Biointec™
blue biotechnology

A SECRET PERSONAL TRAINER

actigym™
marine ingredient

V.0

WISHING A TONED BODY

- **Muscle tone** is responsible for having the desired lean and well-toned body by everyone, as it **supports tightly the above lying structures**:
  - It is the tightness or resistance to stretch of muscles at rest,
  - contributes to silhouette definition and body firmness.

- But it declines due to:
  - Aging (which is inescapable),
  - and/or physical inactivity (sedentary lifestyle, lack of time to exercise,…)

When tone is lost and muscles lengthen, sagginess appears, especially in arms, belly and buttocks.

actigym™ marine ingredient

Lipotec www.lipotec.com

ENDURANCE EXERCISE BENEFITS

Endurance training

Medium to long distance jogging, cycling, swimming,…

- Relatively low intensity.
- Extended periods of time.

vs.

Strength training

Weightlifting, sprinting,…

- High intensity.
- Short periods.

- Enhances general cardiovascular and respiratory condition.
- Provides better oxygen and nutrients supply to skin cells.
- Helps to get in a **better body shape** by:
  - ✓ Lowering subcutaneous fat accumulation.
  - ✓ Enhancing muscle tone.

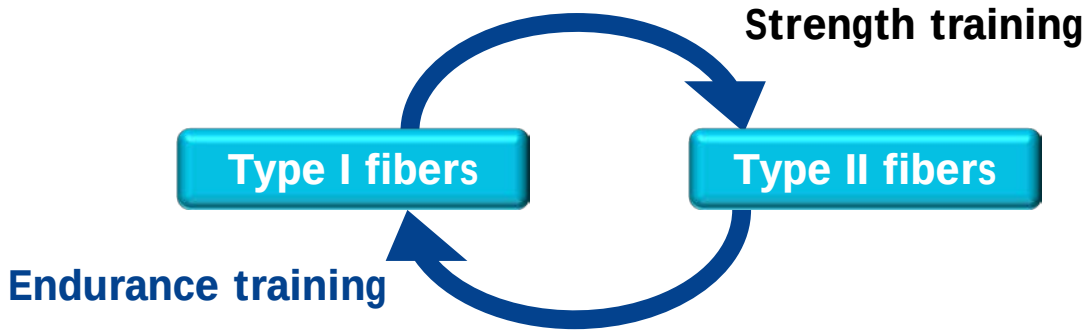
Endurance training is highly beneficial to health and appearance.

actigym™ marine ingredient

Lipotec www.lipotec.com

SWITCH BETWEEN MUSCLE FIBERS

- There is a dynamic conversion between fiber types depending on the type of exercise:



- Skeletal muscles contain varying proportions of muscle fiber cells:
  - **Type I** (or red-slow): slow and prolonged contractions, resistant to fatigue. In high proportion in postural muscles (**tonic or anti-gravity**).
  - **Type II** (or red-white-fast): fast and short contractions, fatigable.

Endurance exercise, through type I muscle fibers, helps to get a better body shape.

actigym™ marine ingredient

Lipotec www.lipotec.com

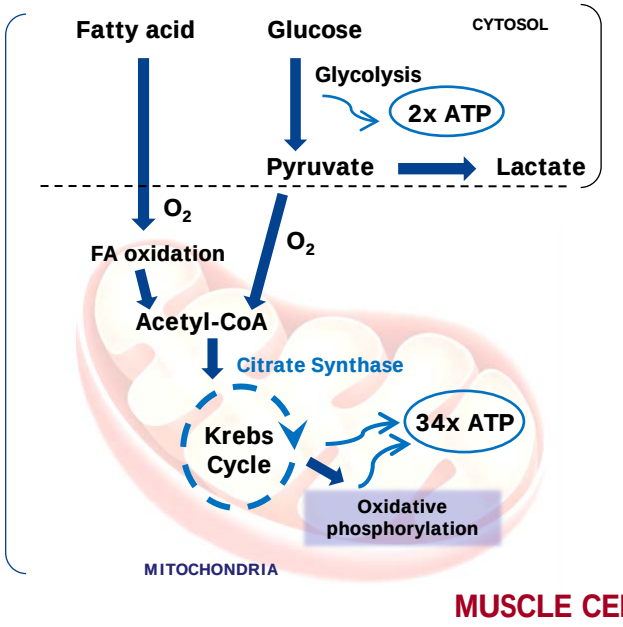
SLOW FIBERS USE AEROBIC METABOLISM TO ENDURE

Type I fibers

- Have a higher content in mitochondria.
- Mainly use **aerobic metabolism** as the principal energy source.

Aerobic metabolism

- Needs O_2.
- Involves complete oxidation of glucose and FAs inside the mitochondria.
- Generates 34 ATPs + 2 ATPs.



Anaerobic metabolism

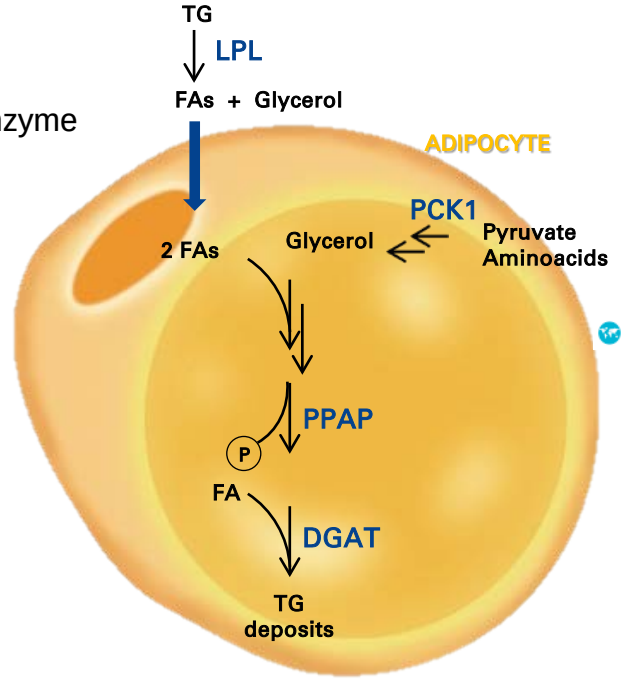
- No use of O_2.
- No use of mitochondria
- Generates only 2 ATPs, but faster.

Greater amounts of energy (ATP) obtained through mitochondrial metabolism in type I fibers allow them provide muscle tone.

STORAGE OF ENERGY IN ADIPOCYTES

FAs uptake

- Circulating TGs are hydrolyzed by the enzyme Lipoprotein Lipase (**LPL**).
- Then FAs can be taken up by adipocytes.



Glycerol synthesis

- Phosphoenolpyruvate Carboxykinase (**PCK1**) is a key enyzme in glycerol synthesis from pyruvate and aminoacids.

TG synthesis

- FAs are reesterfied with glycerol to form TGs, catalyzed by:
  - Phosphatidic Acid Phosphatases (**PPAP2A** and **PPAP2B**).
  - Diacylglycerol Transferase (**DGAT**).

Adipocytes store fat when there is no extra need of energy.

actigym™ marine ingredient

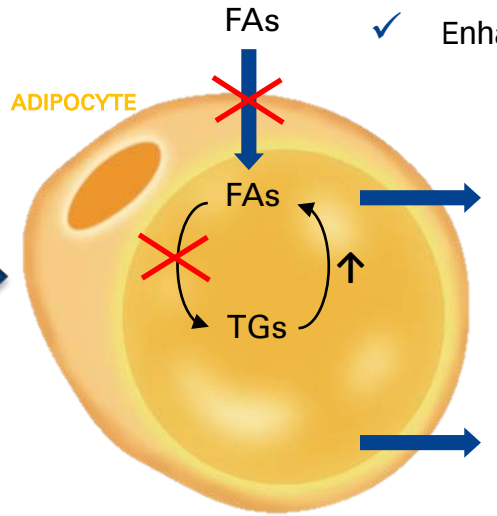
Lipotec www.lipotec.com

ENDURANCE EXERCISE EFFECTS

IN ADIPOCYTES:

- ✓ Reduced fat uptake.
- ✓ Decreased FAs esterification into TGs.
- ✓ Enhanced TGs degradation into FAs.

Endurance exercise training



Mobilization of FAs

Adiponectin release

TO MUSCLE FIBERS:

Delivery to muscles as source of energy, due to an increased energy demand.

- Adipokines are proteins secreted by adipocytes that regulate multiple functions in different tissues.
- Adiponectin regulates energy metabolism in muscle cells.

Endurance training induces adiponectin release, signaling to muscle fibers.

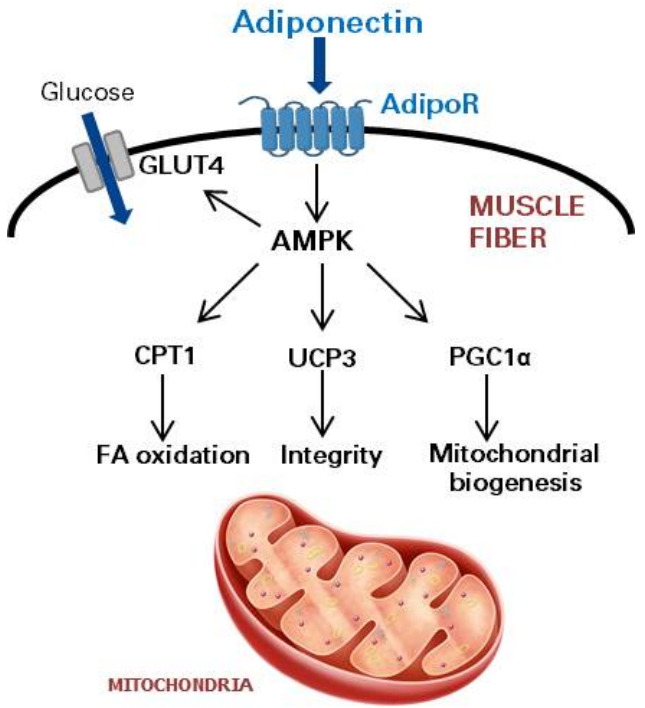
actigym™ marine ingredient

Lipotec www.lipotec.com

ADIPONECTIN EFFECT ON MUSCLES

- **Adiponectin** interacts with receptors AdipoR and activates AMP-activated protein Kinase (AMPK), a regulator of metabolism in muscle fibers that:

  - Increases **glucose uptake** by translocating GLUT4 to the membrane.
  - Boosts **FAs oxidation** by activating Carnitine Palmitoyl Transferase (CPT1) and citrate synthase.
  - Protects **mitochondrial function** by upregulating Uncoupling Protein 3 (UCP3).
  - Increases the **number of mitochondria** by activating Peroxisome proliferator-activated receptor Gamma Coactivator 1-alpha (PGC1α).



Adiponectin increases mitochondrial content and activity, leading to a higher number of tonic muscle type I fibers.

actigym™ marine ingredient

Lipotec www.lipotec.com

BERMUDA, A SMALL BUT GORGEOUS ISLAND

- Subtropical island in the North Atlantic Ocean.
- Around 120 km of impressive coastline, with sand beaches and stunningly clear waters, abound coral reefs, seagrass beds, mangrove swamps and underwater caverns.

- There lives a heavenly sponge (*Dysidea etheria*), from which *Bacillus sp.* was isolated.

Photo taken from the Flower Garden Banks page of the National Marine Sanctuary for the United States National Oceanic and Atmospheric Administration and the United States Department of Commerce, http://flowergarden.noaa.gov/about/spongelist.html

Bacillus sp. produces an enormous number of metabolites able to modulate various metabolic pathways in different biological systems.

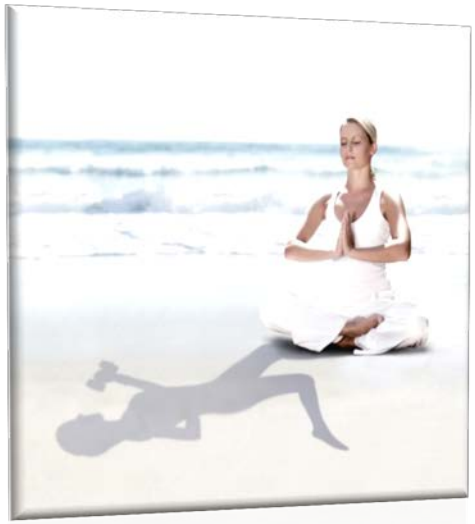
actigym™ marine ingredient

Lipotec www.lipotec.com

A SECRET PERSONAL TRAINER

actigym™ marine ingredient

Low molecular weight substance containing peptidic and glucidic material that mimics the effect of endurance exercise training to improve body tone.



- Active biotechnological ingredient that enhances **adiponectin release** from adipocytes.
- Releases adiponectin that signals **to muscle fibers**:
  - Boosting their mitochondrial metabolism.
  - Promoting the development of slow type fibers.
- In adipocytes, **downregulated the expression of genes** involved in **FA** uptake and **TG** synthesis.
- *In vivo*, 5% ACTIGYM™ *marine ingredient* provided **great results**, increasing **impressively when combined with exercise**:
  - Decreased skin fold and reduced abdominal perimeter.
  - Reduced contour of thighs and perimeter of arms.
  - Induced a diminution in body weight.

ACTIGYM™ *marine ingredient* EFFICACY

IN VITRO EFFICACY

- Induction of adiponectin release by adipocytes
- Enhancement of mitochondrial activity in muscle fibers
- ATP production in muscle cells
- Slow myosin levels in skeletal muscle
- Microarray analysis

IN VIVO EFFICACY

- Resculpting body shape

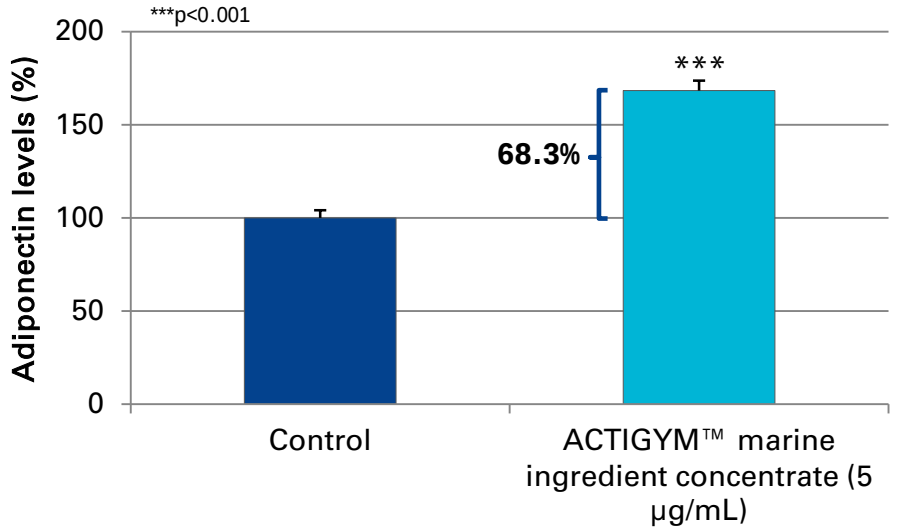
INDUCTION OF ADIPONECTIN RELEASE BY ADIPOCYTES

- Primary human subcutaneous preadipocytes were induced to differentiation while treated with 5 µg/mL ACTIGYM™ *marine ingredient concentrate*.
- Levels of adiponectin secreted by the adipocytes were quantified by ELISA.

Non-treated cells were used as control.

The marine ingredient significantly **increased the levels of adiponectin** produced by human subcutaneous adipocytes **by 68.3%** with respect to control.

ACTIGYM™ *marine ingredient* boosts the release of adiponectin by adipocytes.



actigym™ marine ingredient

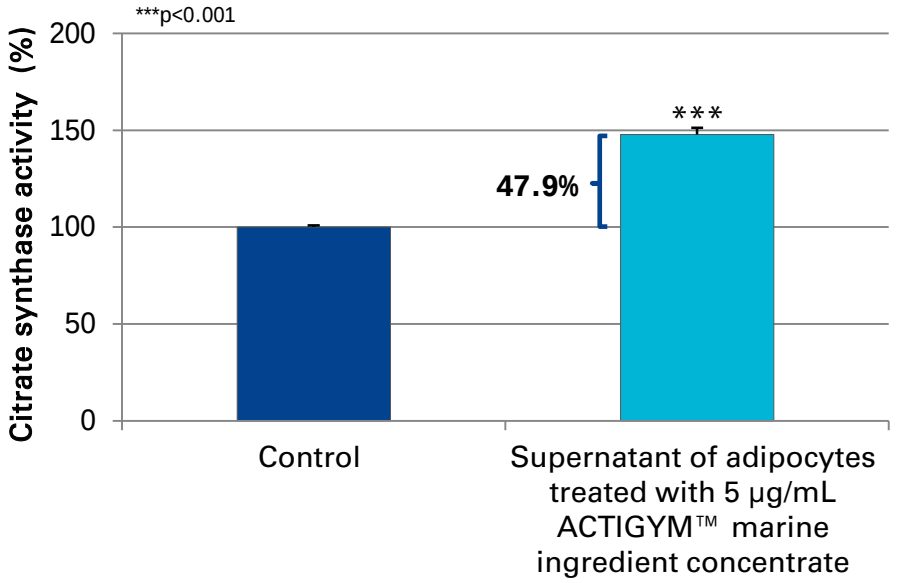
Lipotec www.lipotec.com

ENHANCEMENT OF MITOCHONDRIAL ACTIVITY IN MUSCLE FIBERS

- Human skeletal muscle cells were incubated with supernatants from adipocytes treated with 5 µg/mL ACTIGYM™ *marine ingredient concentrate*.
- Cells were lysed and citrate synthase activity was detected by a colorimetric assay. Citrate synthase activity was used as a quantitative marker of mitochondrial activity.

Cells treated with supernatants from non-treated adipocytes were used as control.

The treatment of muscle cells with adipocytes supernatants, containing high levels of adiponectin due to ACTIGYM™ *marine ingredient*, **increased citrate synthase** and therefore mitochondrial function **by 47.9%**.



By inducing adiponectin in adipocytes, ACTIGYM™ *marine ingredient* mediates an enhancement of mitochondrial activity in muscle fibers.

actigym™ marine ingredient

Lipotec www.lipotec.com

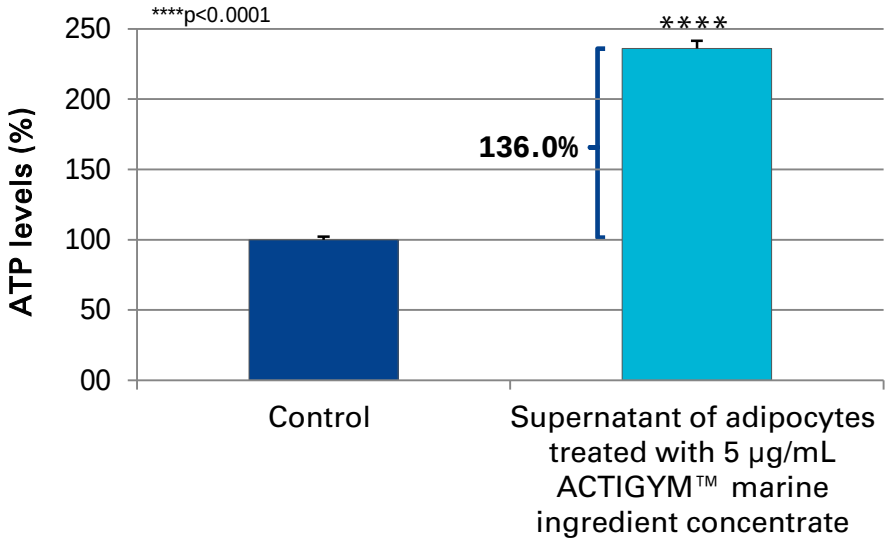
ATP PRODUCTION IN MUSCLE CELLS

- Human skeletal muscle cells were incubated with supernatants from adipocytes treated with 5 µg/mL ACTIGYM™ *marine ingredient concentrate*, thus containing higher levels of adiponectin.
- Then, cells were lysed and the amount of ATP was quantified using a fluorescence assay.

Cells treated with supernatants from non-treated adipocytes were used as control.

Adipocytes supernatants, containing increased adiponectin levels after treatment with ACTIGYM™ *marine ingredient*, **increase by 136% ATP production** in skeletal muscle cells.

ATP levels were significantly increased in muscle fibers, revealing an important improvement of mitochondrial function.



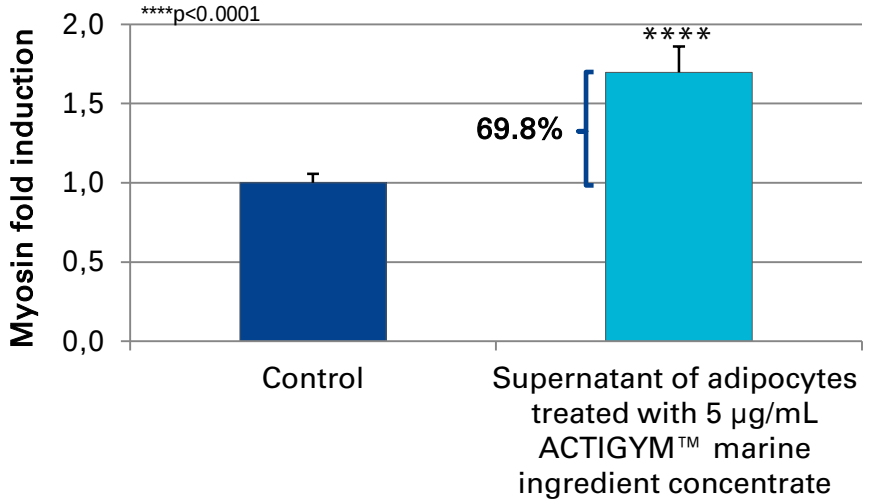
actigym™ marine ingredient

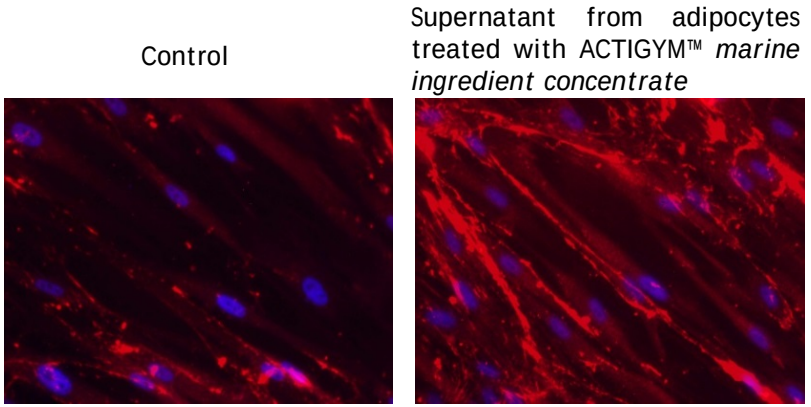
Lipotec www.lipotec.com

SLOW MYOSIN LEVELS IN SKELETAL MUSCLE

- Human skeletal muscle cells were incubated with supernatants from adipocytes treated with 5 µg/mL ACTIGYM™ *marine ingredient concentrate*, thus containing higher levels of adiponectin.
- The expression of slow myosin heavy chain was assessed by immunofluorescence staining and used as a marker of differentiation toward type I muscle fibers.

Cells treated with supernatants from non-treated adipocytes were used as control.





Enhanced expression of slow myosin was observed after incubation with supernatants from adipocytes treated with the active ingredient.

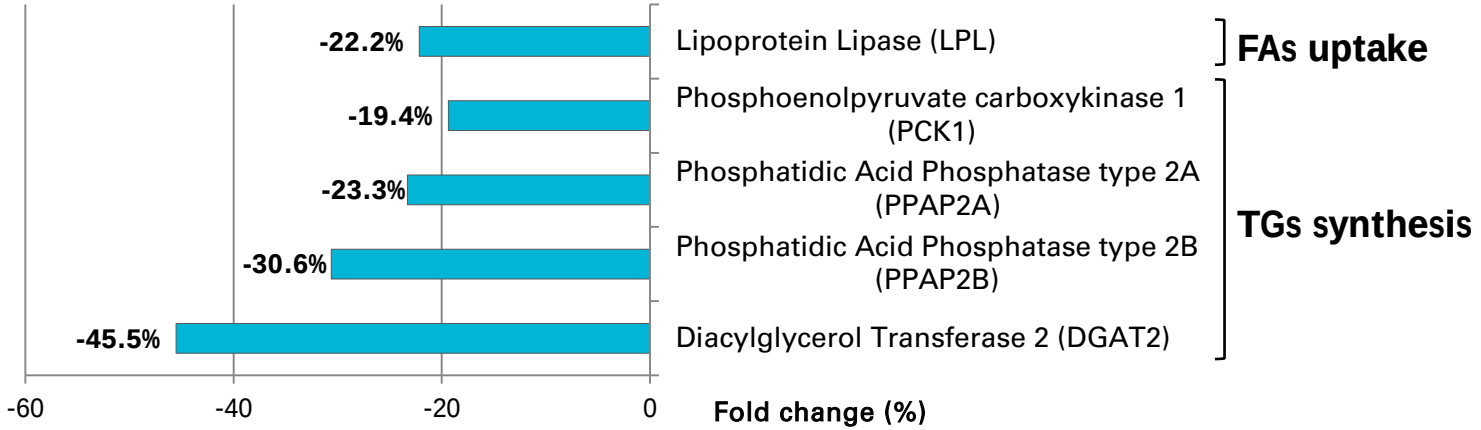
ACTIGYM™ *marine ingredient* enhanced the expression of the type I fibers marker through adipocyte-mediated signaling.

actigym™ marine ingredient

Lipotec www.lipotec.com

MICROARRAY ANALYSIS

- Primary human subcutaneous preadipocytes were incubated for 8 days in differentiation medium alone (control) or in the presence of 14 µg/mL of ACTIGYM™ *marine ingredient concentrate*. Then, cells were lysed and RNA purified.
- A microarray was performed using ASurePrint G3 Human Gene Expression Microarray v2 platform. After normalization and analysis of the data, fold variation in the expression of genes with respect to control values was calculated.



ACTIGYM™ *marine ingredient* downregulated the expression of genes involved in lipid accumulation.

actigym™ marine ingredient

Lipotec www.lipotec.com

RESCULPTING BODY SHAPE (I)

- 60 female volunteers (35-50 years old) with sedentary life styles were split into 3 groups:

  1st - performed a standardized exercise under the supervision of a personal trainer, twice a week, and applied a placebo cream.

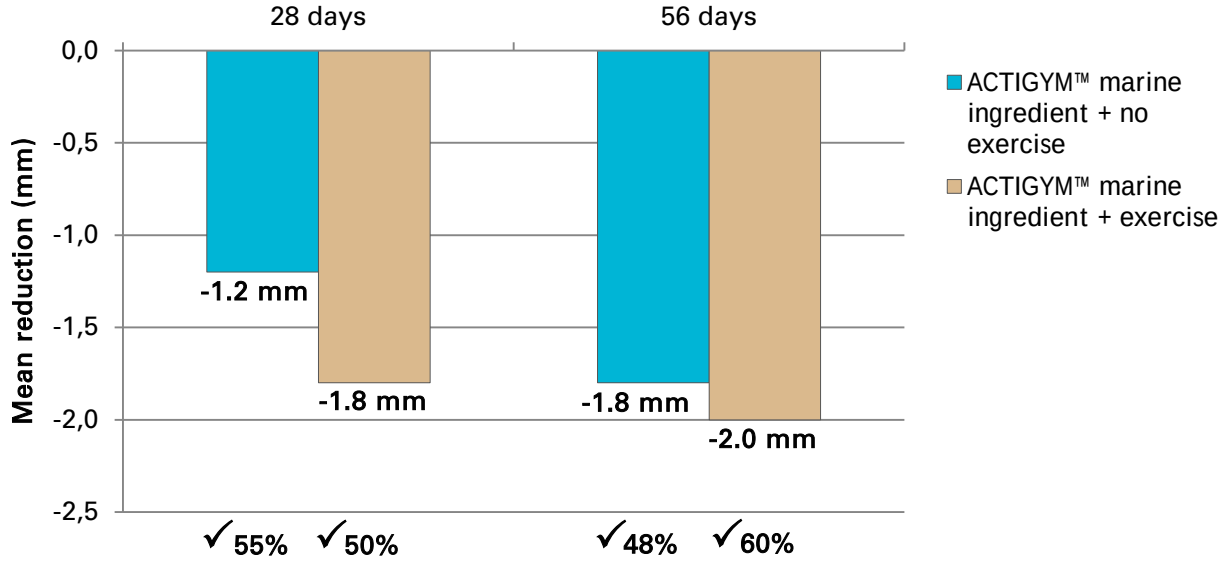
  2nd - applied a cream containing 5% ACTIGYM™ *marine ingredient* twice a day, with no physical activity.

  3rd - complied with the same training as the 1st group and used a cream containing 5% ACTIGYM™ *marine ingredient*, twice a day.

- To evaluate the improvement of body tone, several parameters were analyzed before and after 28 and 56 days, and a self-evaluated questionnaire was completed by the volunteers at the end of the study.

RESCULPTING BODY SHAPE (II)

- **SKIN FOLD**
  - Measurement of suprailiac skin fold on abdomen with a caliper, which is an indirect estimation of subcutaneous adipose tissue.



✓Reactive subjects: % of volunteers reducing a minimum of -0.6 mm.

ACTIGYM™ *marine ingredient* promoted a reduction of the 19.2% in abdomen skin fold after 56 days.

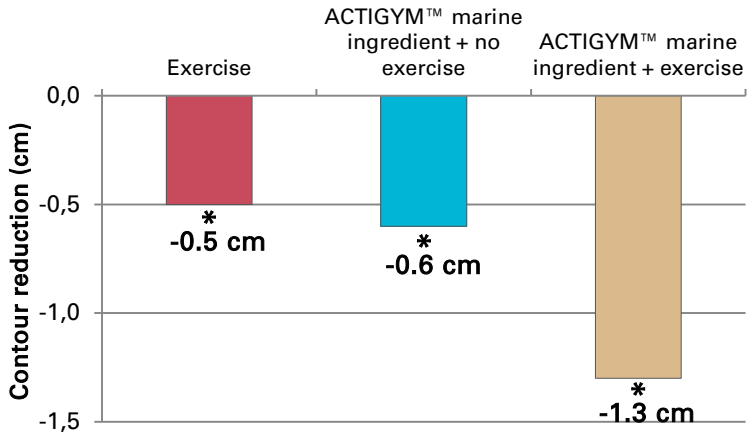
actigym™ marine ingredient

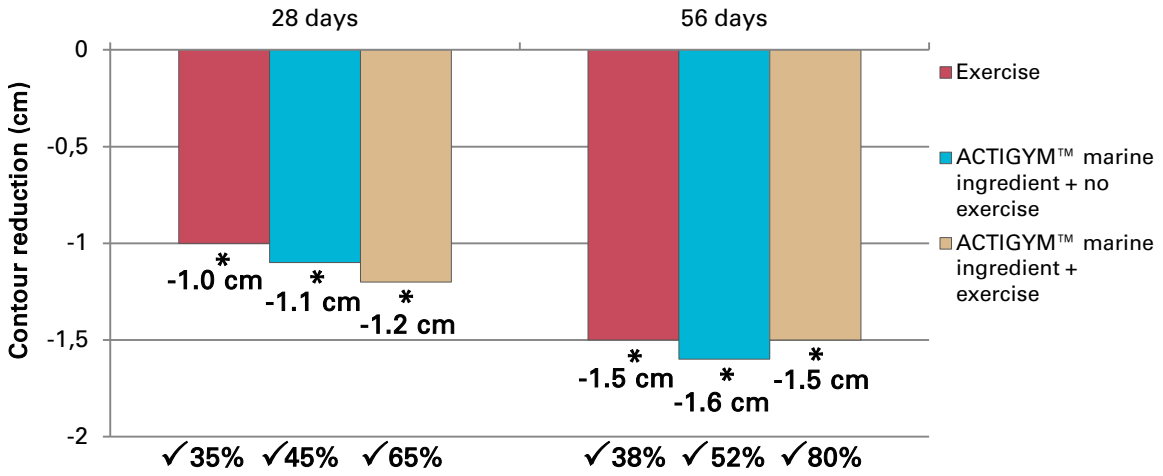
Lipotec www.lipotec.com

RESCULPTING BODY SHAPE (III)

CENTIMETRIC MEASUREMENTS (I)

- Abdomen contour.





All volunteers after 56 days (*p<0.05).

✓ Reactive subjects: % of volunteers reducing a minimum of -0.5 cm, after 28 and 56 days (*p<0.05).

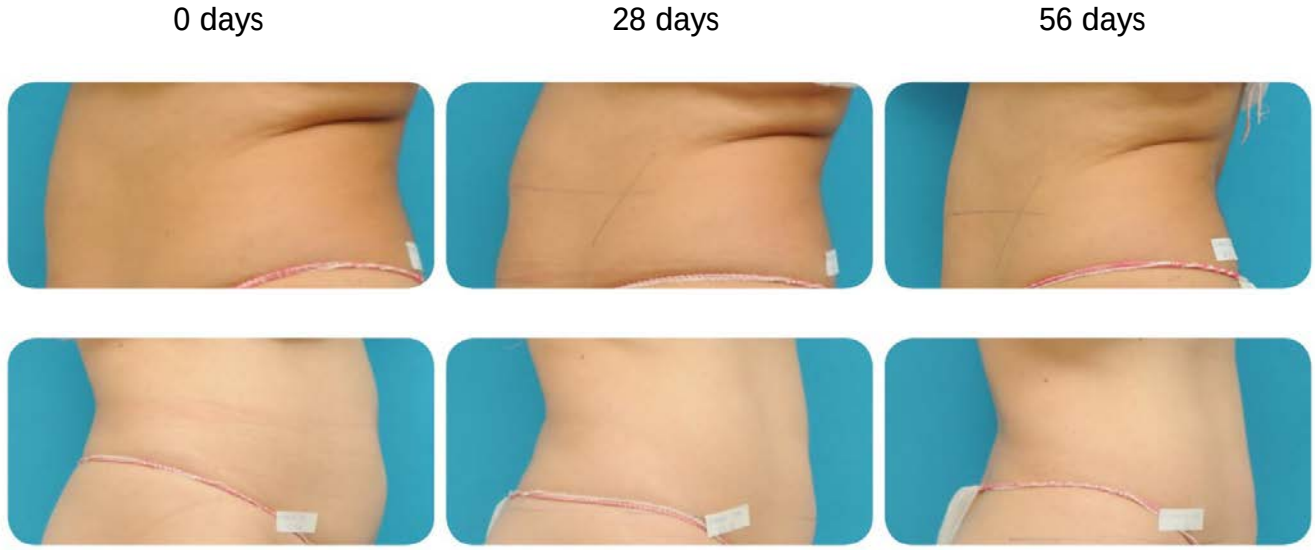
After 28 days, abdomen contour decreased up to -2.8 cm with ACTIGYM™ *marine ingredient*, and up to -3.1 cm when combined with exercise.

actigym™ marine ingredient

Lipotec www.lipotec.com

RESCULPTING BODY SHAPE (IV)

- Macroscopic photographs of abdomen.



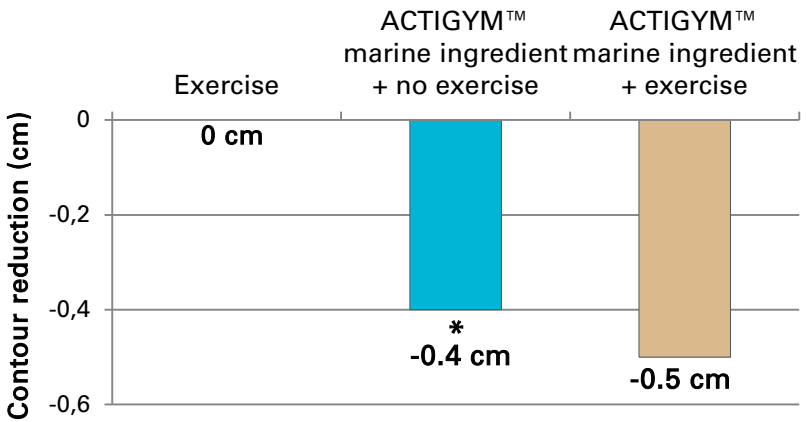
Visibly reduced abdomen contour with impressive results when combined with exercise.

actigym™ marine ingredient

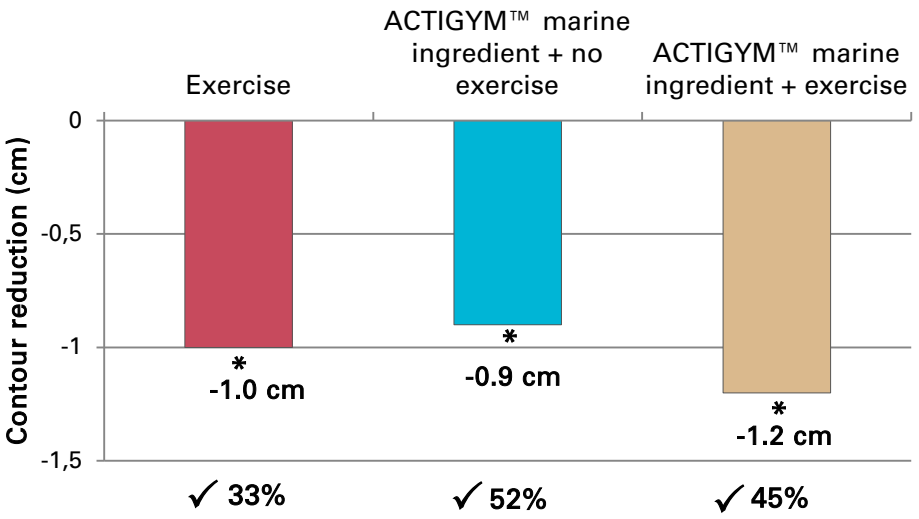
Lipotec www.lipotec.com

RESCULPTING BODY SHAPE (V)

- **CENTIMETRIC MEASUREMENTS (II)**
  - Thigh contour.



All volunteers after 56 days (*p<0.05).



✓ Reactive subjects: % of volunteers reducing at least -0.3 cm, after 56 days (*p<0.05).

ACTIGYM™ *marine ingredient* reduced thigh girth up to -2.1 cm and up to -2.9 cm when combined with physical training.

actigym™ marine ingredient

Lipotec www.lipotec.com

RESCULPTING BODY SHAPE (VI)

- Macroscopic photographs of thighs.

	0 days	56 days
ACTIGYM™ *marine ingredient* + no exercise		
ACTIGYM™ *marine ingredient* + exercise		

ACTIGYM™ *marine ingredient* reduced thighs perimeter, smoothing contours.

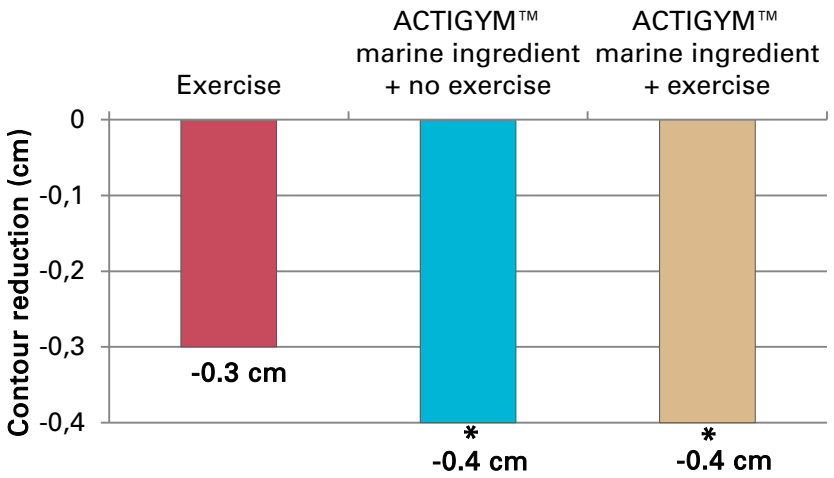
actigym™ marine ingredient

Lipotec www.lipotec.com

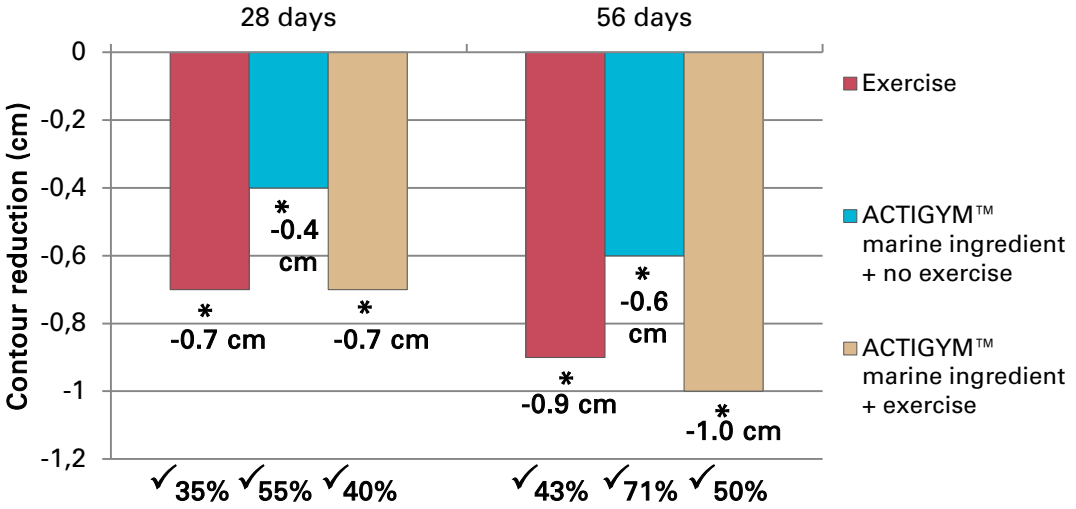
RESCULPTING BODY SHAPE (VII)

CENTIMETRIC MEASUREMENTS (III)

- Arm contour.



All volunteers after 56 days (*p<0.05).



✓ Reactive subjects: % of volunteers reducing at least -0.2 cm, after 28 and 56 days (*p<0.05).

ACTIGYM™ *marine ingredient* reduced arm contour up to -1.3 cm and up to -2.4 cm when combined with physical activity.

actigym™ marine ingredient

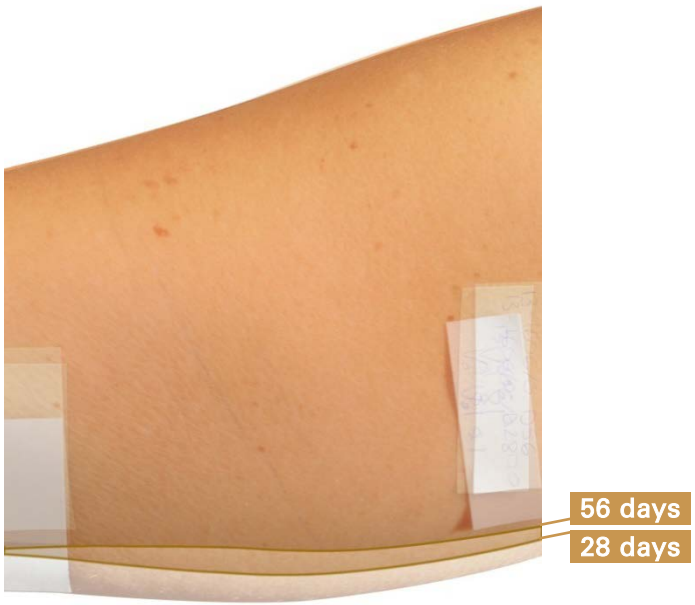
Lipotec www.lipotec.com

RESCULPTING BODY SHAPE (VIII)

- Superimposed macroscopic photographs of arms.

ACTIGYM™ *marine ingredient*
+ no exercise

ACTIGYM™ *marine ingredient*
+ exercise



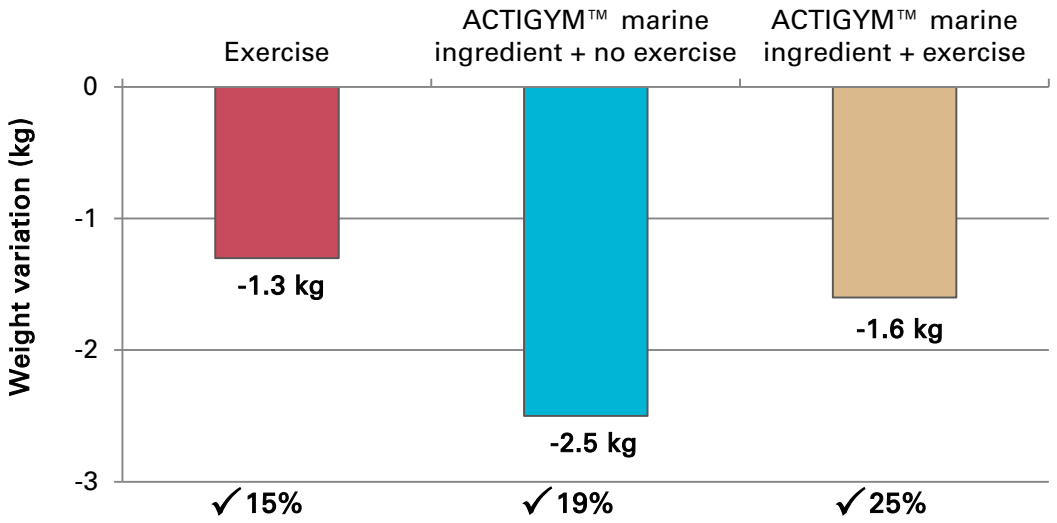
ACTIGYM™ *marine ingredient* visibly decreased arm sagginess, even being more noticeable when combined with exercise.

actigym™ marine ingredient

Lipotec www.lipotec.com

RESCULPTING BODY SHAPE (IX)

- **BODY WEIGHT**



✓ Reactive subjects: % of volunteers reducing at least -1.0 kg, after 56 days.

Body weight decreased up to -3.1 kg with ACTIGYM™ *marine ingredient* and up to -2.2 kg when combined with physical activity, after 28 days.

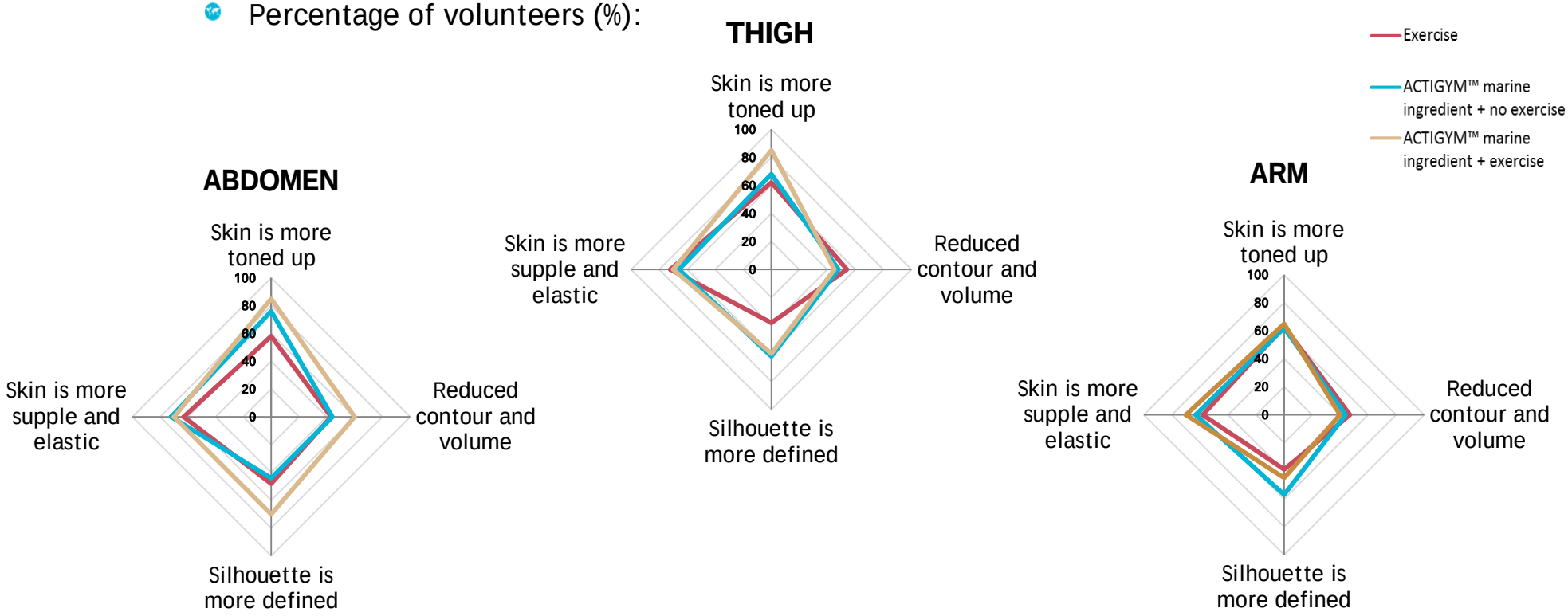
After 56 days, maximal reductions were -4.8 kg and -3.4 kg respectively.

actigym™ marine ingredient

Lipotec www.lipotec.com

RESCULPTING BODY SHAPE (X)

- **SELF-ASSESSMENT OF THE COSMETIC EFFICACY**
  - Scoring equal or higher than 6 was considered for calculations (in a scale 0-10).
  - Percentage of volunteers (%):



Self-evaluation revealed overall satisfaction with the ingredient, and perception of increased tonification and silhouette definition.

actigym™ marine ingredient

Lipotec www.lipotec.com

CONCLUSIONS

actigym™
marine ingredient

- active biotechnological ingredient from Bermuda, that **mimics the effect of endurance training in releasing adiponectin** by subcutaneous adipocytes (68.3%).
- adiponectin signaling boosted **mitochondrial metabolism** in muscle cells, increasing citrate synthase activity by 47.9% and ATP production by 136.0%.
- Promoted the development of the more resistant **type I muscle fibers** (69.8% increase).
- **downregulated** expression of adipocyte genes involved in **FA uptake and TG synthesis**.
- applied at 5% in volunteers, decreased **subcutaneous skin fold,** reduced **contours** of abdomen (up to -2.8 cm), thighs (up to -2.1 cm) and arms (up to -1.3 cm) and **body weight** (up to -4.8 kg).
- provided a general improvement in the **appearance and tone of body silhouette**.

TECHNICAL INFORMATION

DESCRIPTION

Active ingredient obtained by biotechnology from a microorganism inhabiting Bermuda. ACTIGYM™ *marine ingredient* mimics the effect of endurance exercise training by increasing adiponectin release and enhancing mitochondrial activity. It improves body definition that can be further complemented with exercise.

APPEARANCE

Transparent solution containing 0.07% active ingredient.

INCI

Glycerin, Water (Aqua), Plankton Extract.
Preservative free.

PROPERTIES

ACTIGYM™ *marine ingredient* improves body tone and redefines the silhouette by reducing abdomen and thigh contour, arm sagginess, and decreasing body weight. It mimics the effect of endurance training with great results that are increased when combined with physical activity.

APPLICATIONS

ACTIGYM™ *marine ingredient* can be incorporated into daily cosmetic formulations for body care to provide a more toned and redefined silhouette.

DOSAGE 5%

pH Recommended pH range between 3.5 and 8.0.

actigym™ marine ingredient

Lipotec www.lipotec.com

actigym™
marine ingredient

A SECRET PERSONAL TRAINER