

Outreach note of the study reports 20E5076, 21E5272 and 21E5273

According to the study plans D19-732-1, D21-0417 et D21-0412

Exploration of the effect of the hyperionisation of tap water, by a transcriptomic study on human skin explants *ex vivo*

Tested products

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

Sponsor

SUBLIO France

M. Frédéric Esnault

8, Rue René Coty

85018 La Roche-sur-Yon Cedex

Email: frederic@sublio.com

Test facility

BIO-EC

1, Chemin de Saulxier

91160 Longjumeau

FRANCE

Tel: +33 (0)1 69 41 42 21

Email: info@bio-ec.fr

www.bio-ec.fr

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STUDIES

Three studies were carried out in order to evaluate the effect of hyperionisation of tap water with the Sublio Ionic WaterBox Pro device.

A first study, identified as 20E5076, has been realized in 2020 on a first donor and has been subject of a genomic study of the whole genome (23 000 genes) by microarray. After the selection of 10 genes of interest, an analysis by RT-qPCR has been realized.

A second study, identified as 21E5273, has been realized on a second donor and has been subject of a genomic study of the 10 genes of interest by RT-qPCR analysis.

A third study, identified as 21E5272, has been realized on a third donor and has been subject of a genomic study of the 10 genes of interest by RT-qPCR analysis.

These studies were subject to complete and detailed reports under the reference 20E5076, 21E5372 and 21E5173; returned to SUBLIO France, only owner of these results.

Subcontracting partners performing
the genomic phase

Laboratoire Genex
1, Chemin de Saulxier
91160 Longjumeau

TESTED PRODUCTS

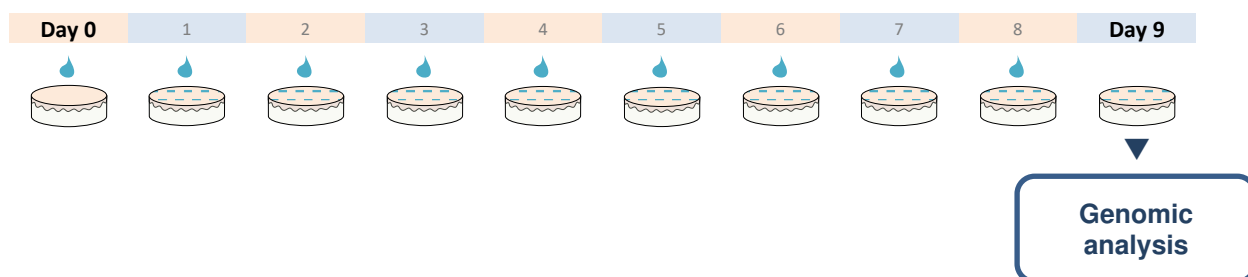
- P1 Water (Longjumeau city), sterilized by filtration at 0.22µm.
- P2 Water (Longjumeau city), sterilized by filtration at 0.22µm and hyper-ionised thanks to the Sublio Ionic WaterBox *Pro* device provided by SUBLIO France company.

MATERIAL & METHODES

The three studies have been realized according to the same *modus operandi*:

- 20E5076 Donor 1: 59-years-old caucasian women
- 21E5373 Donor 2: 45-years-old caucasian women
- 21E5172 Donor 3: 54-years-old caucasian women

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.

RESULTS

1. Control of the morphology

After 9 days of treatment, the tap water (P1) is well tolerated by the skin.

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

These results were the same for the three donors.

The hyperionisation does not result in any change in the skin tolerance of tap water.

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	qPCR efficiency	Function
CXCL2	chemokine (C-X-C motif) ligand 2, exonic	94%	Inflammation Inflammatory mediator
FLG	filaggrin	98%	Cutaneous barrier Involved in keratinocyte differentiation and NMF formation
HMOX1	heme oxygenase 1	90%	Oxidative stress Detoxifying enzyme
IVL	involucrin	96%	Cutaneous barrier Involved in keratinocyte differentiation
KLK8	kallikrein-related peptidase 8, exonic	100%	Cutaneous barrier Involved in desquamation
KRT15	keratin 15, exonic	95%	Epidermal stimulation cytokeratin specific of the basal layer of keratinocytes
LOR	loricrin (exonic)	102%	Cutaneous barrier Involved in keratinocyte differentiation
PADI1	peptidyl arginine deiminase, type I, exonic	98%	Cutaneous barrier Involved in NMF formation
SPRR3	small proline-rich protein 3, exonic	99%	Cutaneous barrier Involved in keratinocyte differentiation
TGM1	transglutaminase 1, intron-spanning	98%	Cutaneous barrier Involved in keratinocyte differentiation
B2M	house keeping	98%	/
GAPDH	house keeping (exonic)	97%	/

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.

We have chosen the following modulation threshold:

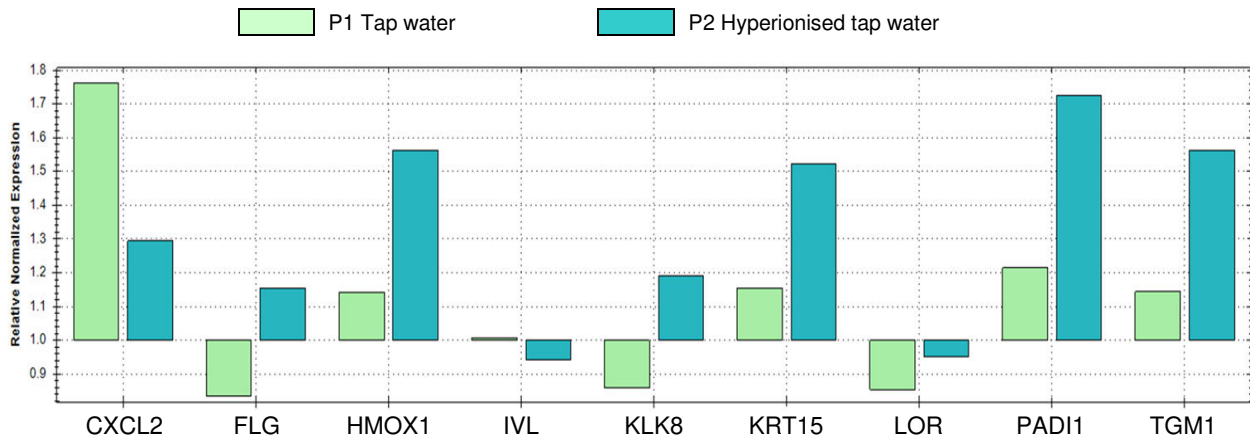
- A value greater than 1.15 to define an induced expression,
- A value lower than 0.8 to define a repressed expression.



SPPR3 was not exploited because its expression was too low.

3. 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.



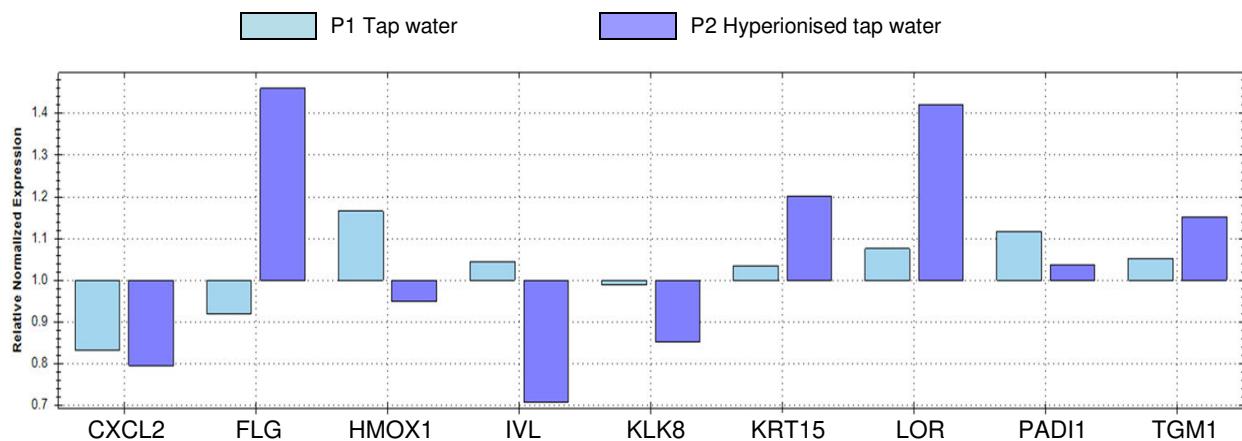
Comparison P2 ■ vs P1 ■ Donor 1								
CXCL2	FLG	HMOX1	IVL	KLK8	KRT15	LOR	PADI1	TGM1
↘	↗	↗	↘	↗	↗	↗	↗	↗

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.

4. 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.



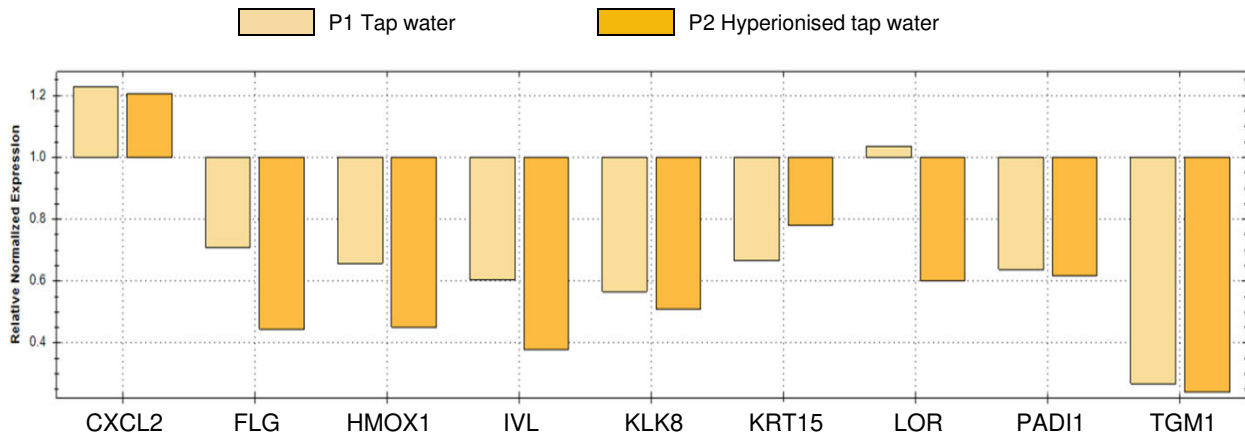
Comparison P2 ■ vs P1 ■ Donor 2								
CXCL2	FLG	HMOX1	IVL	KLK8	KRT15	LOR	PADI1	TGM1
↔	↗	↘	↘	↘	↗	↗	↘	↗

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).

5. Activity of tap water +/- hyperionised, 3rd donor (21E5272)

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 3

CXCL2	FLG	HMOX1	IVL	KLK8	KRT15	LOR	PADI1	TGM1
↔	↓	↓	↓	↔	↑	↓	↔	↔

For the donor 3, the tap water (■) induces a significant increase of the inflammation (CXCL2) et a significant decrease of the genes involved in the formation and the regulation of cutaneous barrier (FLG, IVL, KLK8, PADI1 et TGM1).

Furthermore, it decreases epidermal renewal (KRT15) and reduces the basal level of oxidative stress (HMOX1).

All other genes were not modulated.

The hyperionised tap water (■) amplifies the decrease of the expression of gene involved in the formation and the regulation of cutaneous barrier (FLG, IVL et LOR).

This treatment also amplifies the decrease of oxidative stress (HMOX1) and stimulates the epidermal renewal (KRT15).

It has been shown that the process begins with a moderate osmotic stress which induces an inflammation. Then, the inflammatory state decreases which allows to the establishment of cell differentiation and consequently, regeneration of cutaneous tissue.

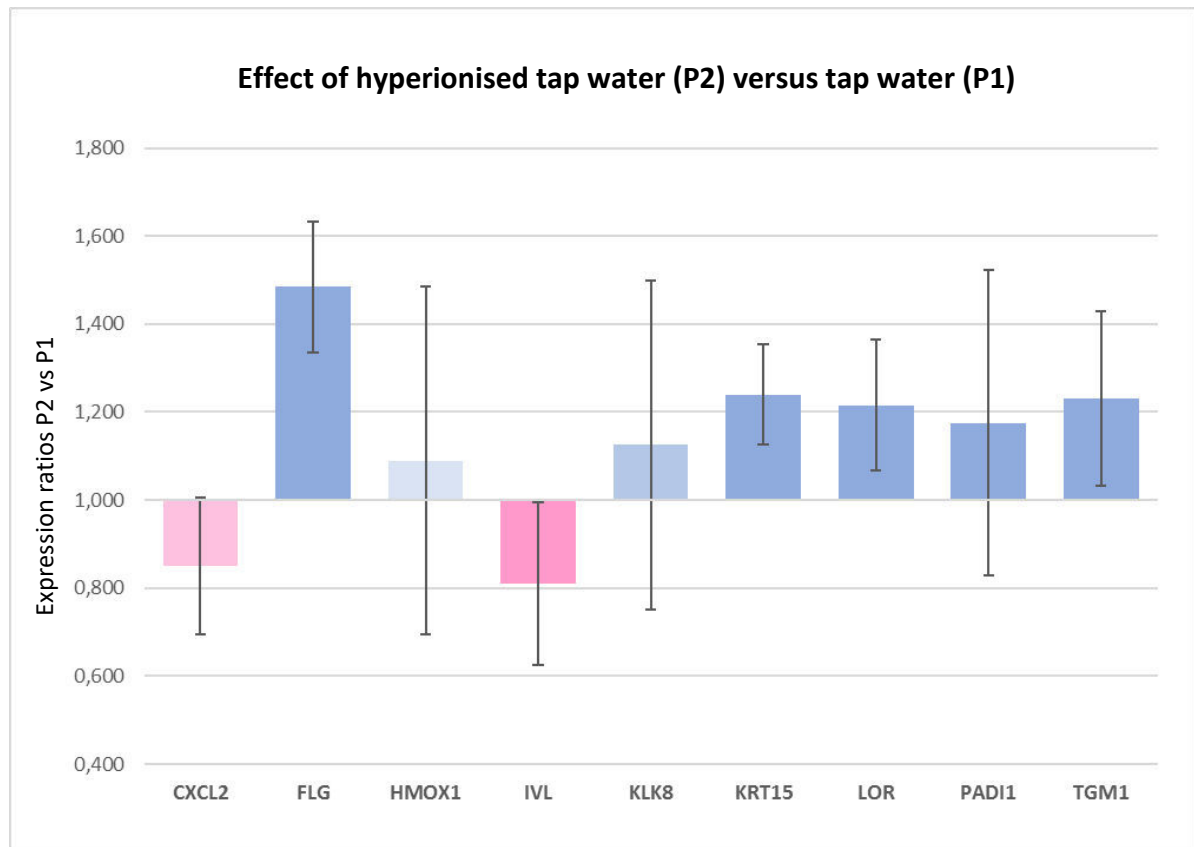
For this donor, the kinetic of the gene modulation are delayed in time. Indeed, the gene modulation profile shows that the initial phase of the inflammation is not yet complete and the activation phase of tissue regeneration is not yet initiated.

DISCUSSION

This study concerns the evaluation of hyperionisation of tap water on three donors. As previously mentioned, the third donor shows a kinetic of gene modulation delayed in time. Consequently, the conclusion includes only the results the first two donors.

**Average of expression ratios
Hyperionised tap water (P2) versus Tap water (P1), à J9.**

	CXCL2	FLG	HMOX1	IVL	KLK8	KRT15	LOR	PADI1	TGM1
Modulation	↘	↗	↔	↘	↗	↗	↗	↗	↗
Average	0,85	1,49	1,09	0,81	1,13	1,24	1,22	1,18	1,23
SD	0,16	0,15	0,40	0,18	0,37	0,11	0,15	0,35	0,20



The hyperionisation of tap water allows to decrease inflammation and to modulate the expression of involucrin.

Besides, the hyperionisation of tap water provides an increase of the genes implied in the formation of the *stratum corneum* which is the main actor of cutaneous barrier. Filaggrin (FLG), loricrin (LOR) and transglutaminase 1 (TGM1) are involved in the last steps of keratinocyte differentiation in corneocytes. Besides, they play a major role in the formation of cornified envelop which is important for *stratum corneum* resistance and flexibility.

The peptidyl arginine deiminase, type I (PADI1) takes part in the formation of NMF by deimination of FLG. This allows the complete degradation of FLG, leading to the production of free amino acids and their derivatives that are essential for moisturizing of the *stratum corneum*.

The kallikrein 8 (KLK8) is an enzyme involved in the regulation of corneocyte desquamation.

The hyperionisation of tap water also increases the expression of the gene of cytokeratin 15 (KRT15) which is specific of the basal layer of keratinocytes (specific localization of cell divisions). The cytokeratin 15 is also considered as an epidermal stem cell marker. Therefore, the cytokeratin 15 is involved in epidermal renewal.

CONCLUSION

According to the experimental conditions described above, the obtained results clearly demonstrate that the hyperionisation of tap water (Longjumeau) with the Sublio Ionic WaterBox Pro device allows to significantly reduce the basal level of inflammation.

Hyperionised water stimulates a lot of genes involved in the formation and the homeostasis of cutaneous barrier.

Besides, hyperionised water significantly stimulates the epidermal renewal.