Pediatric RESEARCH

CLINICAL RESEARCH ARTICLE

Effect of blue LED phototherapy centered at 478 nm versus 459 nm in hyperbilirubinemic neonates: a randomized study

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BACKGROUND: Treatment of choice for hyperbilirubinemic neonates is blue light matching the absorption spectrum of bilirubin-albumin in vitro with maximum absorption at 459 nm. Blue LED light centered at 478 nm was hypothesized as being more efficient than that centered at 459 nm. This study compares the bilirubin-reducing effect of the two light qualities with equal irradiance in a randomized nonblinded clinical trial.

METHODS: Inclusion criteria were healthy hyperbilirubinemic neonates with gestational age ≥33 weeks. Forty-nine neonates included in each group received phototherapy from above for 24 h. Mean irradiances were 9.2×10^{15} and 9.0×10^{15} photons/cm^2/s for the 478 and 459 nm groups, respectively.

RESULTS: Mean [95% CI] decreases in total serum bilirubin were 150 [141, 158] and 120 [111, 130] µmol/L for the 478 and 459 nm groups, respectively; mean difference was 29 [17, 42] µmol/L. Mean [95% CI] percentage decreases in bilirubin were 54.8% [52.5, 57.0] and 41.8% [39.3, 44.3]; mean difference was 12.9 [9.6, 16.3] percentage points. After adjustment this difference was 13.4 [10.2, 16.7] percentage points. All differences were highly statistically significant ($P < 0.001$).

CONCLUSION: Blue LED light centered at 478 nm had a greater bilirubin-reducing effect than that centered at 459 nm with equal irradiance quantified as photon fluence rate.

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IMPACT:

- Blue LED light centered at 478 nm had a greater in vivo bilirubin-reducing effect than blue LED light centered at 459 nm with equal irradiance quantified as photon fluence rate in the treatment of hyperbilirubinemic late preterm or term neonates.
- LED light centered at 478 nm might reduce the duration of phototherapy compared to LED light centered at 459 nm as the same effect can be obtained while exposing the infants to fewer photons.
- Blue light matching the absorption spectrum of the bilirubin-albumin complex in vitro with peak absorption at 459 nm is used worldwide as it is considered to be the most effective light for phototherapy of jaundiced neonates. This study showed that blue LED light centered at 478 nm had a greater bilirubin-reducing effect than blue LED light centered at 459 nm. Therefore, blue LED light centered at 478 nm should be used instead of blue light centered at 459 nm. By this, the risk of potential side effects might be minimized, and the duration of phototherapy potentially reduced.

INTRODUCTION

As a result of the transition from intra- to extrauterine life, the unconjugated bilirubin concentration increases in all neonates. This is visible as jaundice in 60−80% of late preterm or term neonates during the first week of life. At very high bilirubin levels, infants can develop acute intermediate to advanced bilirubin encephalopathy, which can cause kernicterus spectrum disorder.[1] This condition is rarely observed in industrialized countries, but is common in the developing world.[2] The incidence of kernicterus spectrum disorder in Denmark is 1.2 per 100,000 newborns.[3]

Phototherapy has been the treatment of choice for high bilirubin levels through the last five decades. In Scandinavia, 2−3% of late preterm or term infants require phototherapy. In the skin, the native nonpolar and toxic Z,Z-bilirubin molecules absorb photons and are converted to more polar and more easily excretable bilirubin isomers; the configurational isomers Z, E- and E,Z-bilirubin and the structural isomers, the E,Z- and E, E-lumirubin.[4,5] Production of lumirubin through E,Z-bilirubin is the major photochemical process.[4] The quantum yield of the bilirubin isomers in vitro is wavelength dependent; in the spectrum 400−500 nm E,Z-bilirubin is preferentially formed at longer wavelengths, Z,E-bilirubin at shorter wavelengths.[4,5] The variation of the isomerization quantum yield of isomers can be explained by the fact that Z,Z-bilirubin consists of two light-absorbing dipyrrole units.[6]

The bilirubin-reducing effect of phototherapy depends on the spectrum of the light, its irradiance level, the exposed body surface area, and the hemoglobin concentration. The higher the

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irradiance and the greater the body surface area exposed, the greater the therapeutic effect, and the higher the hemoglobin concentration, the lower the effect.[7–9] Regarding the spectrum of light, the worldwide choice through decades has been blue light matching the absorption spectrum of the bilirubin-albumin complex in vitro with a maximal absorption at 459 nm.[10]

In order to minimize potential side effects and reduce the duration of phototherapy, it is important to treat the infants with the most efficient spectral quality (color) of the light. The emission spectrum of the light leading to the greatest bilirubin excretion rate is not known with certainty.[11–13]

Using the optics of the neonatal skin, Lamola et al.[13] calculated an action spectrum of phototherapy for bilirubin in the circulating blood as a function of wavelength; that is, the relative fraction of the exposed light absorbed by bilirubin modified by photochemical reactions. Because absorption by skin melanin and back-scattering of light are only faintly dependent on wavelength, the action spectrum practically is only affected by the concentration of hemoglobin, oxyhemoglobin, and bilirubin. The calculated action spectrum differs from the absorption spectrum of the bilirubin-albumin complex in vitro. The former is mainly situated in the wavelength range 460−490 nm with a peak at 476 nm,[13] and the latter in the wavelength range 445−475 nm with a peak at 459 nm.[14] However, the maximum of the action spectrum shifts 4 nm to shorter and 4 nm to longer wavelengths depending on the content of Z,E-bilirubin and lumirubin, respectively.[13] This information led to our hypothesis that phototherapy using blue light emitting diode (LED) light centered at 478 nm would be more efficient than blue LED light centered at 459 nm.

Therefore, the aim of this randomized nonblinded clinical trial was to compare the bilirubin-reducing effect of LED light with a spectrum centered at 478 nm versus 459 nm with essentially equal irradiance quantified as photon fluence rates. The effect was defined as the decrease in total serum bilirubin concentration (TSB) after exposing the infants to 24 h of light.

METHODS

Study groups

This study was performed at the Department of Pediatrics at Aalborg University Hospital between 1 March 2017 and 31 July 2018. The inclusion criteria were healthy neonates with uncomplicated hyperbilirubinemia, gestational age ≥ 33 weeks, birthweight ≥1800 g, postnatal age > 24 h and ≤28 days, and treatable in a bassinet. The infants were naked except for diapers minimized in size and opaque eye pads. Infants with lower weight might be at risk of hypothermia. Infants with rapidly increasing or very high TSB were excluded because they require double lights or exchange transfusion. The infants were treated according to the National Danish Guidelines. The infants studied were only included one time. Doctors in charge informed the parents, enrolled and assigned the patients to phototherapy. The infants were randomized to either LED light with a spectrum centered at 478 or 459 nm using sequentially numbered sealed opaque envelopes, generated by one of the authors (M.L.D.). The randomization was balanced in blocks of 4, 6, or 8 infants.

The primary endpoint was the difference in the decrease of TSB during 24 h of therapy between infants exposed to blue LED light centered at 478 nm and those exposed to blue LED light centered at 459 nm. The secondary endpoint was the association between the decrease of TSB during the treatment and the hemoglobin concentration in each phototherapy group.

The infants were included in the study for 24 h, in which they were exposed to light from above, only interrupted for feeding and/or nursing up to 30 min every third hour. The body temperature was measured every 8 h. The infants were weighed at initiation and termination of the treatment.

One hundred and fifteen infants were eligible. Parents of 11 infants refused to participate and two infants were not included due to work load in the department. Thus, 102 infants were randomized, 51 to each wavelength. Two infants in the 478 nm group were withdrawn from the study: one because the infant developed respiratory distress and one due to communication problems with the mother. In the 459 nm group, two infants were withdrawn as well: one due to a failed blood sample and another one due to communication problems with the mother. Thus, 49 infants received phototherapy in each group.

Measurements

At the start of phototherapy, TSB (TSB_0) and hemoglobin concentration were measured from capillary blood drawn by heel prick. After 24 h of phototherapy, TSB was measured again (TSB_{24}). TSB was determined with the diazo-method using Cobas 8000 Analyzer (Roche Diagnostics International, Mannheim, Germany)[15] and the hemoglobin concentration by a photometric method using ABL 800 Flex Analyzer (Radiometer, Copenhagen, Denmark).

Phototherapy devices

The emission spectra of the LED lamps are shown in Fig. 1.

The phototherapy devices have been described earlier.[16] The geometry of the devices was identical. The irradiance, quantified as photon fluence rate, at an equal distance from the lamps to the infant skin was higher for the LED light centered at 478 nm than for the light centered at 459 nm. Therefore, the distance from the phototherapy devices to the mattress was adjusted to 35.5 cm for the light centered at 478 nm and to 22 cm for the light centered at 459 nm, so that the light irradiance at skin level was practically the same for the two groups. The distances from the lamps to the mattress were adjusted with wood sticks suspended at the devices.

The flux density of photons was measured with a spectrometer (Model USB2000+; Ocean Optics, Dunedin, FL), which had a constant spectral sensitivity over the range of 200−1100 nm. The irradiance was measured at 9 cm above mattress level representative of the average height over the mattress of the infant's exposed skin. The measurements were performed in a light footprint measuring 28 × 49 cm consisting of a matrix of 4 × 7 = 28 squares of 7 × 7 cm. The mean irradiance of the footprint, measured at the start of the investigation, was 9.2×10^{15} and 9.0×10^{15} photons/cm^2/s for the light centered at 478 and 459 nm, respectively. The individually measured values ranged from 6.3×10^{15} to 11.1×10^{15} photons/cm^2/s. The irradiance did not change during the course of the study. In addition, the irradiance of the light centered at 459 nm was measured by handheld spectrometers, neoBLUE (Natus Medical, San Carlos, CA) and BiliBlanket Meter II (GE HealthCare Technologies, Waukesha, WI). The characteristics of the two LED lights are given in Table 1.

Ethics

The study was approved by The Regional Committee on Health Research Ethics in North Denmark Region, Denmark, N-20160071. Informed consent, verbal and written, was obtained from the parents. The study was registered in the Clinical Trial Registry with number NCT 03183986.

Statistical analysis

Continuous variables are summarized by their medians, minimum, and maximum values. Categorical variables are summarized by the number and percentage of participants in each category. Differences in continuous variables between the phototherapy groups were analyzed using Wilcoxon rank-sum tests. Associations between categorical variables and phototherapy group were studied using chi-squared tests for association or Fisher's exact test depending on the number of observations per combination.

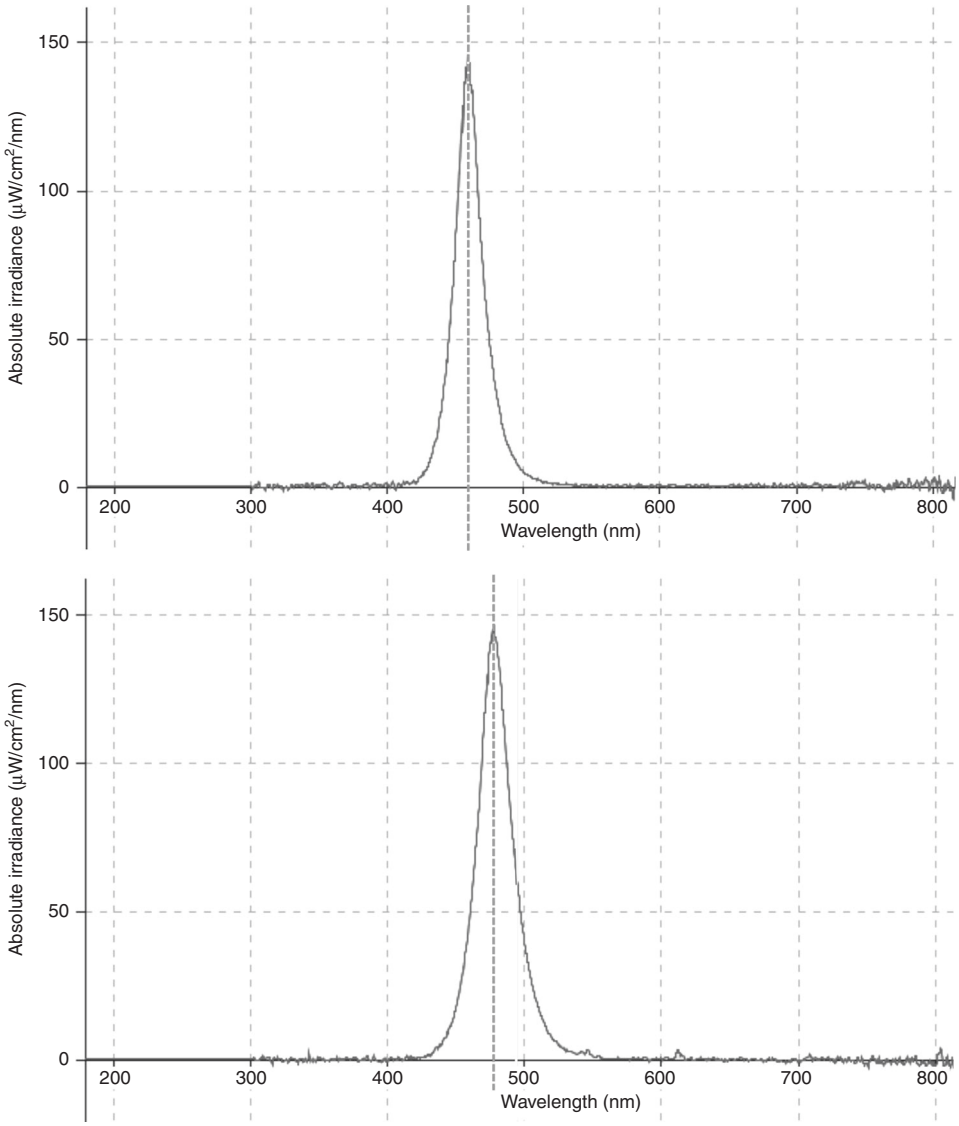

Fig. 1 The emission spectra of the LED lights centered at 459 nm (top) and 478 nm (bottom).

Mean and 95% confidence intervals (CI) were computed for TSB_0 and TSB_{24}, and also for changes in TSB during phototherapy, both in total [ΔTSB_{0-24} (μmol/L)] and percentwise [ΔTSB_{0-24} (%)] in each of the phototherapy groups. Differences between the two groups were analyzed using *t* tests except for ΔTSB_{0-24} (μmol/L), which were adjusted for TSB_0 (analysis of covariance).

A multiple linear regression model was used to adjust the effect of the type of phototherapy on ΔTSB_{0-24} (%) for factors known from the literature: birthweight, postnatal age, hemoglobin concentration, and type of feeding.

Data from an earlier study about decrease in TSB during 24 h of therapy were used for the sample size calculation.[16] To be able to detect a clinically relevant difference between the two groups of 6 percentage points in the decline in TSB during phototherapy, at least 48 infants were required in each group. We assumed the variance was the same in both groups (10.4 percentage points), and we chose a significance level of 0.05 and a power of 0.8.

RESULTS

The demographic and clinical characteristics of the infants are shown in Table 2. No significant differences were found between the two groups, except for an overrepresentation of males in the 478 nm group.

As shown in Table 3, TSB_0 is slightly higher in the 459 nm group, although this difference is not statistically or clinically significant. The mean [95% CI] decrease in TSB during the 24 h of treatment was 150 [141, 158] μmol/L for the 478 nm group and 120 [111, 130] μmol/L for the 459 nm group. Likewise, the mean [95% CI] percentage decrease in TSB was 54.8% [52.5, 57.0] in the 478 nm group and 41.8% [39.3, 44.3] in the 459 nm group. These differences between the groups were highly significant ($P < 0.001$). Thus, when looking at the differences between the two groups, they were 29 (95% CI [17,42]) μmol/L and 12.9 (95% CI [9.6, 16.3]) percentage points in favor of the 478 nm group. In other words, the mean percentage decrease was 31% higher for the 478 nm group (54.8%) than for the 459 nm group (41.8%). The remarkable difference in ΔTSB_{0-24} (%) between the two groups is further illustrated by the boxplots in Fig. 2.

The results from the adjusted regression analysis for the effect of phototherapy on ΔTSB_{0-24} (%) are presented in Table 4. As shown, the mean difference between the groups was 13.4 (95% CI [10.2, 16.7]) percentage points in favor of the 478 nm group, just slightly higher than in the unadjusted analysis presented in Table 3.

Table 1. Characteristics of the blue LED lights centered at 478 and 459 nm.

	478 nm	459 nm
Emission wavelength (nm)		
Range	421−556	416−524
Peak	478	459
Bandwith[a]	464−491	447−470
Emission angle (°C)[b]	90	90
Distance (cm)[c]	26.5	13
Irradiance		
Ocean Optics spectrometer USB2000+		
Photons/cm^2/s	9.2×10^{15}	9.0×10^{15}
μW/cm^2 [d]	3823	3879
neoBLUE		
μW/cm^2/nm[d]	ND[e]	58.9
BiliBlanket Meter II		
μW/cm^2/nm[d]	ND[e]	60.2

[a]At half peak level.
[b]Light emission angle of LED.
[c]Distance from lamp to infant skin (9 cm above mattress level).
[d]Clinically, the irradiance is quantified either as μW/cm^2 (total energy of the spectrum) or as μW/cm^2/nm (energy per wavelength).
[e]Not determined. The irradiance could not be measured properly for light centered at 478 nm with these spectrometers as their sensitivity range was not appropriate for the emission spectrum and peak.

Table 2. Baseline demographic and clinical characteristics of the infants in each phototherapy group.

	478 nm ($n = 49$)	459 nm ($n = 49$)	*P* value
Sex (female/male), *n*, (%)[a]	13/36 (27/73)	25/24 (51/49)	0.01
Non-Caucasian ethnicity, *n*, (%)[b]	3 (6)	8 (16)	0.20
Maternal diabetes mellitus/ gestational diabetes, *n*, (%)[b]	7 (14)	3 (6)	0.32
Cephalhematoma, *n*, (%)[b]	2 (4)	3 (6)	1.00
Feeding, *n*, (%)[b]			0.92
Breast milk exclusively	23 (47)	24 (49)	
Formula exclusively	7 (14)	5 (10)	
Mixed	19 (39)	20 (41)	
Age			
Gestational age (days)[c]	266 [238, 293]	268 [239, 293]	0.75
Postnatal age (h)[c]	90 [41, 250]	95 [38, 225]	0.90
Weight			
Birthweight (g)[c]	3350 [1820, 5280]	3240 [2280, 4360]	0.64
Weight change during phototherapy (%)[c]	0.44 [−3.4, 7.53]	1.06 [−3.17, 4.42]	0.30
Hemoglobin[c] (mmol/L)	12.1 [9.2,14.9]	11.9 [9.3,15.2]	0.93

[a]*P* value from chi-square test for association.
[b]*P* value from Fisher's exact test.
[c]Median [min, max]. *P* value from Wilcoxon rank-sum test.

Table 3. Total and percentage decrease of serum bilirubin concentrations in the two groups after 24 h of phototherapy.

	478 nm ($n = 49$)	459 nm ($n = 49$)	Difference	*P* value
TSB_0 (μmol/L)[a]	274 [261, 287]	286 [273, 299]	−11 [−30, 7]	0.22
TSB_{24} (μmol/L)[a]	125 [116, 133]	166 [156, 175]	−41 [−54, −28]	<0.001
ΔTSB_{0-24} (μmol/L)[a]	150 [141, 158]	120 [111, 130]	29 [17,42]	<0.001
ΔTSB_{0-24} (%)[a]	54.8 [52.5, 57.0]	41.8 [39.3, 44.3]	12.9 [9.6, 16.3]	<0.001

17 μmol/L bilirubin = 1 mg/100 mL.
TSB_0 total serum bilirubin concentration at the start of phototherapy, TSB_{24} total serum bilirubin concentration after 24 h of phototherapy, ΔTSB_{0-24} difference in total serum bilirubin concentration from the start of phototherapy to 24 h of phototherapy.
[a]Mean [95% confidence interval], *P* value from a *t* test.

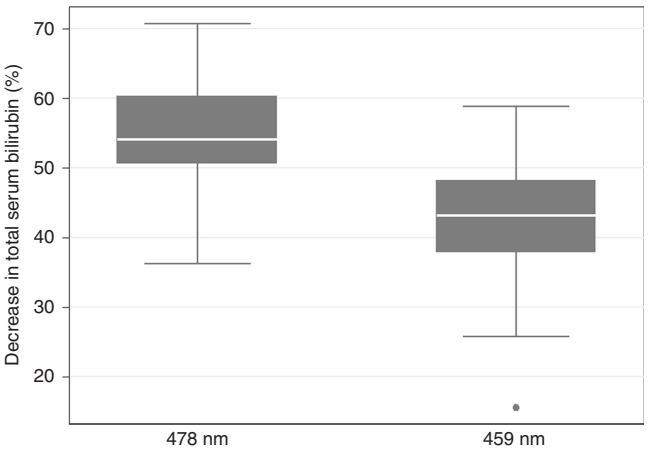

Fig. 2 Boxplots showing the percentage decrease in total serum bilirubin after 24 h of phototherapy in each of the phototherapy groups. The percentage decreases of total serum bilirubin are shown as medians, 25–75 percentiles and ranges.

Table 4. Multiple linear regression with percentage decrease in total serum bilirubin during 24 h of phototherapy as outcome.

	Coefficient [95% CI]	*P* value
478 nm group[a]	13.4 [10.2, 16.7]	<0.001
Birthweight (kg)	−3.64 [−6.0, −1.27]	0.003
Postnatal age (h)	0.04 [0, 0.09]	0.046
Hemoglobin (mmol/L)	−0.52 [−1.72, 0.68]	0.39
Feeding[b]		0.86
Formula exclusively	−1.30 [−6.62, 4.03]	
Mixed	−0.81 [−4.51, 2.89]	

[a]Reference level is the 459 nm group.
[b]Reference level is exclusive breast milk. *P* value from Wald test for all levels simultaneously.

We looked at the effect of hemoglobin on ΔTSB_{0-24} (μmol/L) for each phototherapy group separately. In the unadjusted analysis, the coefficient in the 478 nm group was 4.41 (95% CI [−1.81, 10.63], *P* value 0.16), and −5.44 (95% CI [−13.00, 2.12], *P* value 0.15) in the 459 nm group. After adjusting for TSB_0 and postnatal age, the coefficients were 1.52 (95% CI [−2.98, 6.02], *P* value 0.5), and −5.07 (95% CI [−10.03, −0.10], *P* value 0.046), respectively.

The only side effect observed was loose stools. No cases of hypothermia were observed.

DISCUSSION

The aim of this study was to determine whether LED light with a wavelength range centered at 478 nm is more efficient than LED light centered at 459 nm for the treatment of neonatal hyperbilirubinemia. The results of the study show that the former was indeed significantly more efficient. The average decrease in TSB during 24 h of treatment was 31% greater for the light centered at 478 nm than that at 459 nm. This increase in efficacy is clinically relevant.

Additionally, photons with wavelengths longer than 459 nm may be safer as they have been found to cause fewer potential side effects in vitro. For instance, turquoise light directed at cell cultures containing bilirubin caused fewer DNA changes[17] and was less toxic to cells than blue light.[18]

For evaluation of phototherapy light, the level of irradiance should be quantified as photon fluence rate (photons/cm^2/s), since the photochemical processes are promoted by photons being absorbed by bilirubin. The irradiance must be measured with a spectrometer with equal sensitivity across the measured spectral range.

At bedside, the levels of irradiance are quantified as energy units (μW/cm^2 or μW/cm^2/nm) using handheld spectrometers. Their level of sensitivity is set for measurements of a wavelength range around a peak (usually 460 nm) and therefore cannot be used to compare the efficacy of lights with different emission spectra.[12]

In 1993, Agati et al.[11] suggested that turquoise light with peak irradiance in the wavelength range 485−515 nm would be more efficient towards lowering bilirubin level in newborns than blue light with peak emission around 460 nm. We have previously performed two studies comparing the effect of light with peak emission around 460 nm with light of longer wavelengths.[16,19] In the first one, a randomized clinical trial of preterm infants, we compared the effect of turquoise fluorescent light (broad spectrum) with peak emission at 490 nm with blue fluorescent light with peak emission at 452 nm with equal spectral irradiance at the infants quantified as energy units. The decrease in TSB was on an average 18% greater in the infants treated with the turquoise light,[19] but the number of photons delivered to the skin was also greater, because at an equal spectral energy level, light at wavelength 490 nm delivers 8% more photons than at wavelength 452 nm. Therefore, had the spectral irradiance been quantified as photon fluence rate, the difference in efficacy between the two groups would have been less than 18%.

In the subsequent randomized clinical trial, we used LED lights as light sources. They have a narrower emission spectrum, whereby the infants are exposed to less unnecessary, nontherapeutic light. We compared the effect of turquoise LEDs centered at 497 nm versus blue LEDs centered at 459 nm with equal irradiance quantified as photon fluence rate, and an equal bilirubin-reducing effect was observed.[16]

Altogether, our previous[16] and present investigations, both using LED lights comparing the effect of light centered at 459 nm with those of lights with longer wavelengths, show that light centered at 478 nm had a greater effect, while light centered at 497 nm had an equal effect. At wavelengths longer than 478 nm, the absorption of light by bilirubin decreases rapidly,[13] while the production of lumirubin still increases.[5] At a wavelength of 497 nm, the bilirubin-reducing effect had decreased to the 459 nm level. The result of our study using turquoise fluorescent light with peak emission at 490 nm appears to support these results.

Seidman et al.[20] compared the decrease of TSB in infants exposed to turquoise LED light centered at 505 nm versus blue LED light centered at 459 nm with the same irradiance at the infants, and they found an equal effect. However, the irradiance of the turquoise light was severely underestimated, because it was measured by a spectrometer with peak sensitivity at 460 nm in the blue spectral range (Olympic Medical Bilimeter).

To our knowledge, our two previous randomized clinical trials[16,19] and the present one are the only ones comparing blue light with maximum emission around 460 nm with light with longer wavelengths with the same irradiance, either quantified as energy units or photon fluence rate, and measured by a spectrometer with the same sensitivity in the used wavelength range.

It is essential to use the most effective light quality for the treatment, in particular in infants with extremely low birthweight, as aggressive phototherapy was shown to cause increased mortality in sick ventilated neonates with birthweight 500−750 g.[21,22] Furthermore, phototherapy can produce more subtle short-term adverse effects in form of transient DNA changes, oxidative stress, changes in the erythrocyte membrane and plasma interleukin concentrations, and a drop in the plasma riboflavin level.[23] Further, it was shown that lumirubin in vitro could upregulate several proinflammatory genes.[24] Regarding long-term adverse effects, the results of the epidemiologic studies on cancer risk are divergent.[23]

As the decrease in TSB was greater for infants exposed to light centered at 478 nm versus 459 nm, the duration of the phototherapy can be reduced through the use of LEDs centered at 478 nm. This is essential, as the first days after birth are beneficial towards the development of a well-structured parent−infant attachment and establishment of breast-feeding.

Like in our earlier study[9] we could demonstrate a significant negative association between the hemoglobin concentration and ΔTSB_{0-24} (μmol/L) in infants exposed to LED light centered at 459 nm. The same negative association could not be shown in infants exposed to LED light centered at 478 nm. This difference may be explained by the facts that hemoglobin in the skin competes with bilirubin regarding absorption of photons, and that it absorbs more photons with wavelengths around 459 nm than of those with wavelengths around 478 nm.[13]

The results are generalizable, as the patients received phototherapy according to guidelines.

The strengths of the study are the use of LEDs with narrow emission spectra, the light irradiance quantified as photon fluence rates and measured by a spectrometer with constant spectral sensitivity, the homogeneous study population, and, finally, the highly significant results that are in agreement with Lamola et al.'s[13] calculations.

CONCLUSION

Blue LED light centered at 478 nm had a greater in vivo bilirubin-reducing effect than blue LED light centered at 459 nm with equal irradiance quantified as photon fluence rate in treatment of hyperbilirubinemic late preterm or term neonates. This clinical investigation corroborated the theoretical calculations. The increase in efficacy was clinically relevant. LED light centered at 478 nm should be used instead of light centered at 459 nm as the same effect can be obtained while exposing the infants to fewer photons, thereby minimizing the risk of potential side effects, and also potentially reducing the duration of phototherapy.

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AUTHOR CONTRIBUTIONS

F.E. conceptualized and designed the study, drafted the initial manuscript, reviewed and revised the manuscript. M.L.D. conceptualized and designed the study, collected

the data, reviewed and revised the manuscript. H.J.V. designed, produced, and characterized the LED devices, designed the study, reviewed and revised the manuscript. M.R.-D. performed the statistical analyses, reviewed and revised the manuscript. A.M.M. included patients, reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ADDITIONAL INFORMATION

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Competing interests: The authors declare no competing interests.

Patient consent: Informed consent, verbal and written, was obtained from the parents.

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