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ORIGINAL ARTICLE

# Re-oxygenation of haemoglobin in livores after post-mortem exposure to a cold environment

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**Abstract** When a body is exposed to a cold environment, the livid colour of livor mortis changes to cherry red. This colour change is due to an increase in the concentration of oxygenated haemoglobin. The chronological course and the extent of haemoglobin re-oxygenation associated with the exposure to low ambient temperatures have not been understood so far. The relations between refrigeration time under a constant ambient temperature (5°C), skin temperature, body mass index (BMI), spectral reflectance curve and $O_2$–Hb concentration in livor mortis were systematically investigated in 84 bodies brought to the Institute of Legal Medicine of the Freiburg University Hospital shortly after death. In the first measurements performed shortly after death, the reflectance curves of the livores of all bodies showed a broad minimum at 555 nm. After a refrigeration time of 44.9±17.9 h, the spectrum changed to the typical picture of $O_2$-rich blood with 2 minima at 541 and 576 nm and a maximum at 560 nm in between. This qualitative change of the reflectance spectra was observed for a skin temperature of 10.3±2.7°C. With the help of a physical skin model it was possible to calculate that due to the post-mortem exposure to cold the $O_2$–Hb concentration in the livores rose from 0–1% to a value of up to 89.3%. The change in the reflectance curve was discernible from an oxygen saturation of 25±13.8%.

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## Introduction

Livor mortis is the first reliable sign of death, which is ascertainable with the naked eye. When the circulation ceases, the blood settles in the lower parts of the body due to gravitational forces first forming blotchy discolourations by dilating the skin vessels, which then become larger and finally coalesce. The colour of the blood changes from red to a purple livid tone due to the consumption of oxygen in the agonal phase. When a body is exposed to low ambient temperatures, however, the colour of hypostasis gradually changes to pink [12]. The most important differential diagnosis in the presence of pink hypostasis is carbon monoxide (CO) poisoning. The colour qualities of pink livores seen after exposure to a cold environment and in CO poisoning are very similar, and spectral reflectance curves show hardly any difference. Nevertheless, we were able to demonstrate in previous studies that by means of spectrometric measurements and statistical data analysis based on a physical skin model it is possible to distinguish between the two possibilities [2, 3, 31].

It is generally known that livores change in colour after post-mortem exposure to cold. In Anglo-American textbooks, this is only described as a phenomenon without explaining the underlying mechanism [6, 24, 28]. In German textbooks [5, 7, 21, 23, 27], it is assumed that this colour change is caused by a shift of the oxygen-binding curve of haemoglobin to the left under low ambient temperatures. Moistness of the skin seems to support the diffusion of oxygen into the skin [12, 23]. Due to the cold, $O_2$ binds more easily to haemoglobin and its

release to the tissue is impeded. Oxygen diffuses from the ambient air through the skin of the hypostatic areas and binds to haemoglobin there. Although this explanation seems plausible, it has not yet been verified by experiments, as far as we know, and the chronological order and the extent of re-oxygenation have not been systematically investigated.

Altogether, there are only a few studies dealing specifically with the colour of livor mortis [2, 12, 16, 25], although several attempts have been made to provide an objective way to determine the colour of livores in particular with regard to the chronological order of occurrence, intensity, shifting and displaceability [13, 14, 26, 29, 30].

We systematically investigated the relationship between the reflectance spectra of livores, the extent of re-oxygenation, the refrigeration time in a constant ambient temperature and the skin temperature.

## Materials and methods

By means of reflectance spectrometry, post-mortem lividity was investigated over a period of 4 days in 84 Caucasians brought to the Institute of Legal Medicine of the Freiburg University between April 2005 and April 2006 shortly after death. Measurements were performed at intervals ranging from 6 to 12 h. Exclusion criteria were unknown time of death, presence of late post-mortem changes, sparse hypostasis, carbon monoxide poisoning, extensive burns and mechanical destruction.

The bodies were stored unclothed and face up at a constant ambient temperature of 4–6°C. Measurements of post-mortem lividity were performed on each body in the posterior lateral region of the thorax. For each measurement, the local skin temperature was also recorded using a thermometer with a surface probe. Further parameters recorded were time of death, start of refrigeration, body mass index (BMI), age and gender. Measurements were performed with a diode array spectrophotometer MCS 400 (Carl Zeiss Jena, Jena, Germany) with a halogen bulb as light source (standard illuminant D65). The measuring head allowed recording of the directed surface reflection of a 5 mm wide measuring spot (measuring geometry 45°/45°). Compressed barium sulphate was used as the white standard according to DIN 5033. The measurements were controlled and evaluated with the help of a personal computer and stored in an electronic laboratory notebook [1]. Reflectance was measured in the range of 350 to 750 nm. In the data analysis, however, only the range of 500–600 nm was taken into account because this range contains the typical minima and maxima for de-oxygenated and oxygenated haemoglobin. For describing the curve, the curvature of the local minima and maxima in the investigated spectral range was used.

The $O_2$–Hb content in the livores was estimated with the help of the previously described Monte Carlo skin model using the same parameters [3]: the Monte Carlo based calibration model was estimated for a semi-infinite half-space with a refractive index of $n_{cp}$=1.36 [4, 22, 32]. The reflectance was calculated for a detector radius of $r_c$= 0.5 cm. The effective particle size distribution $h(d)$ of the scatterer (collagen fibres and mitochondria) was calculated from $d$=0.1 to 0.8 μm with a discretisation of $\Delta d$=0.1 μm. The extinction spectra of Hb, $O_2$–Hb and CO–Hb were taken into account for the modelling of the absorption coefficient. The desired parameters were estimated by a least squares fit of the measured reflectance spectrum in the 500–600 nm range, standard deviations (SD) were calculated under the assumption of Gaussian error propagation.

## Results

The 84 deceased Caucasians included 58 males and 26 females aged 22–92 years (mean 54 years). In 28 cases, there was a natural cause of death (coronary heart disease, pneumonia, acute pancreatitis, pulmonary embolism, massive cerebral haemorrhage) and 56 individuals had died from an unnatural cause of death (craniocerebral trauma, polytrauma, gunshot wound, intoxication by medical or addictive drugs). Refrigeration of the body at 4–6°C was started 2 h after death at the earliest, usually within the first 12 h post-mortem. In 13 individuals, the BMI was <20, in 25 between 20 and 25 and in 46 it was >25.

From these bodies, a total of 360 spectra were recorded. In the first measurements, the typical course for de-oxygenated haemoglobin with a local minimum at 555 nm was found in all cases except 2. Within the next days, the spectrum changed as follows (Fig. 1): the positive curvature of the reflectance curve at the local minimum (555 nm) decreased slowly at first (Fig. 1: curves 2 and 3) until a plateau (0 curvature) had formed at that site of the reflectance curve (Fig. 1: curve 4). At the same time, there was a new local minimum directly adjacent to the initial local minimum. The newly formed local minimum was usually located at a wavelength of 576 nm. This local minimum became more distinct, whilst the plateau of the reflectance spectrum developed into a local maximum (negative curvature) of the reflectance curve and another local minimum formed on its left side (Fig. 1: curve 5), usually at 541 nm. Occasionally, the new local minima were seen to form in reverse order.

The chronological development of the reflectance spectrum in the range between 500 and 600 nm suggests an inherent non-linear process. The related qualitative change of the reflectance curve can be characterised by computing the curvature of the reflectance curve at the position of the central local extremum, such that a vanishing curvature

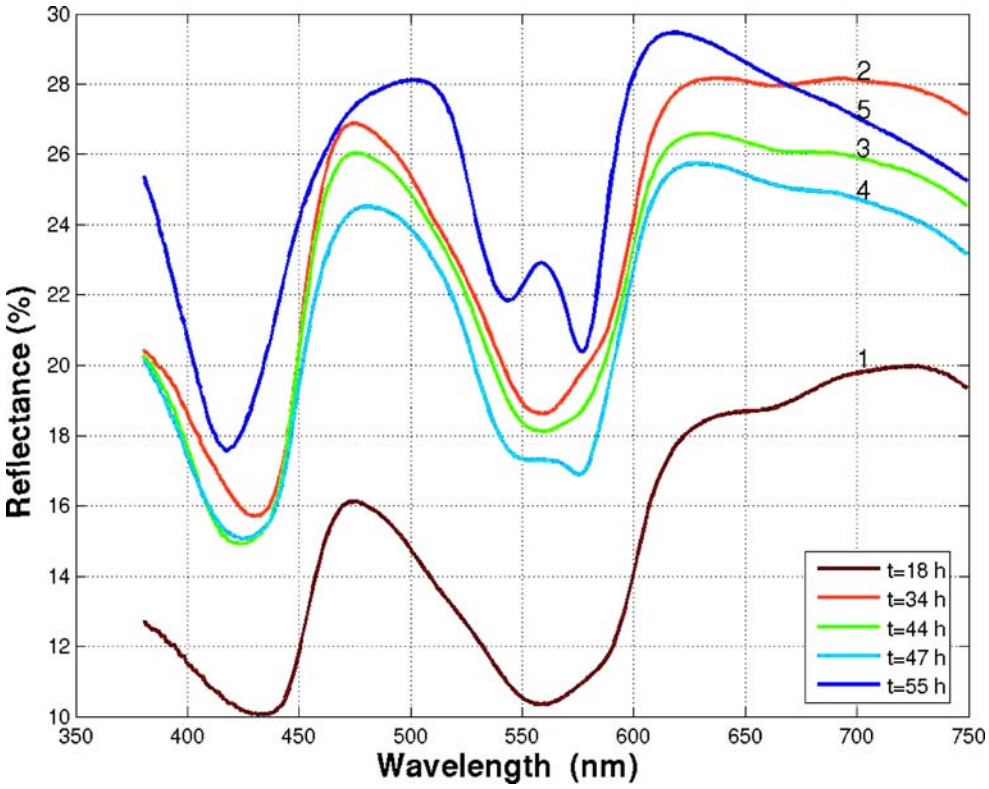


**Fig. 1** Spectral reflectance curves of livor mortis of a 65-year-old man (craniocerebral trauma) with measurements taken between 18 and 55 h after starting refrigeration at 4–6°C

indicates the transition from one to three local extrema. This transition occurred after 44.9±17.9 h with the earliest transition being observed after 12 h and the latest transition after 75 h.

We called this time the "critical time" (Fig. 2). After the beginning of the curvature change, the curve developed the typical picture for oxygenated haemoglobin within 32± 24 h. Afterwards, the reflectance curves did not show any changes of their shape except for a shift in parallel towards the reference line corresponding to increasing darkening of the livores. Cause of death, age and gender showed no correlation with the shape of the spectral reflectance curve or its temperature-dependent chronological change.

The time from the start of the curvature change to the full picture was dependent on the body mass index. In underweight individuals (BMI <20), it was 28±8.5 h; in individuals with a BMI between 20 and 25, it was 32±17.7 h and in individuals with a BMI above 25, it was 51±27.5 h.

The curvature change was directly related to the skin temperature. When the measurements were started, the skin temperature ranged between 9.3°C and 29.4°C (mean: 17.4± 4.8°C). With the exception of 2 cases in which the skin temperature had been 9.3°C and 10°C, respectively, at the beginning of the measurements, the reflectance curves showed a broad minimum at 555 nm. From the very beginning, the two exceptional cases showed spectra with the typical course for oxygenated blood. In all the other cases, the described qualitative change of the spectral curves was observed for a skin temperature of 10.3±2.7°C. We called this temperature the "critical temperature" (Fig. 3). After approximately 60 h, the skin temperature reached the ambient temperature of 4–6°C in all cases.

There was a correlation between the body mass index, the skin temperature and the critical time. In underweight individuals, the spectral curves started to change qualitatively somewhat earlier than in individuals of normal weight, whereas in overweight individuals it occurred somewhat later. The difference was not statistically significant, however. The same is true of the skin temperature, which was 9.2°C on an average in underweight individuals and 11.8°C in overweight individuals (Figs. 2 and 3) when the reflectance curve undergoes the qualitative change from 1 to 3 local extrema.

In the 2 exceptional cases, the proportion of oxygenated blood was already 41.6% and 70.6%, respectively, in the first measurement. In all the other cases, the measurements performed within the first 10 h after starting refrigeration produced oxygen saturation values of 0–1%. In the interval of 10 to 20 h after the start of refrigeration, the value rose to 5.1±11.1%. In the period of 20 to 40 h after starting refrigeration, shortly before the curve changed qualitatively, the proportion amounted to 22.9±16.9%. The mean value at the time when the curve changed qualitatively was 25% with a SD of 13.76%. After the curve ceased to change, the proportion of $O_2$-rich blood was 58.9±26.9%. The highest concentration reached was 89.3%. There was no correlation between the body mass index and the $O_2$–Hb concentration when the reflectance curve undergoes the transition.

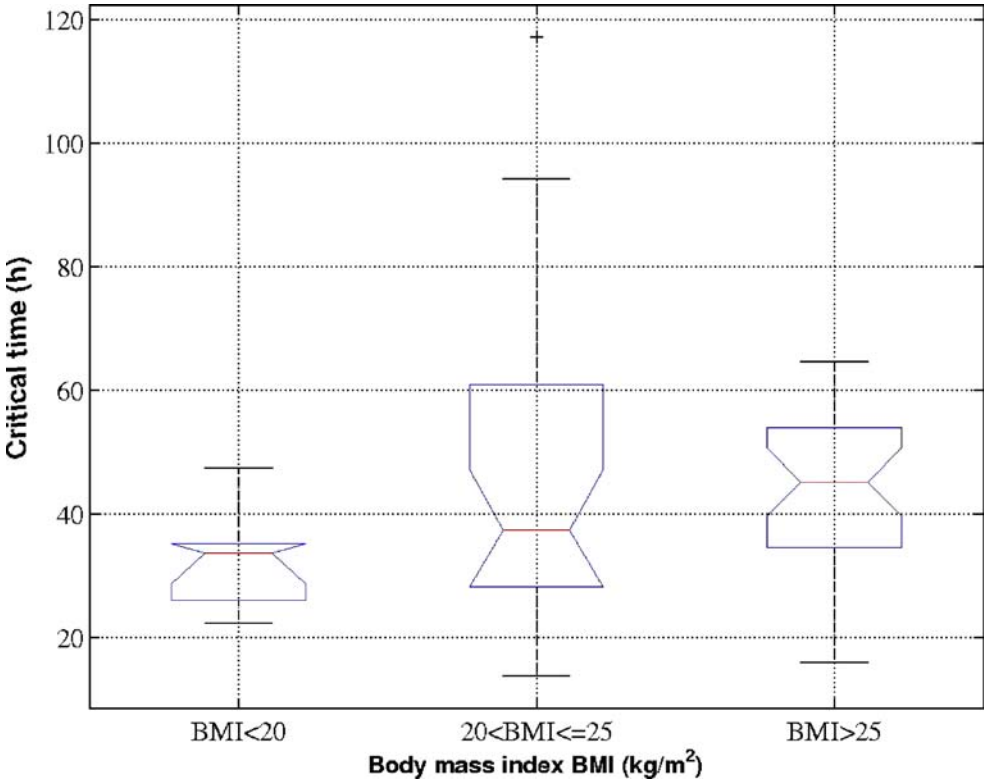


**Fig. 2** Box plots of the critical time (qualitative change of the reflectance curve after refrigeration of the body) in relation to the body mass index (BMI)

## Discussion

Our investigations were based on the hypothesis that the colour change of livor mortis seen after exposing a body to low ambient temperatures is due to a cold-related shift of the oxygen-binding curve of the haemoglobin in the livores to the left. Such a shift to the left occurs when the temperature of the tissue in the hypostatic areas drops

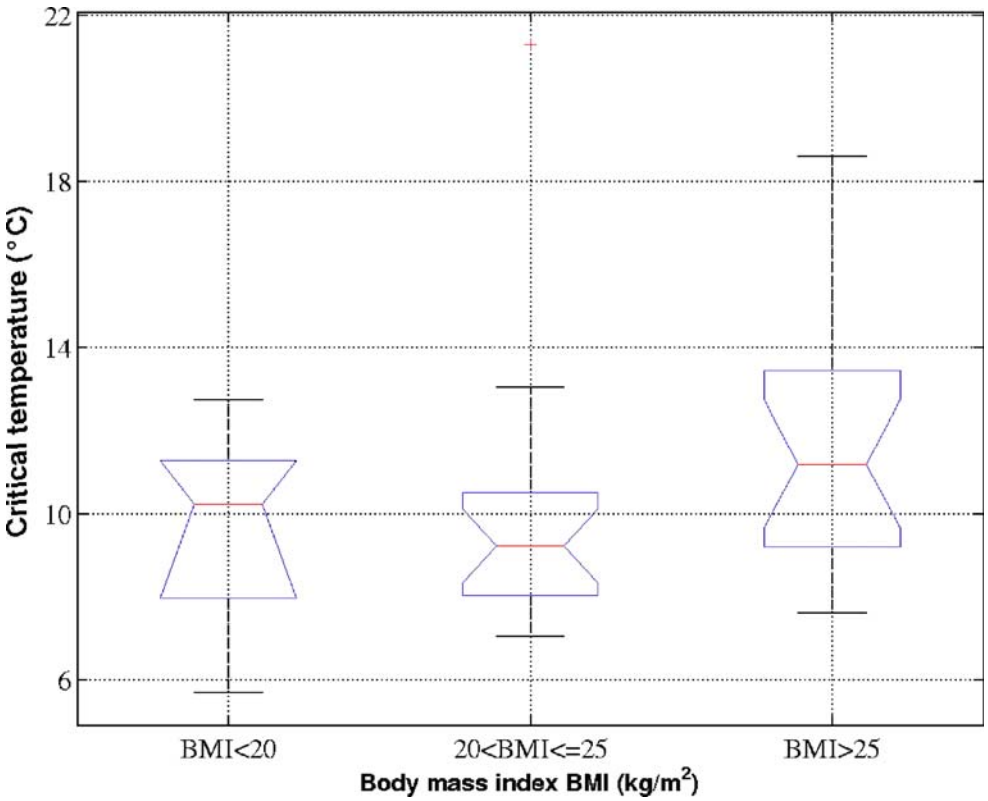


**Fig. 3** Box plots of the critical temperature (skin temperature at the critical time) in relation to the body mass index (BMI)

below a critical temperature. On the other hand, the cooling of a body depends on the duration of the exposure to cold, the body mass, the body surface and the gradient between body temperature and ambient temperature [9, 10, 18–20]. However, all attempts to describe the cooling behaviour of a body made so far used the core temperature of the body, not the skin temperature, which is influenced by many external factors and is thus much more variable. As the cooling of a body is mainly due to the loss of heat via the skin, the skin temperature is involved in this process.

A surprising result was the long time of up to 60 h it took the skin temperature to drop to the ambient temperature of 4–6°C. In contrast, the relations between BMI, critical skin temperature and chronological order did not come as a surprise. It is sufficiently known that the cooling behaviour of a body depends on its mass [9–11]. As in an overweight individual, the cooling of the body core takes longer; it is natural that the skin temperature will also drop more slowly than in a body of normal weight or underweight individual.

After exposure to low ambient temperatures, livores are usually not cherry red in all parts of the body, but show bluish margins adjacent to the sites of pressure-induced blanching of livor mortis. It is assumed that in those areas the access of air is impaired and thus there is not enough oxygen diffusing from the ambient air into the livores to effect a change of colour. In those cases in which the ground beneath the body shows good thermal conductivity, post-mortem lividity often changes to cherry red beside this bluish zone after a short interval already. An explanation for this finding may be that in these areas the local temperature has dropped below the critical temperature faster than in the remaining parts of the body. However, this phenomenon was not systematically investigated by us.

According to our studies, the qualitative change of the reflectance curve occurred in the wave range between 500 and 600 nm when the skin temperature was about 10°C. This is consistent with the results reported by Kessler [15] who demonstrated that the colour change of livor mortis occurs when the ambient temperature is lower than 15°C. Subsequently, the spectral curve changed and the broad band at 555 nm typical of $O_2$-depleted blood was replaced by 3 bands at 541, 560 and 576 nm. These are characteristic of oxygenated haemoglobin. The change of the curve was observed in all cases, only the chronological course differed. The measurements confirmed the hypothesis that the colour change of livor mortis is caused by the re-oxygenation of haemoglobin. Moreover, they allow us to draw the conclusion that re-oxygenation is related to the increased binding affinity of haemoglobin to oxygen under low temperatures because the change of the curve was dependant on the temperature of the skin. Finally, the values for de-oxygenated and oxygenated haemoglobin calculated with the help of the skin model also showed that the colour change was due to a change in oxygen saturation.

The values of oxygen saturation in the blood of livor mortis calculated by means of the previously described skin model [3] were in the same range as they are found in the venous blood of the living body [8]. This is all the more remarkable because the $O_2$-uptake from the environment does not occur by active transport, but in a purely passive way by diffusion from the surrounding air into the skin. This "passive" oxygenation, which is caused only by the increased oxygen-binding capacity of haemoglobin under the influence of the cold, provides an explanation for the long interval between the beginning and end of the curvature change. The estimated baseline values for oxygen saturation are quite realistic. In oxymetric studies of post-mortem heart blood and femoral blood, Maeda et al. found values below 10% [17] in over 60% of the cases. Saturations above 50% in the left heart blood were seen in deaths due to hypothermia, saturation of more than 60% was observed in those cases in which pink livores were present. Maeda et al. stated that they could not find correlations with the post-mortem interval or the rectal temperature [17] although the publication does not indicate the environmental conditions under which the bodies had been stored post-mortem and the temperatures measured.

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