Outreach note of the study report 21E5273 SUBLIO
According to the study plan **D21-0412**

Exploration of the effect of the hyperionisation of tap water and thermal water from Balaruc-les-Bains, by a genomic study on human skin explants *ex vivo*

Tested products

Tap water (Longjumeau) +/- hyperionised with the Sublio Ionic WaterBox *Pro* device

Thermal water (Balaruc-les-Bains) +/- hyperionised with the Sublio Ionic WaterBox *Pro* device

Sponsor

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STUDY

This study was subject to complete and detailed reports under the reference 21E5273 ; returned to SUBLIO France, only owner of these results.

Date of the beginning of the study	25th June 2021
Date of the beginning of the technical phase of the study	21st September 2021
Subcontracting partners performing the genomic phase	**Laboratoire Genex** 1, Chemin de Saulxier 91160 Longjumeau

This study includes an *ex vivo* study with the products P1 and P2 (corresponding to the second donor for tap water) and with the products P3 and P4 (thermal water from Balaruc-les Bains) and a genomic study with a RT-qPCR analysis of the different samples of this study and the samples of the study 20E5076 (P1 and P2 of the first donor).

TESTED PRODUCTS

P1 Water (Longjumeau city), sterilized by filtration at 0.22µm.

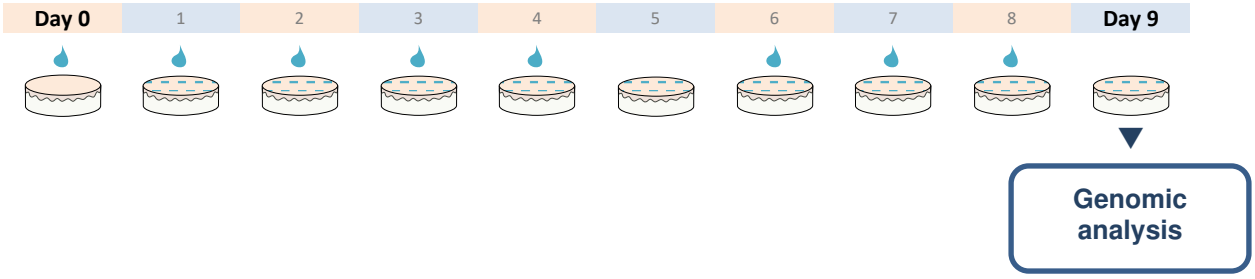
P2 Water (Longjumeau city), sterilized by filtration at 0.22µm and hyper-ionised thanks to the Sublio Ionic WaterBox *Pro* device provided by SUBLIO France company.

P3 Thermal water (Balaruc-les-Bains) provided by SUBLIO France company and sterilized by filtration at 0.22µm (thermal water is stored at 4°C and must be used within 48 hours after the collect).

P4 Thermal water (Balaruc-les-Bains) provided by SUBLIO France company, sterilized by filtration at 0.22µm and hyper-ionised thanks to the Sublio Ionic WaterBox *Pro* device provided by SUBLIO France company (thermal water is stored at 4°C and must be used within 48 hours after the collect).

MATERIAL & METHODES

The aim of the study is to apply water (hyperionised or not) daily on human skin explants kept in survival for 9 days. Then, a genomic study was conducted by the Genex laboratory to analysis the expression of 10 genes stimulated or repressed by the treatment



After 9 days of treatment (2µL/explant), the explants are harvested and fixed in RNAlater to preserve RNA.

After extraction, the quantity and the quality of RNA was controlled.

The extracted RNA were retro-transcribed (iScript, Bio-Rad). They were analyzed and semi-quantified by qPCR (iTaq, Bio-Rad) in order to evaluate the 10 gene of interest.

A histological study has been realized simultaneously in order to check cell and tissue morphology.

The samples from the study 20E5073, performed in the same conditions (tap water ± hyperionised on the 1st donor), have been added to the genomic analysis of this study (2nd donor).

RESULTS

1. Control of the morphology

After 9 days of treatment, the tap water (P1) and the thermal water (P3) are well tolerated by the skin.

After 9 days of treatment, the tap water or the thermal water hyperionised with the Sublio Ionic WaterBox Pro device (P2 or P4 respectively) are also well tolerated by the skin.

The hyperionisation does not result in any change in the skin tolerance of tap water (Longjumeau) and thermal water (Balaruc-les-Bains).

2. Genomic study

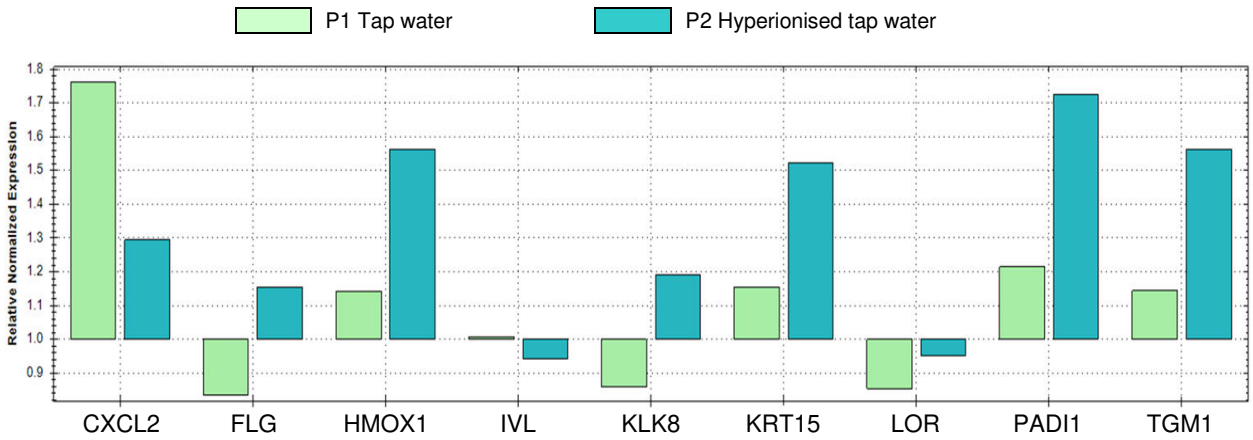
The following table summarizes the symbol and the name of each gene of interest (selected for the study from the results of the previous genomic study 20E5076) with their respective qPCR efficiency and their biological function. Two housekeeping genes (B2M et GAPDH) and a RT (reverse transcriptase) control have also been included in this analysis.

gene	name	Function
CXCL2	chemokine (C-X-C motif) ligand 2, exonic	**Inflammation** Inflammatory mediator
FLG	filaggrin	**Cutaneous barrier** Involved in keratinocyte differentiation and NMF formation
HMOX1	heme oxygenase 1	**Oxidative stress** Detoxifying enzyme
IVL	involucrin	**Cutaneous barrier** Involved in keratinocyte differentiation
KLK8	kallikrein-related peptidase 8, exonic	**Cutaneous barrier** Involved in desquamation
KRT15	keratin 15, exonic	**Epidermal stimulation** cytokeratin specific of the basal layer of keratinocytes
LOR	loricrin (exonic)	**Cutaneous barrier** Involved in keratinocyte differentiation
PADI1	peptidyl arginine deiminase, type I, exonic	**Cutaneous barrier** Involved in NMF formation
TGM1	transglutaminase 1, intron-spanning	**Cutaneous barrier** Involved in keratinocyte differentiation
B2M	house keeping	/
GAPDH	house keeping (exonic)	/

The analysis of the housekeeping genes B2M and GAPDH shows a stable amplification with high quality.
An average was calculated from the values of the three explants from the same donor (biological groups) after normalization with the values of the housekeeping genes.
For each gene of interest, a ratio between the different treatment conditions was calculated. Thus, these ratios allow to identify the effect of hyperionisation of tap water and thermal water.
We have chosen the following modulation threshold: a value greater than 1.15 to define an induced expression and a value lower than 0.8 to define a repressed expression.

Activity of tap water +/- hyperionised, 1st donor (20E5073)

Expression ratios between the two averages of the expression values for tap water (P1) or hyperionised tap water (P2) versus control (T), on day 9.



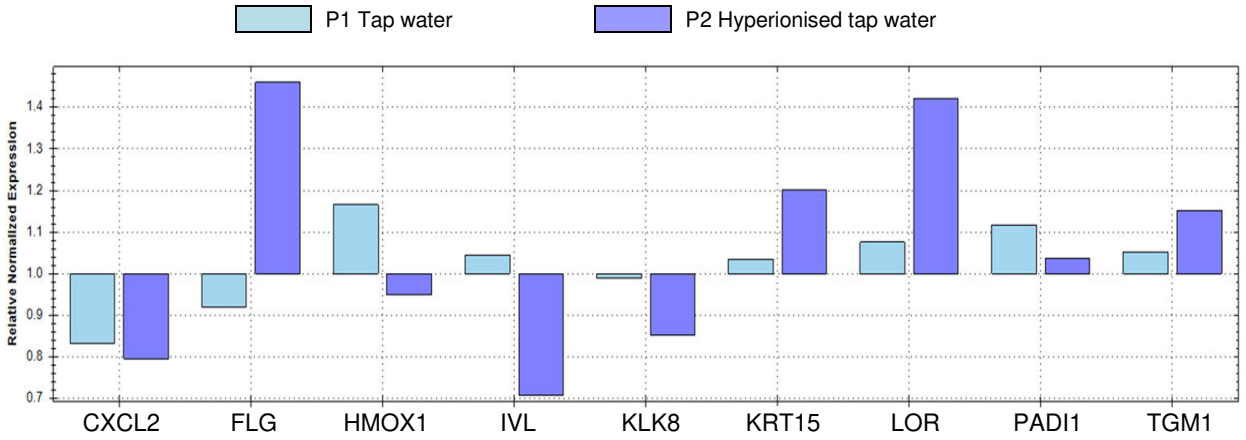
Comparison P2 ■ vs P1 ■ Donor 1								
CXCL2	**FLG**	**HMOX1**	**IVL**	**KLK8**	**KRT15**	**LOR**	**PADI1**	**TGM1**
↘	↗	↗	↘	↗	↗	↗	↗	↗

For the donor 1, the tap water (■) induces an increase of inflammation (CXCL2) and an increase of the genes involved in the formation of NMF (Natural Moisturising Factor) by increasing PADI1 expression. It also stimulates the epidermal renewal (KRT15). All other genes were not modulated.

The hyperionised tap water (■) induces a significant decrease of inflammation (CXCL2) and a significant increase of the genes implied in the regulation of epidermal barrier (FLG, KLK8, PADI1, TGM1).
It also stimulates epidermal renewal (KRT15) even more. This treatment also increases the expression of HMOX1 which shows antioxidant properties. Furthermore, as HMOX1 is able to metabolize the heme in carbon monoxide, it also has anti-inflammatory properties.

Activity of tap water +/- hyperionised, 2nd donor (21E5273)

Expression ratios between the two averages of the expression values for tap water (P1) or hyperionised tap water (P2) versus control (T), on day 9.



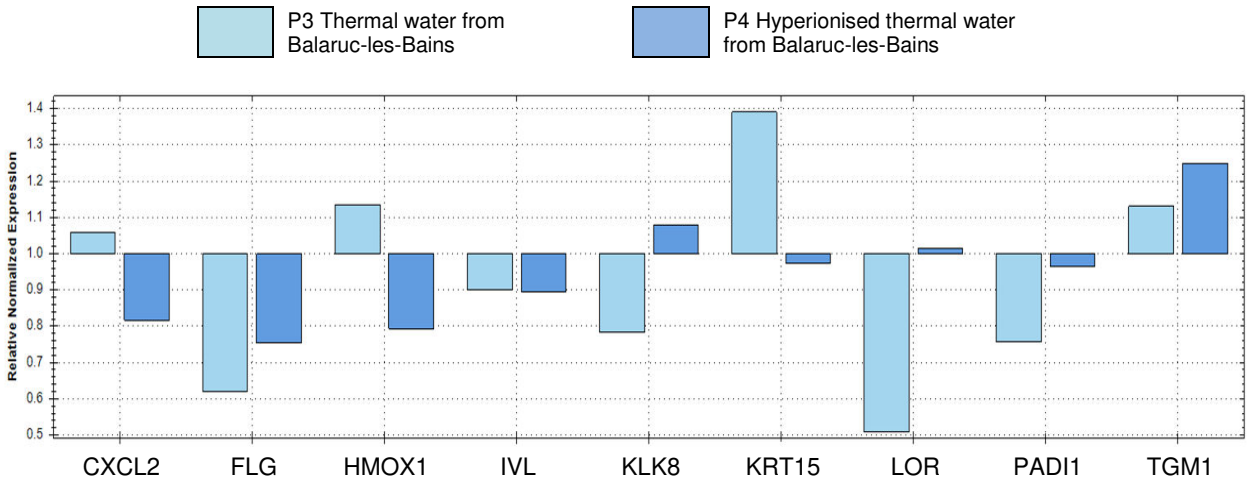
Comparison P2 vs P1 Donor 2								
CXCL2	**FLG**	**HMOX1**	**IVL**	**KLK8**	**KRT15**	**LOR**	**PADI1**	**TGM1**
↔	↗	↘	↘	↘	↗	↗	↘	↗

For the donor 2, the tap water (■) slightly increases HMOX1 which shows antioxidant properties. All other genes were not significantly modulated.

The hyperionised tap water (■) significantly increases the expression of genes implied in the formation and the regulation of cutaneous barrier (FLG, LOR). It also stimulates the epidermal renewal (KRT15). However, it decreases the expression of involucrin (IVL).

Activity of thermal water from Balaruc-les-Bains +/- hyperionised, 2nd donor (21E5273)

Expression ratios between the two averages of the expression values for thermal water (P3) or hyperionised thermal water (P4) versus control (T), on day 9.



Comparison P4 ■ vs P3 Donor 2								
CXCL2	**FLG**	**HMOX1**	**IVL**	**KLK8**	**KRT15**	**LOR**	**PADI1**	**TGM1**
↘	↗	↘	↔	↗	↘	↗	↗	↗

The thermal water from Balaruc-les-Bains (■) decreases the expression of the genes involved in the formation and the regulation of cutaneous barrier (FLG, LOR et PADI1). It also stimulates the epidermal renewal (KRT15).

The hyperionised thermal water from Balaruc-les-Bains (■) induces a significant decrease of the inflammatory basal level (CXCL2) which is not impacted by the treatment with the un-ionised thermal water from Balaruc-les-Bains.
This treatment significantly decreases the basal level of oxidative stress (HMOX1) which is slightly increase with the un-ionised thermal water from Balaruc-les-Bains.
It significantly increases the expression of the genes involved in the formation and the regulation of cutaneous barrier (FLG, LOR et PADI1).
Furthermore, the hyperionised thermal water from Balaruc-les-Bains increases the kallikrein 8 (KLK8) which is an enzyme involved in the regulation of corneocyte desquamation.
However, it reduces the stimulation of epidermal renewal (KRT15).

CONCLUSION

Tap water

This study concerns the evaluation of hyperionisation of tap water on a first and a second donor.
Please refer to the global analysis of the results of the three donors.

However, according to the experimental conditions described above, the obtained results clearly show that the hyperionisation of tap water (Longjumeau) with the Sublio Ionic WaterBox Pro device leads to a decrease of inflammatory basal level.
The hyperionised tap water notably stimulates some of the genes involved in the formation and the homeostasis of the cutaneous barrier. Furthermore, it significantly stimulates the epidermal renewal.

Thermal water from Balaruc-les-Bains

This first study analysis shows that the treatment of the skin explants with the thermal water from Balaruc-les-Bains stimulates the epidermal renewal. But it also decreases a part of the cutaneous barrier markers.

However, these results cover only 10 of the 23 000 human genes. These 10 genes only represent a small part of the many biological effects developed during thalassotherapy.

The repression of filaggrin, KLK8, loricrin and PADI1 clearly shows that the prolonged contact with thermal water induces a cutaneous barrier alteration.

The hyperionisation of thermal water from Balaruc-les-Bains allows to inhibit these side effects by reducing the skin inflammation, by limiting the decrease of filaggrin and by totally preventing the decrease of loricrin and PADI1, while offsetting the decrease of KLK8.

By reducing skin inflammation and by regulating skin hydration, the hyperionised thermal water from Balaruc-les-Bains allows to significantly improve the beneficial effects of thalassotherapy.