

Neuregulin-4: a key BATokine serving as a central hub for slimming efficacy

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Introduction:

White adipose tissue (WAT) is the primary store of lipids of the body and is naturally innervated reacting to different stimuli by secreting important signaling molecules. Neuregulin-4 (NRG4) is a Brown Adipose Tissue adipokine (or 'BATokine') with robust effects on lipid metabolism [1-2]. NRG4 release in WAT is downregulated in obesity [3] and increases upon cold/exercise stimuli. As main functions, it stimulates brown adipocyte differentiation, modulates thermogenic markers positively increasing lipolysis, regulates glucose metabolism and promotes neurite outgrowth to improve adipose tissue innervation [4]. Obesity resulting from WAT excess is considered a prevalent health issue. The browning of WAT through the regulation of NRG4 levels, a central hub involved in the regulation of multiple biological processes, has been proposed as a promising therapeutic strategy in the treatment of obesity-associated metabolic disturbances. After exhaustive research we have found a novel blend of botanical extracts, *Arctostaphylos uvae-ursi* leaf extract and *Silybum marianum* extract (BL3BTE0030_0041) with impressive slimming efficacy. The blend exerts this activity via a unique mechanism of action, the increase in the release of NRG4, through which, a decrease in lipidic content, at both lipolysis and adipogenesis levels can be observed. Moreover, the novel blend is able to reduce the uptake of glucose, as well as, act as an inductor of sympathetic innervation of adipose tissue, needed to activate thermogenic functions.

Materials & Methods:

MODULATION OF GENES BY qPCR
Gene expression was determined by RT-qPCR in differentiated human subcutaneous adipocytes after treatment with the blend BL3BTE0030_0041 during differentiation (9 days, adipogenesis protocol) or once the preadipocytes were terminally differentiated into mature adipocytes (the last 48 h, lipolysis protocol).

MODULATION OF THE RELEASE OF NRG4
The levels of NRG4 released by human subcutaneous adipocytes after treatment with BL3BTE0030_0041 was assessed by means on an ELISA test and by immunofluorescence.

LIPID CONTENT QUANTIFICATION
Using Adipored assay, lipid content was quantified in two different assays: for adipogenesis, treatment was performed during the differentiation of preadipocytes into mature adipocytes for 9 days while for lipolysis, the blend was added during the last 48 h.

GLUCOSE UPTAKE
Glucose uptake was measured by luminescence, in differentiated human subcutaneous adipocytes using a commercial kit (Glucose Uptake-Glo™ assay (Promega)).

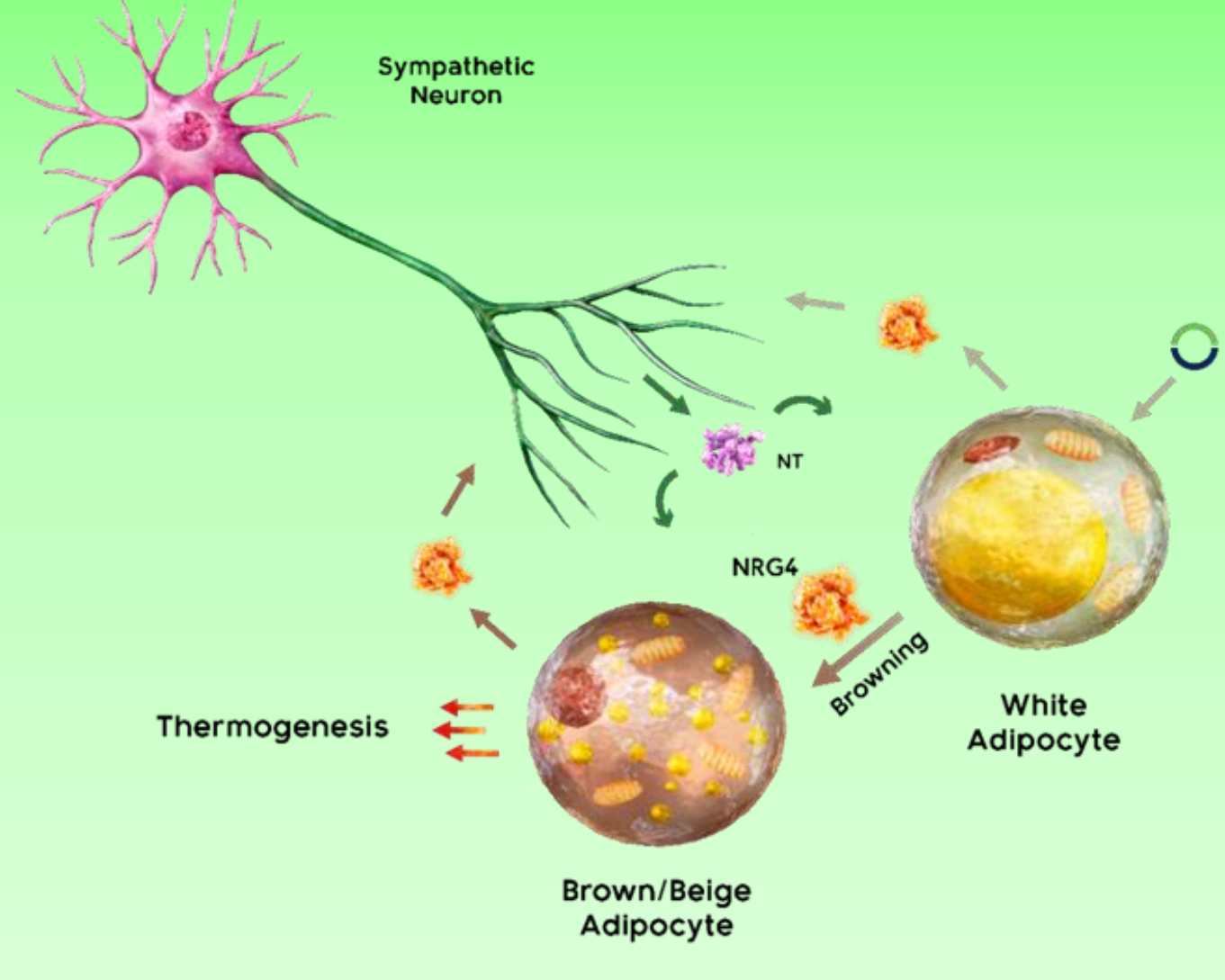
NEURITE OUTGROWTH
NRG4-rich conditioned medium from treated adipocytes was used to measure the induction of neurite outgrowth on SH-SY5Y cells by optical microscopy and Image J analysis.

CLINICAL EFFICACY
The slimming efficacy of the blend was measured on the body of 30 healthy female volunteers (50% Caucasian and 50% Latin American). The study was conducted versus placebo: volunteers applied the active product, a cream containing the blend at a concentration of 2%, on one hemi-body (thigh and arm) and the placebo formulation on the other side, according to a previously defined randomization list; moreover, active product was also applied by all the subjects on the abdomen. Product efficacy was evaluated after 14 (T14), 28 days (T28) and 56 (T56) days of daily use. Thigh, arm and abdomen circumference of the volunteers was measured using a flexible millimetered meter. Skin profilometry was assessed by means of Primos 3D (GF Messtechnik GmbH). In this study the Ra parameter was evaluated, which is a measurement of roughness of the skin (in this case, cellulite), therefore a decrease in Ra indicates an improvement in skin smoothness.

All data was normalized versus basal. Statistical data analysis was performed using Student t-test by comparison of % of response from test substance vs response of the damage or untreated control condition wells (Basal) where ***p<0.001, **p<0.01 and *p<0.5. All graphics show the mean ± SEM

Conclusions:

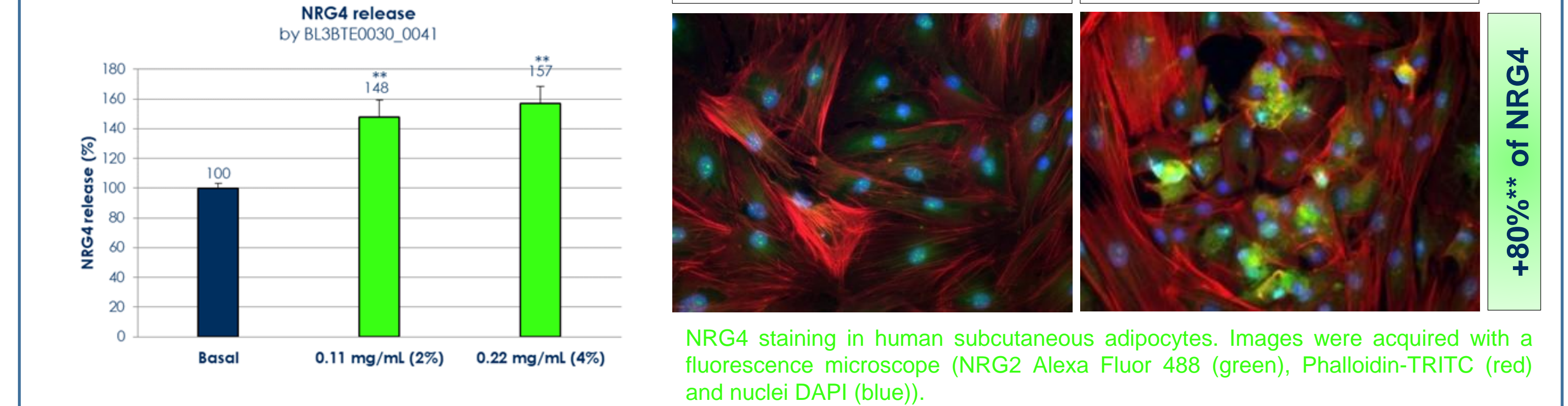
The novel blend presented here, formed by *Arctostaphylos uvae-ursi* leaf extract and *Silybum marianum* extract, through its action on NRG4, appears to exhibit a novel and highly innovative action at the nervous-adipose tissue communication level for the highly demanded application of slimming and anti-cellulite, and consequently it is of high interest for the cosmetic industry.



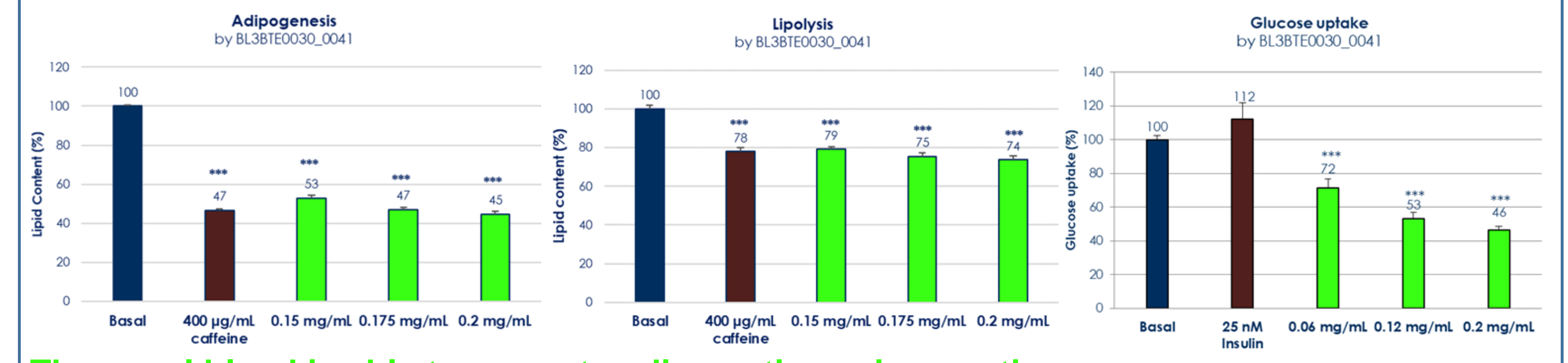
Results & Discussion:

The novel botanical blend BL3BTE0030_0041 modulates slimming-related genes:
Adipogenesis (9 day treatment, 0.2 mg/mL):
SCD: -1.71-fold FABP4: -2.15-fold
PDK4: 3.55-fold CEBPA: -1.69-fold
LPL: -2.66-fold GLUT4: -13.62-fold
Lipolysis (48 h treatment, 0.2 mg/mL):
NRG4: 1.93-fold GLUT4: -1.81-fold
PDK4: 2.08-fold CD36: -1.68-fold

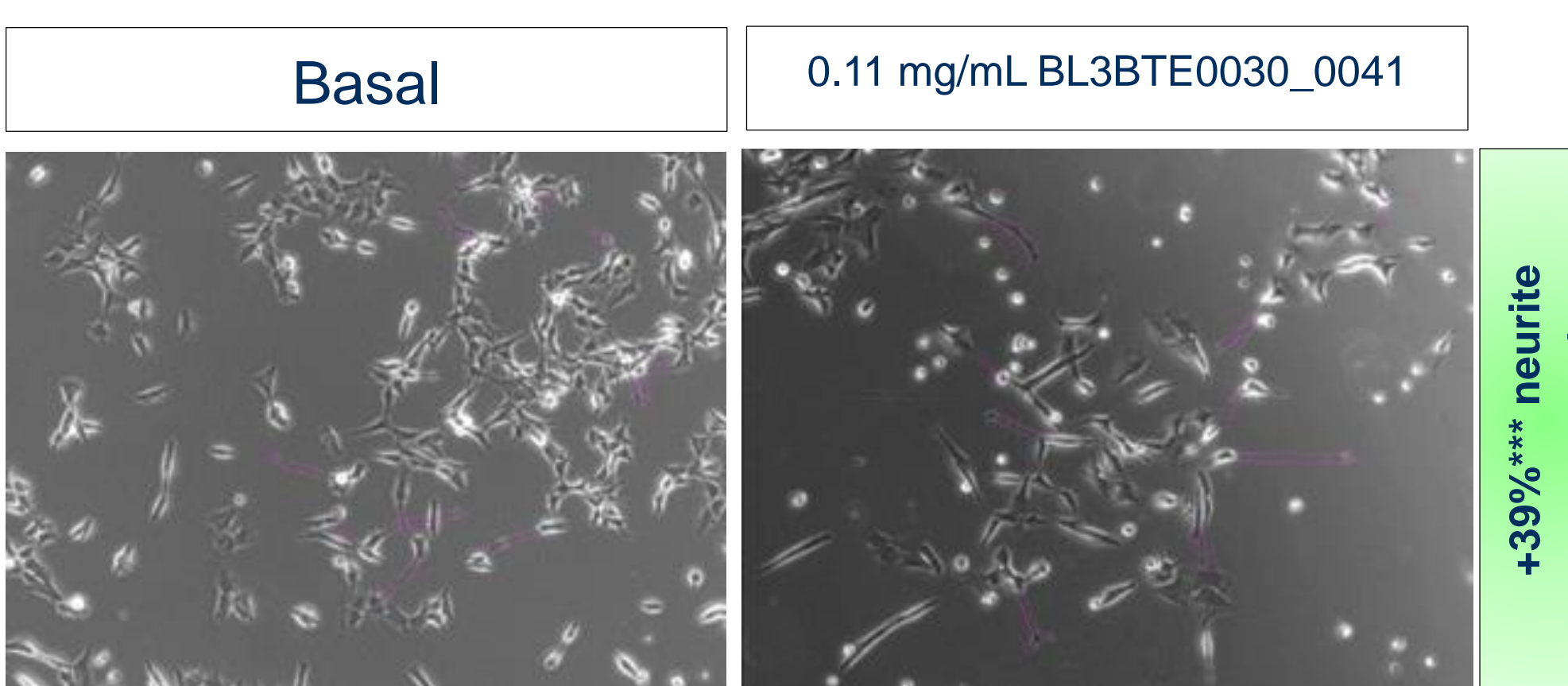
The blend BL3BTE0030_0041 increases NRG4 release:
Using an ELISA assay, the significant increase in NRG4 in human subcutaneous adipocytes due to treatment with the blend could be evaluated. Additionally, this increase was further demonstrated by immunofluorescence.



The novel blend BL3BTE0030_0041 shows a clear lipid modulation and decrease in glucose uptake:
The blend was able to significantly decrease both adipogenesis and lipolysis at all the concentrations tested, at the same level as the positive control caffeine. This modulation of lipid content is accompanied by a decrease in glucose uptake in a concentration-dependent manner.



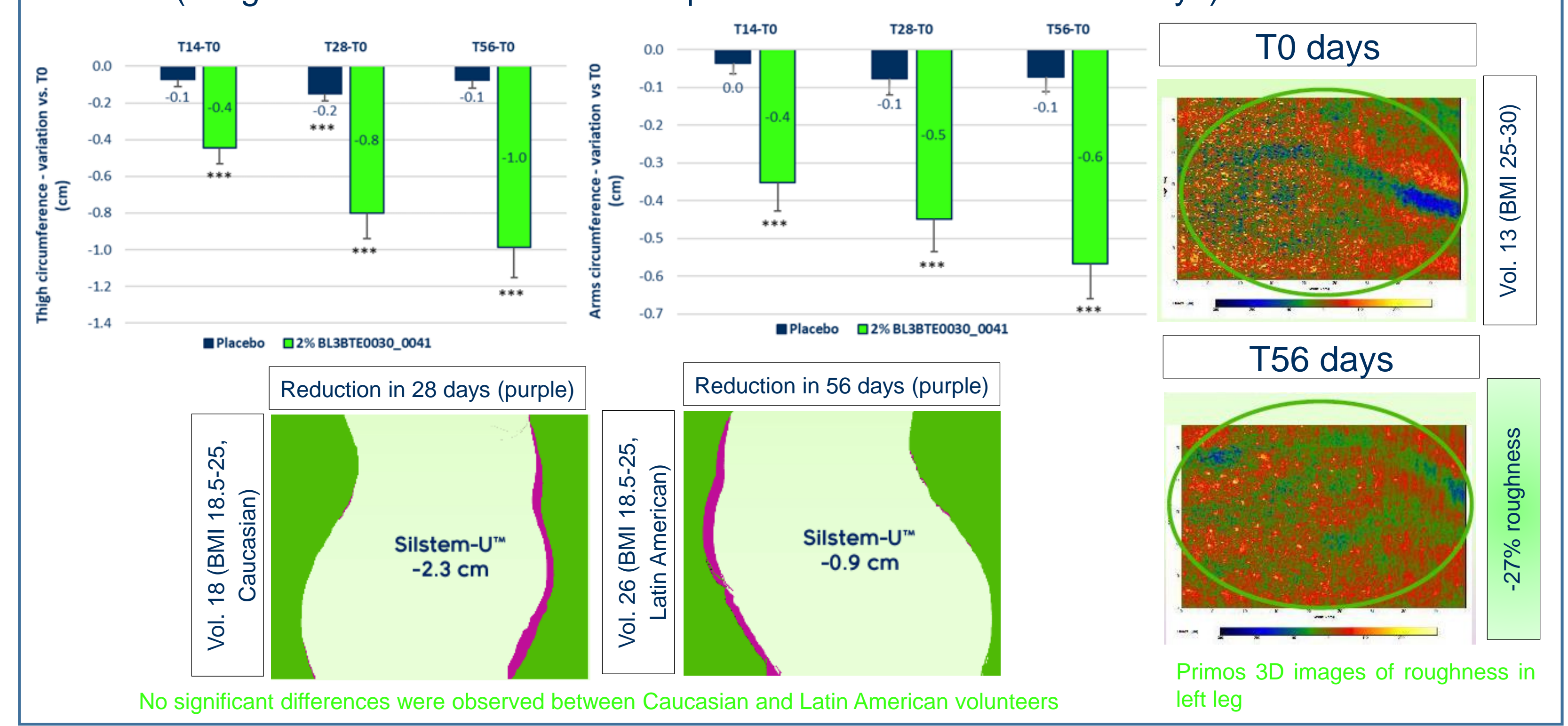
The novel blend is able to promote adipose tissue innervation:



Optical microscopy and Image J analysis of neurite length (pink).

Conditioned medium from adipocytes treated with BL3BTE0030_0041 was used to treat SH-SY5Y cells and measure neurite outgrowth. The length of the neurites was significantly increase 39% and 48% at 0.11 and 0.22 mg/mL of active. Norepinephrine was used as positive control, increasing neurite outgrowth by 18%.

The novel botanical blend BL3BTE0030_0041 shows a clear slimming efficacy in vivo:
A significant decrease in the thigh, arm, and circumference of the volunteers treated with a cream containing 2% of the commercial solution of BL3BTE0030_0041 was observed, and in all cases higher for the active than placebo. In addition, a clear improvement in the skin surface was detected (a significant decrease in the Ra parameter of 13.2% after 56 days).



References:

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