

Effects of the KN95 mask on the skin microbiome and blood oxygen saturation

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Significance statement

Wearing face masks has become necessary in the last couple of years with the surge of the SARS-CoV-2 virus. What kind of effect wearing a face mask for a prolonged time has on a person's health still needs further research. This study was conducted to show the effects of wearing N95 face masks over a prolonged period on the skin flora and the oxygen saturation in the blood. To measure these changes, DNA was taken from the facial area from subjects with swabs and the subsequent DNA was extracted and sequenced. Furthermore, oxygen saturation was measured with an oximeter at set times. This approach could be used in subsequent studies to show the health effects of wearing different kinds of face masks.

Abstract

This study measures the effects of wearing a face mask on a person's health, specifically the face microbiome and blood oxygen saturation. In this study five subjects participated, going through a baseline and intervention period. In the baseline period the subjects tried to avoid wearing a face mask and during the intervention the subjects wore a KN95 face mask for 8 hours a day, 5 days a week. Each subject also kept a diary, noting down various points like the amount of sleep, stress and oxygen saturation level we measured each day. We concluded that there was no significant change in the oxygen saturation levels in the blood. We observed more significant changes in the microbiome with multiple species related to the forming of acne having grown in number in the intervention period. Even so, there was no significant change in the amount of acne spots of the subjects. Our approach sets a basis for others to continue and improve upon.

Keywords

Microbiome, facial microbiome, skin flora, blood oxygen saturation, SpO₂, masks, face masks, N95 mask, acne, acne vulgaris, COVID-19, SARS-CoV-2 virus, corona.

Introduction

Since the new severe acute respiratory syndrome coronavirus (SARS-CoV-2), that causes coronavirus disease 2019 (COVID-19), measures were taken to limit the spreading of the virus (1). One of the measures taken is the use of face masks. Surgical masks reduce the emission of virus particles in the environment (2). Therefore, masks have become recommended or mandatory in Western-Europe. Due to surgical mask use, several skin changes and other negative effects have been observed (3). Negative effects include discomfort, a changing microbiome balance and a decrease in oxygen saturation, which can cause headaches and breathing problems (4) as well as other symptoms that are related to cognitive decline (5). A surgical mask can influence the blood oxygen saturation levels when resting and when exercising (5,6). The blood oxygen saturation levels can decrease because CO₂-rich exhaled air is trapped in the mask and does not disperse, resulting in an environment richer in CO₂, and poorer in O₂ (7, 8). Several studies report a change in the microbiome (9 - 11); although the composition can differ. Wearing a mask for a prolonged time can lead to an increase in skin temperature, redness and a marginal increase in the secretion of sebum content (2), which can influence the pH-value of the skin, which in turn, influences the skin barrier. This may also be the reason why some people observe the development of acne (12).

In this study the effects of certified surgical masks on the skin microbiome and oxygen saturation with a baseline measurement were compared. Samples were taken from the cheek to analyze the microbiome composition. The dominance of *Cutibacterium acnes* (*C. acnes*) was of particular interest. We also wanted to see whether the blood oxygen levels in the index fingers of the left and right hand would differ. In addition, the relation between skin microbiome changes and oxygen saturation has been studied.

- Met opmerkingen [RPP1]: Do we need a title page?
- Met opmerkingen [LD2R1]: No, that's not part of a paper
- Met opmerkingen [RPP3]: In what order?
- Met opmerkingen [LK4R3]: We can discuss it today.
- Met opmerkingen [LD5R3]: I'd propose to do it based on relative contribution. This is what is normally done when writing papers.
- Met opmerkingen [LK6R3]: How can we measure the relative contribution of each person?
- Met opmerkingen [LD7R3]: That's not that hard. There are several aspects of the whole experiment:
 - Biology part
 - Programming part
 - Writing the paper
- Met opmerkingen [LD8R3]: It's something we have to agree on
- Met opmerkingen [LK9R3]: Can we use co-authors? I think everyone make a lot of effort on this report.
- Met opmerkingen [LD10R3]: It not just the paper, its the relative contribution to the whole experiment. Ive no idea about co-authors
- Met opmerkingen [LK11]: have or has?
- Met opmerkingen [LK12R11]: @Riesebos PC, Peter
- Met opmerkingen [RP13R11]: good question :/
- Met opmerkingen [LD14R11]: "My parents have owned their boat since I was born."
- Therefore, wearing face masks have become
- <https://grammar.yourdictionary.com/vs/has-vs-have-proper-grammar-rules.html>
- Met opmerkingen [LD15R11]: Although that's what I think
- Met opmerkingen [RP16R11]: the face masks might be plural but the wearing is not and that's what has refers to, hence it's has
- Met opmerkingen [RP17R11]: dat is mijn uitspraak en daar zult u het mee moeten doen.
- Met opmerkingen [RP18R11]: zoals de rijdende rechter het altijd zo mooi zegt
- Met opmerkingen [LK19]: Microbiome, blood oxygen saturation, masks
- Met opmerkingen [LD20R19]: KN95
- Met opmerkingen [LK21]: @Riesebos PC, Peter uses?
- Met opmerkingen [RP22R21]: use is correct
- Met opmerkingen [LK23]: Did we did anything for this species?

Results

Comparing SpO₂

There appears to be no statistically significant difference between wearing a face mask and not wearing one regarding the measured oxygen saturation levels. The average oxygen saturation level comes down to 97.94% and 98.05% for both wearing and not wearing the face mask respectively. We also performed a t-test which showed us that there was no significant difference in blood SpO₂ levels between wearing and not wearing a face mask as well as there being no significant difference between the left and right index finger's measurements. This can be seen when looking at the p-value of 0.156, which is higher than the cut-off value of 0.05. The results are summarized in the table below:

Subject	Baseline		Intervention	
	Right Index Finger Average	Left Index Finger Average	Right Index Finger Average	Left Index Finger Average
1	98.59 %	98.71 %	98.41%	98.45 %
2	97.71 %	97.90 %	97.95%	97.87 %
3	97.86 %	97.88 %	97.70%	98.04%
4	97.64 %	97.45 %	97.37 %	97.29 %
5	98.40 %	98.30 %	98.04 %	98.20 %
Mean	98.05%		97.94%	

Table 1. Average SpO₂ values of each subject

Microbiome Comparison

In figure 1 the sequencing results of the baseline and intervention are shown. The figure reveals that the percentage of *Staphylococcus saccharolyticus* (*S. saccharolyticus*) decreased while the *Staphylococcus capitis* (*S. capitis*) increased. For subject 1, the percentage of *S. saccharolyticus* declined from 27% to 1%. Moreover, for subject 2, 3, 4 and 5 the percentage of *S. saccharolyticus* was reduced to almost 0% from 17%, 20%, 19% and 45% respectively. For subject 1, 2, 3 and 5 the average count of the *S. capitis* has increased to 40%, while for subject 4 this rise was from 0% to

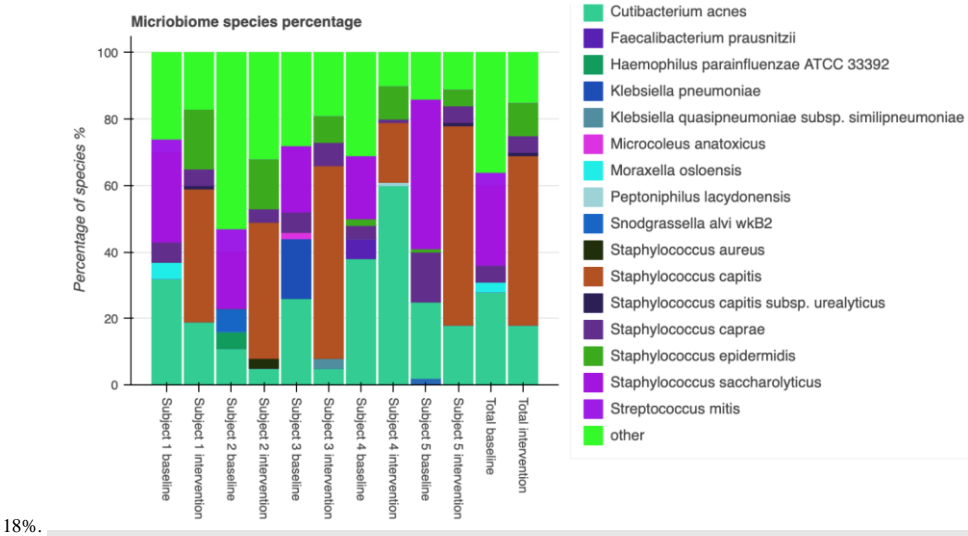


Figure1. Microbiome species count for each subject (for both the baseline and intervention)

Met opmerkingen [RP24]: Martijn would like us to add two more relevant graphs which are now missing. Perhaps in a supporting document with references to it.

Met opmerkingen [RP25R24]: @Hossain MS, Shahadat

Met opmerkingen [HS26R24]: @Riesebos PC, Peter can you please check it and let me know

Met opmerkingen [RPP27]: Maybe also talk about the acne spots count

Met opmerkingen [LK28R27]: @Hossain MS, Shahadat can you also write the spots count on results? And there is no significant different between wearing and without masks

Met opmerkingen [HS29R27]: @Lin K, Kai @Riesebos PC, Peter I've already added about acne spots in microbiome part.

Met opmerkingen [LK30]: @Hossain MS, Shahadat where can we get this number(97.94,98.05)? Is it in the table? If not please add it to the table< thanks

Met opmerkingen [HS31]: @Riesebos PC, Peter can you please check this line? Is it correct or not?

Met opmerkingen [RP32]: this basically says the same things twice now

Met opmerkingen [HS33R32]: yeah.. this is same. how can we combine this two?

Met opmerkingen [RP34R32]: like this?

Met opmerkingen [HS35R32]: we have to mention that we also compared the left and right index finger to answer this two hypothesis
H0: There is no significant difference in blood oxygen saturation between the right and left index finger.
H1: There is a significant difference in blood oxygen saturation between the right and left index finger

Met opmerkingen [RP36R32]: Where are these hypothesis written down?

Met opmerkingen [RP37R32]: they seem to be missing from this report

Met opmerkingen [HS38R32]: beginning of results section i mentioned about these two

Met opmerkingen [RP39R32]: I think it's fine now

Met opmerkingen [PA40]: there is no need a table

C. acnes is one of the bacteria that can cause acne (13). After having worn the surgical mask, the amount of observed *C. acnes* saw a decrease, only for subject 4 was this not the case. The amount of *C. acnes* in subject 4's facial microbiome grew by 21.75% (Supplementary Figure 1). The change in the observed amount of *C. acnes* between the baseline and the intervention is depicted in Table 2 below, where the delta is the difference between the baseline and intervention.

Subject	Baseline	Intervention	Delta
1	31.7%	19.3%	-12.33%
2	10.6%	5.15%	-5.45%
3	25.5%	5.27%	-20.23%
4	38.1%	60.1%	+21.75%
5	23.1%	18.1%	-5.05%

Table 2: *Cutibacterium acnes* percentage of each subject

The nature of the changes that took place between the baseline and the experiment can be seen in Tabel 3. After the Simpson's Diversity Index and the Shannon Diversity Index have been applied to everyone, where delta is the difference between the baseline and experiment data.

Subject	Simpson's Diversity Index			Shannon Diversity Index		
	Baseline	Experiment	Delta	Baseline	Experiment	Delta
1	0.818	0.765	-0.053	4.056	3.255	-0.801
2	0.939	0.8	-0.139	5.697	3.937	-1.76
3	0.857	0.648	-0.209	4.496	2.948	-1.548
4	0.809	0.597	-0.212	4.3	2.35	-1.95
5	0.721	0.599	-0.122	2.704	2.454	-0.25

Table 3: Simpson's Diversity Index and Shannon Diversity Index of each subject

Before beginning this experiment, we expected that there would be a considerable change in the number of acne spots present on the subjects' faces between wearing and not wearing a mask. We used paired sample t-test for statistical analysis and determined that there was no significant change in acne spot count since the p -value was 0.39, which is more than the threshold value of 0.05 (Supplementary Figure 2).

Discussion

SpO₂ reflection

Our research set out to find if wearing a face mask would influence the facial skin microbiome and blood oxygen saturation. When looking at our statistical analysis data we can see how little the oxygen saturation changes between the baseline measurements and intervention. Thus, it can be said that no significant change is happening and that the specific mask used for our research is not a cause for lower blood oxygen saturation. It can also be said that there is no significant difference between measuring the left or right hands' index finger. The blood oxygen saturation does not show any significant decrease while wearing a KN95 mask compared to that while not wearing a mask.

Microbiome reflection

The facial skin microbiome seems to show more changes. Looking at the count of the species, large shifts can be observed. One such observation shows us that there is a species that decreases in the intervention period which can be a cause for acne (*C. acnes*). Another species that shows a substantial change is *Staphylococcus capitis*. The decrease in *C. acnes* stands in stark contrast with the fact that four out of the five subjects had more acne spots during the intervention period compared to fewer observed spots during the baseline. This lets us to believe more factors must be at play. We conclude that wearing a face mask causes a shift in the microbiome which could be a cause for more observable acne spots.

Points of discussion

Given the results, we have the following suggestions to improve our study design. First off, oxygen saturation was only measured when the subject had been resting for a minimal duration of 15 minutes. This was done to mimic the daily life of the subjects, in this case students during the day on campus. Unfortunately, this way of measuring does not tell us if the subject would have had a lower oxygen saturation during more physically intense labour since the respiratory system would have had enough time to get the oxygen saturation back to normal levels, if the level had dropped at all.

Met opmerkingen [RP41]: add reference

Met opmerkingen [LK42]: @Hossain MS, Shahadat this is for discussion not here

Met opmerkingen [HS43R42]: @Rieschbos PC, Peter

Met opmerkingen [LK44R42]: My fasle. It can be here.

Met opmerkingen [HS45R42]: Then we can remove this comment

Met opmerkingen [RPP46]: Needs more sources

Met opmerkingen [LK47]: @Rieschbos PC, Peter C.acne is decline after experiment except me.

Met opmerkingen [LK48]: @Rieschbos PC, Peter the percentage relate with acne decline

Met opmerkingen [PA49]: good point

Furthermore, due to time constraints only one type of mask was used, with 5 subjects participating. Therefore, we cannot say if our findings hold true for any other type of mask. Other mask related aspects to consider are the duration for which we wore the mask, how often we removed the masks, taking of the mask to eat, the personal hygiene of the mask wearer, in which environments and circumstances the mask was worn and the ethnicity of the subject. These all might have had an impact on how the facial skin microbiome was influenced but are hard to change or regulate. One way of trying to regulate these things is to make more protocols and have a more lab like environment. Some protocols were made for how to store the mask, how to put it on, how to wash our hands and a plethora of other things to keep these factors into check.

Also of note is the difference in length of time for the baseline and the intervention period. The baseline ended up lasting 13 days and the intervention 8 days. One would have to do further research to see how different durations of mask wearing would influence the facial skin microbiome.

Lastly, as in most other research, there often appear to be some outliers. In our case this would be the species count for subject 4, where subject 4 was the only one where the amount of *C. acnes* species increased during the intervention. This goes against our expectations of the face mask causing an increase in acne related species. Unfortunately, we could not find a reason as to why this happened. We suspect this might be caused by an increase in oxygen saturation between the subjects' skin and face mask. Furthermore, subject 5 seems to be the only one to have a significant change in SpO₂ values. There does not seem to be a reason behind this significant change compared to the other subjects.

Seeing a great shift in the microbiome, no significant shift in the SpO₂ levels as well as no significant shift in the acne spot counts leaves us wondering what other effects the change in microbiome may have on a person's health. We also wonder what exactly caused the shift in the microbiome. This shows us we are just starting to grasp the effects of face masks on the facial microbiome. At the same time, this shows us the importance of the continuation of studies being performed in this field.

Material and methods

Five subjects (4 male and 1 female) participated in this study with differing ethnicities. All subjects fit in the age range of 24 to 36 years and the subjects did not have a preexisting skin disease. No antibiotics were used, and no infections have been observed in the month prior. The subjects were not immune-compromised or suffering from other pre-existing medical conditions that would make wearing a facemask impractical. However, one subject used hormonal supplements (Levothyroxine) during the entirety of the experiment.

Subjects were given a KN95 (melt blown cloth 29%, filter cotton 29%, and non-woven fabric 42%) mask from KUBAOYA and wore them for 8 hours 5 days a week. The subjects were requested to clean their hands with alcohol-based sanitizer for 20 to 30 seconds or with water and soap for 40 to 60 seconds. They were given instructions on how to wear the mask, to take off the mask, and how to store the mask. The masks were replaced daily with new clean masks. The subjects were also asked to keep up a journal (referred to as diary). In this way, we were able to keep track of potential factors that could interfere with the experiment.

To examine the influence of wearing face masks on blood oxygen saturation, data was measured and collected by using a CMS50D1 pulse oximeter from CONTEC. To make exact measurements, subjects followed the protocol that was provided by CONTEC. The subjects measured both the left and right index finger to detect discrepancies in the results. Measurements were taken three times a day (at the start of the day, after 4 hours and after 8 hours of mask wearing). Subjects did not bathe or shower 12-24 hours before the sample would be taken, and the same oximeter was used throughout the whole experiment.

To collect samples, sterile gloves were worn to minimize cross-contamination and soaked sterile cotton swabs were used in a 1.5 ml PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, de-ionized water from Milli-Q® Water) with 0.1% Tween® 20 from Sigma in microcentrifuge tube.

A soaked cotton swab was rubbed on a pre-measured skin area (4cm X 4cm) with zigzag strokes and constant pressure. The swab heads were cut one to three cm in length to fit in the microcentrifuge tubes. The tubes were then vortexed in a microcentrifuge for one minute and centrifuged for 17,000g, 15 minutes in room temperature.

One ml of supernatant was removed, and the sample was transferred to a new microcentrifuge tube without swab head. Then the sample got resuspended by transferring and storing it in liquid nitrogen. QIAamp® was used as DNA Micro Kit (LOT:169032985) for DNA extraction following the tissue protocol to isolate the DNA from the December third edition 2014 QIAamp® DNA Micro Handbook. To get a higher concentration of DNA, 1 ul of carrier RNA was added at step five and 20 ul of distilled water was applied at step 12. Using Qubit® dsDNA BR Assay Kit from Intitrogen (LOT: 1975497) and nano drops, the quantities of the samples were detected.

Met opmerkingen [PA50]: ?

Met opmerkingen [RP51R50]: What is wrong with this?

Met opmerkingen [PA52]: is not it need to mention the oxygen percentage of subject2 increased during use of surgical mask?

Met opmerkingen [LK53]: We already did the experiment for microbiome after experiment.

Met opmerkingen [RP54R53]: What do you mean? this talks about how wearing a mask for (as example) 3 weeks might have different results on the microbiome than our 8 days of mask wearing.

Met opmerkingen [LK55R53]: ok

Met opmerkingen [LK56]: Subject 4 increased acne species during intervention.

Met opmerkingen [RP57R56]: Which acne species was it that changed only for subject 4 in the intervention?

Met opmerkingen [LK58]: @Riesebos PC, Peter I am the only one who follow our expectation.... If the oxygen decline, *C. acne* should increase.

Met opmerkingen [LK59R58]: That's why I said maybe the oxygen between skin and mask increase. We expect it decline, and cause *C. acne* incline. The result is this incline and we do not know why. So, if someone want to re-do this experiment, they can measure the oxygen concentration between skin and mask.

Met opmerkingen [RP60]: You need to make some final statements in a last paragraph here about how interesting/important this research was, don't just "fall of the edge" of the paper here!

Met opmerkingen [RP61]: So in the discussion, you should discuss *why* you think this is the case: a simple factual statement like this one should otherwise be in the results. Also, this would be a good point to comment on the fact the the well known bacterium associated with acne (the cutibacterium) actually decreased for most subjects! What could that mean?

Met opmerkingen [LK62R61]: *S. capitis* increased if this species enter our body that will cause inflammation of inner heart(heart valves).
C. acne decreased this species is one of the species that will cause acne on our face.
S. saccharolyticus decreased this species will cause inflammation of inner heart(heart valves).

Met opmerkingen [RP63]: Could you look at this @Lin K, Kai

Met opmerkingen [RPP64]: Needs more sources

Met opmerkingen [RP65]: kit number

Met opmerkingen [RP66R65]: @Lin K, Kai

Met opmerkingen [RP67]: Manufacturer, catalog nr. ?

Met opmerkingen [RP68R67]: @Lin K, Kai

To analyze the acne difference between wearing and not wearing face masks, we calculated the average acne number for each subject. Then we used a Q-Q plot to make sure it is normal distribution. Finally, we used a paired sample t-test to analyze. This method is used for measuring two different points. An essential approach, like alpha diversity, is frequently used for analyzing the microbiome in a sample based on sequencing data. In this experiment we used the alpha diversity method. Alpha diversity, which is frequently used interchangeably with the word species diversity, is a single number that describes the distribution of species abundances in each sample and is dependent on species richness and evenness (14). We used the Simpson's diversity index and the Shannon Diversity index to check the diversity of species. The Simpson's diversity index is a measure of species diversity (15). It provides a value between 0 and 1, with 1 indicating total variation and 0 indicating complete homogeneity. The Shannon diversity index is a method of measuring species diversity in a community. The greater the value, the greater the species variety. The smaller the number, the lower the diversity (16). In this case, we utilize the index on both the baseline and experimental data.

To sequence the microbiome, Oxford Nanopore 16S Barcoding Kit (SQK-RAB204) was used for 16S sequencing and followed the protocol version RAB_9053_v1_revN_14Aug2019. The sequencing data was assigned to a taxonomy species level using the Oxford Nanopore EPI2ME platform, Fastq 16S Workflow revision 2021.09.09. Finally, the data was analyzed using Python (Anaconda distribution version 3.8.12, Pandas (1.3.5), Numpy (1.22.1), scipy(1.7.3), scikit_bio(0.5.6)) were the most utilized modules, and the data was visualized using Bokeh (2.4.2) /Holoviz /Panel (0.12.6) to create an informative dashboard.

The dashboard is available at <https://github.com/PRiesebo/sigma>. Contact the owner to get access to the repository.

Acknowledgments

We would like to thank Martijn Herber and Marcel Kempenaar for guiding us throughout the project, furthermore we would like to thank Fenna Feenstra and Peter Kroon for teaching us how to use a variety of python libraries for our data analysis and dashboard building. Lastly, we would like to thank Edzard Geertsema for guiding us in writing our report.

- Met opmerkingen [RP69]: You need to refer to your analysis scripts here, either as a link to your github repo or as supplementary info files.
- Met opmerkingen [RP70R69]: @Lin K, Kai
- Met opmerkingen [LK71R69]: @Latumalea DD, Djakim
- Met opmerkingen [LD72R69]: What do you mean with 'analysis' scripts?
- Met opmerkingen [LK73R69]: @Riesebo PC, Peter Can we just make a branch to put all of the code for statistics inside? Then add the link to the supplementary info files.
- Met opmerkingen [RP74R69]: This was Martijn's comment I don't know what he means exactly @Latumalea DD, Djakim
- Met opmerkingen [RP75R69]: but I think a link to our codebase
- Met opmerkingen [RP76]: Please reformat more neatly.

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Met opmerkingen [RPP77]: Needs fixing