 2: Skin colour over time in response to LPS administration (solid lines) or saline placebo (dashed lines). Error bars represent 95% confidence intervals for the paired difference between two means at each time point.

Table 1: Delta E (ΔE^*) values showing magnitude of total colour difference (redness, yellowness and lightness) at each time point in both arm and face locations. Values represent colour in LPS condition relative to placebo.

	T0	T1	T2	T3	T4	T5	T7
Face	0.34	1.31	1.74	0.93	0.45	0.79	0.46
Arm	0.35	1.29	1.18	0.75	0.35	0.18	0.37

3.4. Plasma carotenoid changes in response to LPS administration

3.4.1. Treatment effects

A 2-way repeated ANOVA revealed a significant interaction between condition and time ($F(3,60)=9.78$, $p<.001$, $\eta p^2 = 328$). Although error bars around the paired difference suggested that pairwise comparisons at any given time point would not be significant (see supplementary Figure 1), planned contrasts revealed that the change was significantly different by condition at 3 ($F(1,20)=11.86$, $p=.003$) and 7 ($F(1,20)=17.04$, $p=.001$) hours post injection. The relative change in carotenoids over time and by condition can be viewed in Figure 3, which shows that the drop in plasma carotenoids following LPS was greater than that in response to placebo at 3 and 7 hours post injection.

Plasma Carotenoid Drop from baseline
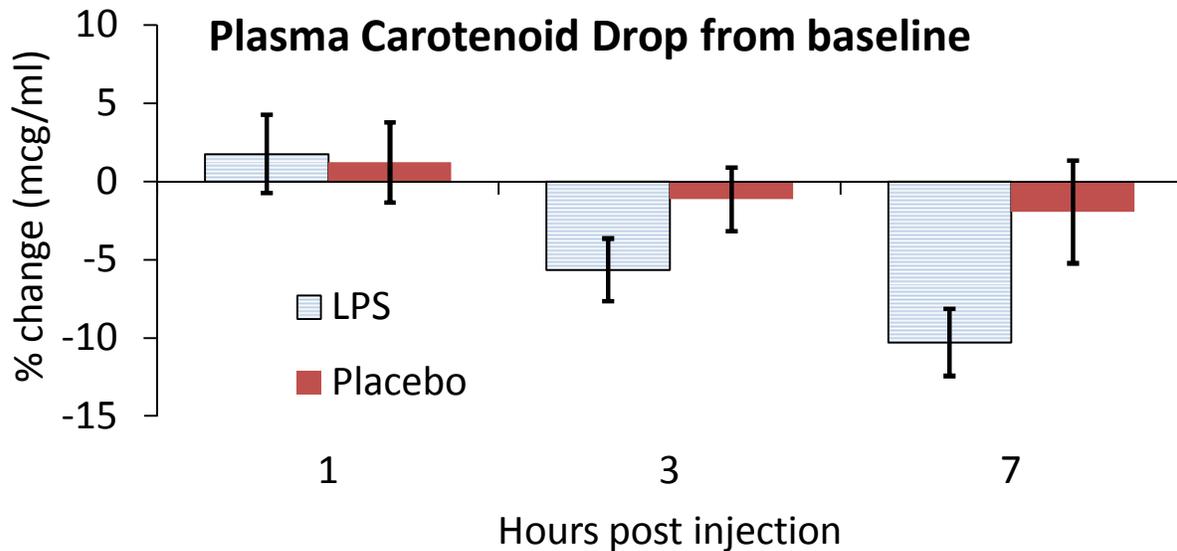
Figure 3: Relative drop in plasma carotenoids over time and by condition. Error bars show 95% confidence intervals around change values. Relative to baseline, the drop in carotenoids after LPS was significantly greater than that after placebo at 3 and 7 hours post injection. Error bars also suggest that change was significantly different from zero only in response to LPS at 3 and 7 hours post injection,

4. Discussion

Within one hour of the activation of an innate immune response stimulated by the injection of LPS, we found clear changes in skin colouration. Facial skin became lighter and less red whilst arm skin became darker, less red and less yellow. Maximum colour changes occurred around the same time as cytokine production and self-reported sickness but ahead of maximum changes in body temperature, blood pressure and plasma carotenoids. Colour changes are consistent with a less healthy appearance as perceptual studies of health show that low levels of red and yellowness in the skin is perceived as less healthy (Re et al., 2011; Stephen et al., 2009a, 2009b). Attending to colour cues that are informative of an acute inflammatory response to microbial

stimuli would have provided evolutionary benefits. Here we show that skin colour does change with illness on a time scale that would be particularly informative of sudden onset of illness such as a contagious infection.

Contrary to our predictions, the colour changes recorded are not well explained in relation to carotenoid loss for two reasons, First, a drop in skin yellowness was only observed in the arm, and there is no reason to predict that skin carotenoids should be reduced with illness in one body location but not another. Second, and more importantly, we did not find evidence that plasma carotenoids changed on a timescale consistent with change of skin yellowness. We do note a significant drop in plasma carotenoids with LPS from three hours post injection, but at this time point skin yellowness had returned to baseline levels. Carotenoids arrive in the skin from the blood either by diffusion through lower layers of skin, or though sweat glands (Darvin et al., 2010). Therefore, although a rapid decrease and recovery in skin carotenoid levels has been reported using Raman spectroscopy (Vierck et al., 2012); in our study it is unclear how carotenoids from the skin could recover without plasma levels also showing a rebound. Prior work shows that plasma carotenoids generally correlate highly with skin carotenoids (Stahl et al., 1998) which in turn correlate highly with skin yellowness (Alaluf et al., 2002; Stephen et al., 2011). Our results however suggest that rapid changes in plasma carotenoids (i.e. at 3 and 7 hours post injection) are not immediately reflected in skin colouration.

With LPS, the most prominent colour change recorded was a drop in skin redness at both locations 1-3 hours post injection. This change in colour peaked around the same time as cytokine production and self-reported sickness but ahead of peak body temperature. It is likely

that these colour changes reflect changes in blood perfusion, or status. Such mechanisms were not directly tested in this study and so cannot be confirmed but we postulate the following potential mechanisms for future investigations. Given that maximum body temperature closely followed maximum drop in redness, vasoconstriction as part of the body's attempt to conserve heat during a the early stages of fever production (Anochie, 2013) seems a plausible explanation for the reduction in skin redness. A reduction in skin redness could occur based on an increased mobilisation of white blood cells that can happen for example in localised wounds but this mechanism is unlikely to account for skin colour change over the whole body given that white blood cells are normally 1/1000 less numerous than red blood cells. he colour change is also unlikely to reflect a reduction in blood pressure as colour recovered before any noted drop in blood pressure. Further studies are needed to comprehend physiological mechanisms underlying skin colour changes during sickness.

It is also noteworthy that although skin redness fell both in the face and arm, the colour change in terms of lightness and yellowness differed by location. Whilst facial skin became less red and lighter, arm skin became less red, darker and less yellow.

The colour change at the arm (across all three colour channels) could be explained in relation to changes in oxygenation of the blood. As testing of oxygenation status of blood (particularly in the superficial levels of the skin) was not possible in the present study, this possible mechanism awaits further research. Deoxygenated haemoglobin reflects more blue light relative to oxygenated haemoglobin and so a shift in the oxygenation status of blood is consistent with a drop in yellowness (given that b^* values represent a scale from blue to yellow). Deoxygenated

blood is also darker in colour and so this explanation is consistent with the noted darkening of arm skin.

Perceptual studies have shown that not only are high levels of blood perfusion judged to be more healthy in appearance, but human observers have a specific preference for the colour of oxygenated blood in human skin relative to deoxygenated blood (Re et al., 2011; Stephen et al., 2009a). This suggests that the change in colouration in both the face and arm have the potential to be perceived as becoming less healthy. If threshold values determined by Re and colleagues in relation to facial stimuli are applied, colour change in the arm could be considered sufficient to be detectable, but not to influence judgements of health (Re et al., 2011). Based on these same threshold values, it would only be in the face, 2 hours post injection that the colour change was sufficiently large to influence judgements of health, a prediction that could be addressed in future experiments.. The face then, which is particularly relevant for social judgements, shows colour changes with the acute inflammatory response which is likely to influence perceived health negatively, and arm shows a colour change that is likely to be judged as less healthy if larger in magnitude.

Humans are attuned to changes in the health status of others; prior work using LPS as a model of sickness find that changes in body odour and movement within a few hours of injection are perceived as less healthy (Olsson et al., 2014; Sundelin et al., 2015). Here we report the first experimental study characterising the skin colour changes associated with acute illness and argue that changing skin colouration, particularly in the face, is an additional cue that could be informative in identifying sudden onset illness. This is further supported by the fact that paler faces appear more fatigued (Sundelin et al 2013). Being able to detect such cues could form part

of a behavioural immune response aiding healthy individuals to avoid infection through contact with sick individuals. Alternatively, observable cues to ill-health could benefit sick individuals themselves if they prompt care-giving behaviours from others.

A limitation of the present study is that skin colour change was measured only in a model of acute and temporary sickness induced by a bacterial by-product. The innate inflammatory response constitutes a first line of defence against a variety of infections, and our results should be relevant for a variety of acute infections. However, the impact of a more established infection upon skin colour has not been addressed and should be further investigated. It is likely that with prolonged or more severe illness colour change could be greater and longer lasting. Mechanisms underlying the recorded colour change also require further investigation although we can confidently rule out carotenoids as a mediator of change in skin yellowness during the acute response. A further limitation of the study is that it was conducted with only a small sample of young and of lightly pigmented individuals. Future work will be necessary to confirm whether the same pattern of results is seen in older populations and amongst those with more deeply pigmented skin tones. Finally, although the measured magnitude of skin colour change suggests that recorded colour change in the face is sufficient to influence judgements of health, this is yet to be confirmed empirically.

In summary, we found that an acute immune response, elicited by LPS administration, was associated with an early change in skin colouration. Facial skin blanched (i.e. became lighter and less red) whilst arm skin became darker, less red and less yellow. The transient change in skin colour reported here is most likely caused by the physiological changes during an acute systemic

inflammation and is likely related to blood status. We found no support for the hypothesis that a reduction in carotenoids would reduce skin yellowness during the immune response. We do however note a reduction in plasma carotenoids that could be reflected in skin yellowness over a longer time period. The pattern of colour loss witnessed in response to LPS is consistent with perceptions of reduced health (less yellow and red skin is judged as less healthy) and these colour changes occurred early in the development of sickness, skin colour change may therefore have served as an important cue to health for others; motivating evolutionarily relevant actions in terms of disease avoidance or care provision.

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Plasma Carotenoids
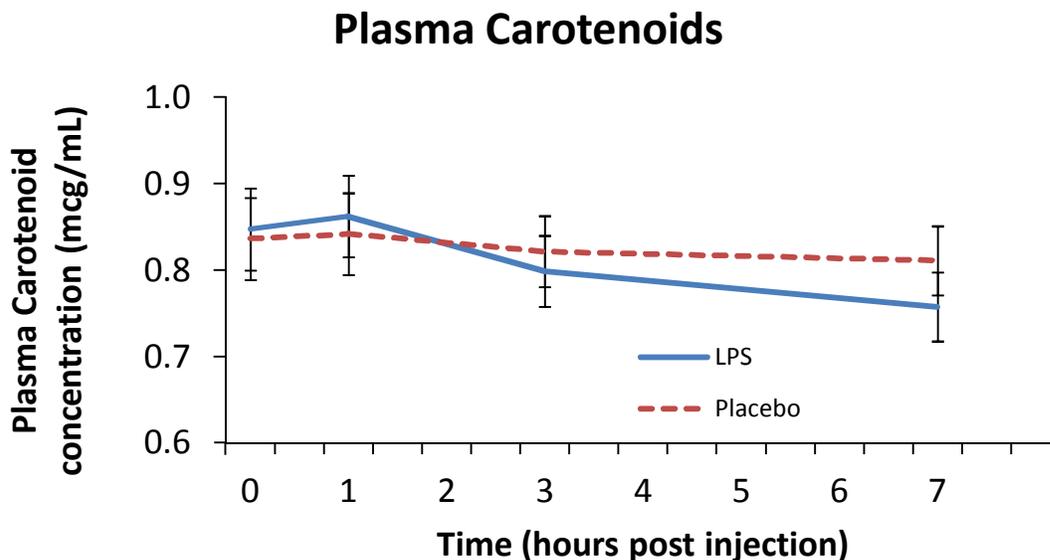
Supplementary Figure 1: Total plasma carotenoid levels over time in response to LPS (solid line)

and placebo (dashed line). Error bars represent 95% confidence intervals for the paired

difference between two means at each time point.

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Highlights

- How skin colour changes in response to acute sickness was investigated

- Illness is manifest in skin colour differently depending on body region

- The face lost redness and became lighter with acute sickness

- The arm lost redness and yellowness but became darker with acute sickness

- Skin colour changes likely provide a cue to health status of others