



Mobilee[®]: Hyaluronic acid matrix ingredient A scientific summary

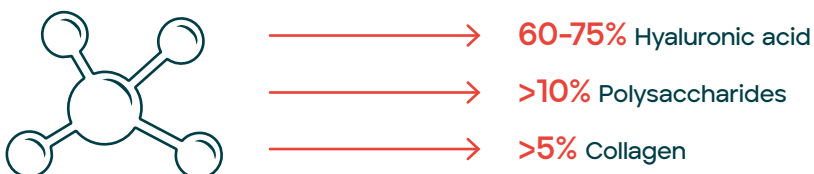
Abstract

The conversation around mobility is changing. A growing number of consumers – of all ages – are taking a more holistic and proactive approach to their health; looking to remain physically active as a way to boost their overall quality of life. At the same time, they want convenient solutions that are both suitable for a busy lifestyle and target multiple aspects of mobility.

Introducing Mobilee® – a hyaluronic acid matrix ingredient that supports joint *and* muscle health. Explore the benefits and science behind Mobilee® and make your next move in the mobility market.

Do more with Mobilee®

Mobilee® is a patented science-based ingredient for joint and muscle health, authorised for innovation in dietary supplements and foods and beverages. It contains:



Mobility: The opportunity

Hyaluronic acid (HA) is naturally found in many tissues and fluids in the body – but most abundantly in the articular cartilage and synovial fluid found in the joints.¹ HA is partially responsible for lubrication and viscoelasticity of the synovial fluid, facilitating joint mobility. But the quantity and quality of HA in the synovial fluid reduces as we age; leading to pain and stiffness, and consequently affecting mobility. This is usually attributed to age-related conditions, like osteoarthritis. However, younger populations may experience joint disorders too, due to injury or overuse of their joints.

As individuals age, muscle mass is also naturally lost over time. This process begins in our 40s and eventually affects an individual's physical ability and quality of life.

47% of people globally say they experience joint discomfort.²

From reactive to proactive

There has been a notable shift from a reactive to preventive mindset, and more people than ever are taking a proactive approach to their health and wellbeing. As such, there is increasing demand for solutions that meet longer-term, sustainable health goals. This is true when it comes to mobility too, with consumers progressively thinking less 'joint health' and more 'overall mobility'.

There is therefore growing opportunity to appeal to these consumers by developing products that support all mobility-related aspects, including bone, joint, tendon and muscle health. This can be achieved by bringing together multiple ingredients that offer different benefits.

Target market



Younger adults

Embracing a healthy lifestyle



Active individuals

Addressing joint discomfort, supporting recovery and maintaining an active lifestyle



Senior adults

Addressing mobility issues and supporting independence

The power of 3

Mobilee®'s unique synergistic effect

Because of its natural role in joint and muscle health, many individuals – especially those with joint disorders – seek support in the form of HA supplementation. Globally, **10%** of consumers who have heard of HA look for it in products to help **boost their joint health and mobility.**³

HA supplementation helps to support joints and muscles through the following actions:

1. Boosts endogenous production of HA (the HA produced naturally in the body) by synoviocytes⁴, increasing lubrication in the joints
2. Enhances muscle cell proliferation⁵, supporting muscle strength
3. Plays an important role in regulating the body's inflammatory response⁶

Fermented HA vs Mobilee®

HA can be commercially manufactured in two ways, the most common method being bacterial fermentation. HA can also be obtained from animal tissues with a high concentration of HA, like rooster comb. Mobilee® – Bioiberica’s hyaluronic acid matrix ingredient derived from rooster comb extract – contains HA, as well as collagen and polysaccharides.

The combination of these three naturally-occurring components in a single ingredient elicits a synergistic effect, leading to an efficacy superior to the sum of its parts.⁴ For example, Mobilee® stimulates the synthesis of endogenous HA by **200-fold** in synoviocytes – much higher amounts than produced by fermented HA at the same tested dose.⁴ Mobilee®’s recommended daily dose is only **80 mg/day** too, lower than the dose needed to support joint health when using fermented HA. This enables manufacturers to meet consumer preferences for more convenient products. Plus, as Mobilee® can be combined with other ingredients, formulators have the option to develop solutions that support overall mobility – not just joint and muscle health.

**Increases endogenous production
of hyaluronic acid 200-fold**

Efficacious at 80 mg/day

Meets demand for convenient solutions

Mobility benefits

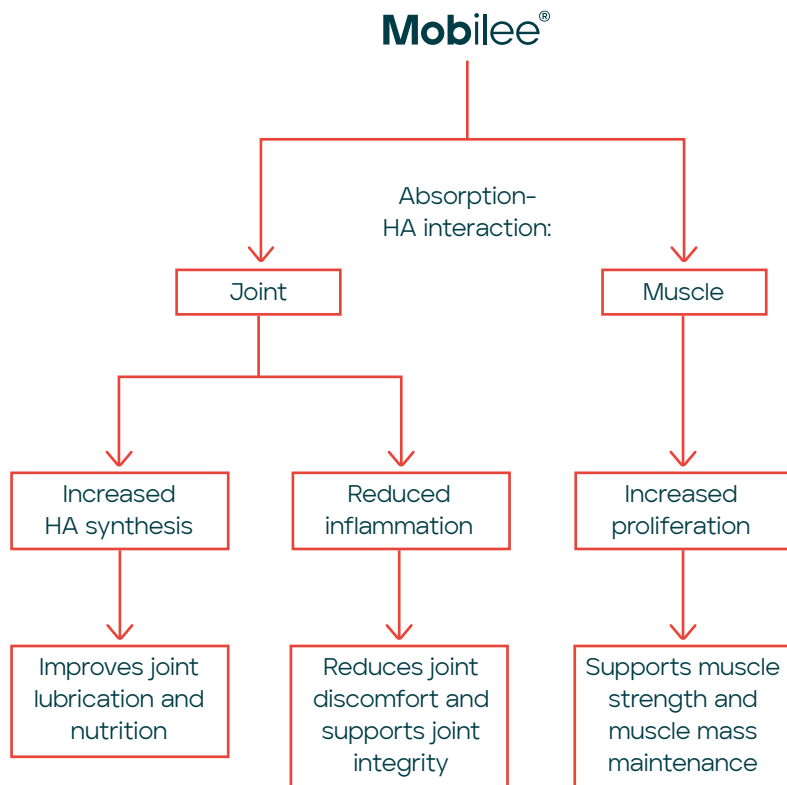


Figure 1: Through its actions, Mobilee® has benefits at the joint and muscle levels

Data suggests that daily Mobilee® supplementation for three months supports overall mobility in the following ways:

Reduces joint discomfort⁷ and inflammation⁸

Increases muscle strength (up to +17%)⁹

Lowers synovial effusion⁷

The science behind **Mobilee®**

Mobilee® is the result of **15+ years**
of development and research.

1. Clinical study: Meta-analysis of clinical trials in healthy individuals with mild knee pain¹⁰

Objective

Evaluate the efficacy of daily consumption of a low-fat dairy product supplemented with Mobilee® (80 mg/day) in healthy volunteers with knee discomfort across a three-month period.

Methods

Pooled analysis of the individualised results from 148 volunteers included in two randomised, controlled, double-blind, parallel trials performed in healthy patients with mild knee pain (VAS 30–50 mm).

The primary outcome was muscle function determined by peak torque, total work and mean power using an isokinetic dynamometer Biodex System 4. Secondary parameters were the ultrasonographic evolution of the affected joint using an osteoarthritis risk parameter scale, and the level of joint discomfort assessed using VAS scale.

Results

Individuals taking the supplemented yoghurt showed greater improvements, with the difference between groups being statistically significant for the total work of the affected joint measured in flexion at 180°/s ($p=0.039$).

The ultrasonographic evaluation showed a significantly greater reduction in the synovial effusion in the volunteers supplemented with Mobilee® in comparison to the non-supplemented group ($p=0.038$).

The pain perceived by the volunteers who consumed the supplemented yoghurt decreased throughout the trial, reaching a 24.6% reduction ($p=0.007$) compared to the control group at the end of the study.

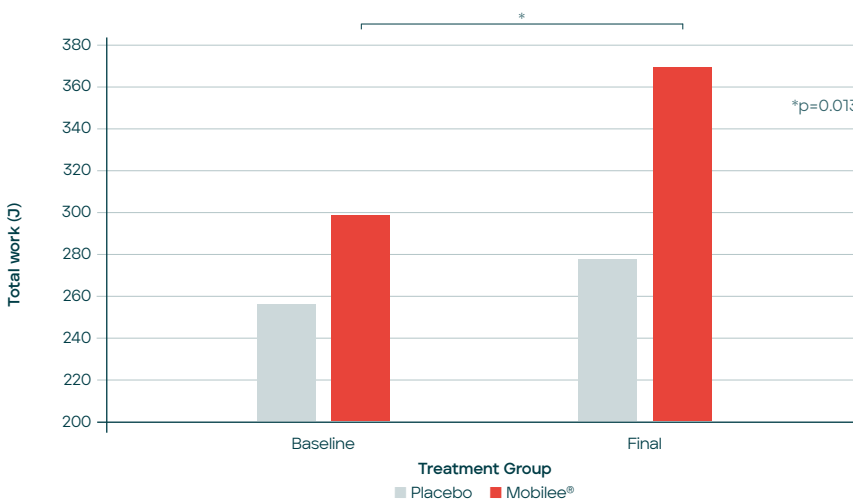


Figure 1: Total work of the affected knee in flexion in males

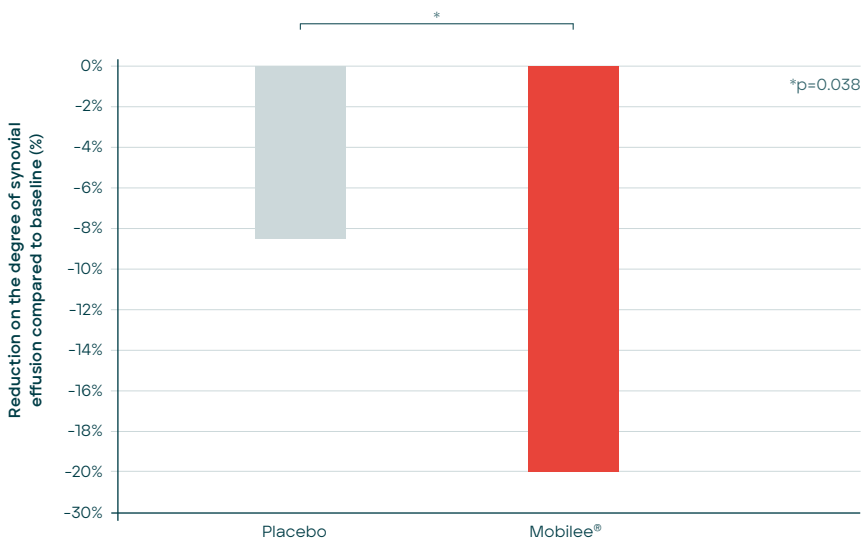


Figure 2: Relative change in synovial effusion after three months compared to baseline values

Conclusion:

Long-term consumption of a yoghurt supplemented with Mobilee® improves muscle strength, synovial effusion and reduces pain, providing clinical benefits in healthy people with mild knee pain.

2. Clinical study: Randomised double-blind, placebo-controlled study exploring efficacy of Mobilee® in joint pain relief and muscle strength¹¹

Objective

Determine the efficacy of a yoghurt supplemented with Mobilee® in healthy individuals with mild joint discomfort.

Methods

80 participants with mild knee pain (VAS 30–50 mm) were included in the trial. 40 participants received a low-fat dairy product with 80 mg of Mobilee® daily for a total of three months, and 40 participants consumed the same low-fat dairy product without Mobilee®.

Knee muscle function was determined by peak torque, total work and mean power in flexion, plus extension at angular velocities of 180°/s and 240°/s using an isokinetic dynamometer Biodex System 4. Synovial liquid volume was measured by an echography, and pain evolution was assessed by VAS.

Solá R et al. A low-fat yoghurt supplemented with a rooster comb extract on muscle joint function in adults with mild knee pain: a randomised, double-blind, parallel, placebo-controlled clinical trial of efficacy. *Food Funct.*, 2015, vol. 6, pg. 3531–3539.

Results

Results showed that participants supplemented with Mobilee® had a general tendency to greater improvement.

In a sub-analysis, men supplemented with Mobilee® demonstrated significantly increased muscle strength in the affected knee joint (in flexion and extension), improving the mainly isokinetic variables measured at 180°/s and also at 240°/s compared to placebo.

In the Mobilee® group, the percentage change in all isokinetic parameters was more than 19% from baseline and 11% versus the placebo. Differences over 10% are considered functionally significant, so these data demonstrate clinical importance.

At three months, synovial effusion was reduced in the supplemented group, whereas it increased in the placebo group.

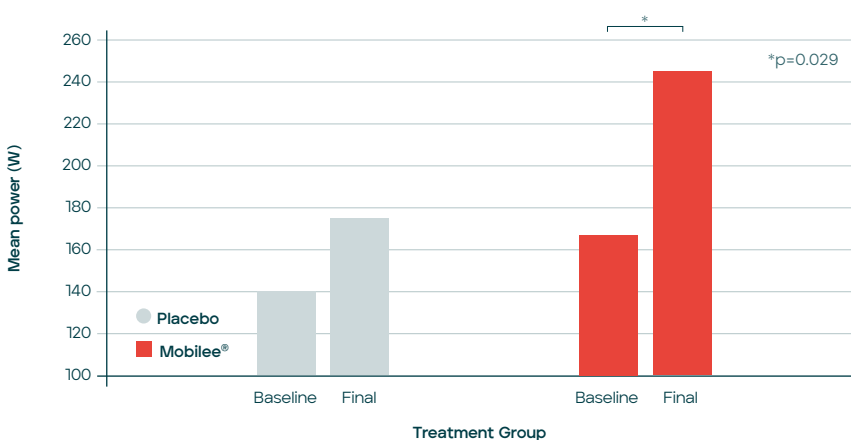


Figure 3: Mean power in knee extension in males at 180°

Conclusion:

Long-term consumption of a low-fat dairy product supplemented with Mobilee® improved muscle function in men and may reduce synovial effusion in individuals with mild knee pain.

3. Clinical study: Randomised double-blind, placebo-controlled trial examining effect of Mobilee® on joint pain, muscle strength, effusion and gene expression⁷

Objective

Determine the efficacy of a yoghurt supplemented with Mobilee® in healthy individuals with mild joint discomfort.

Methods

This randomised, double-blind, placebo-controlled nutritional intervention included 77 participants with mild knee pain (VAS 30-50 mm) who were randomised into two groups. The study group consumed one yoghurt per day for a total of three months supplemented with 80 mg of Mobilee®. The control group ate the same yoghurt without any supplement.

Clinical assessment included isokinetic test of thigh muscles, ultrasonographic evaluation of the knee and pain assessed using the VAS scale. Whole-genome microarray analysis of blood samples from a subset of 20 subjects was collected pre- and post-intervention and assessed to explore the feasibility of using total human blood RNA as a source of biomarkers of articular health improvement.

Results

Daily supplementation with Mobilee® reduced pain intensity, reaching significantly lower values compared to the placebo from the second month of treatment (32.5 ± 4.96 vs 34.0 ± 3.85 mm respectively; $P=0.005$), and especially at the third month (21.1 ± 12.36 vs 31.9 ± 15.81 mm; $P=0.0005$).

The ultrasonographic assessment revealed a significant reduction on the degree of synovial effusion associated with Mobilee® versus placebo (44% vs 22%; $P<0.05$).

The sub-analysis of the muscular strength evolution, excluding those participants with a pathologic degree of synovial fluid at baseline, showed a reduction in muscular strength on the placebo group after three months of study (-2.3 ± 2.71 Nm), while in the Mobilee® group it was significantly increased ($+2.9 \pm 1.67$ Nm; $P<0.05$).

Transcriptomic analysis revealed that 157 known genes were differentially expressed in blood cells between Mobilee® and placebo groups post-intervention, but not pre-intervention ($P<0.05$; fold-change ≥ 1.2). Some of them are related to GAG metabolism and extracellular matrix dynamics. In particular, lower expression of cartilage degrading enzymes as glucuronidase-beta and matrix metalloproteinase 23B were found in the Mobilee® group.

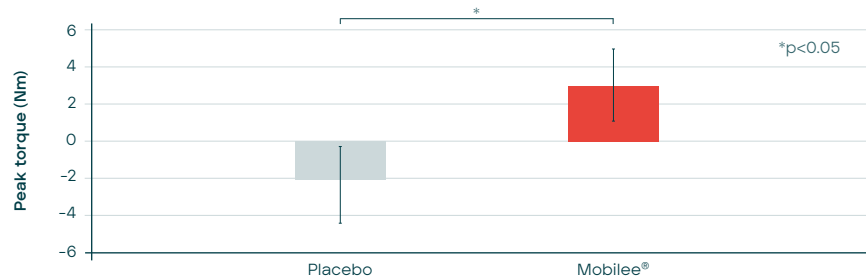


Figure 4: Change in muscular strength (peak torque) in extension at 240°/s in participants without synovial effusion at baseline

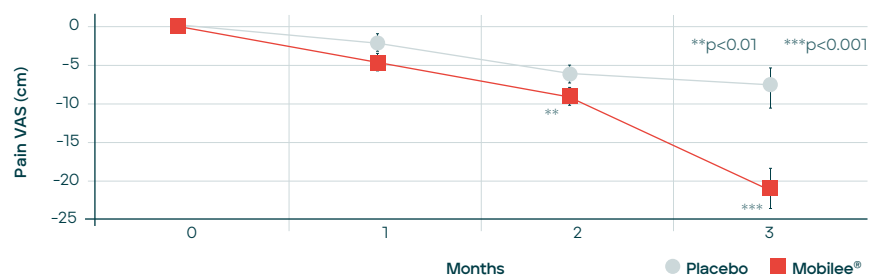


Figure 5: Time evolution of pain intensity according to VAS (mm)

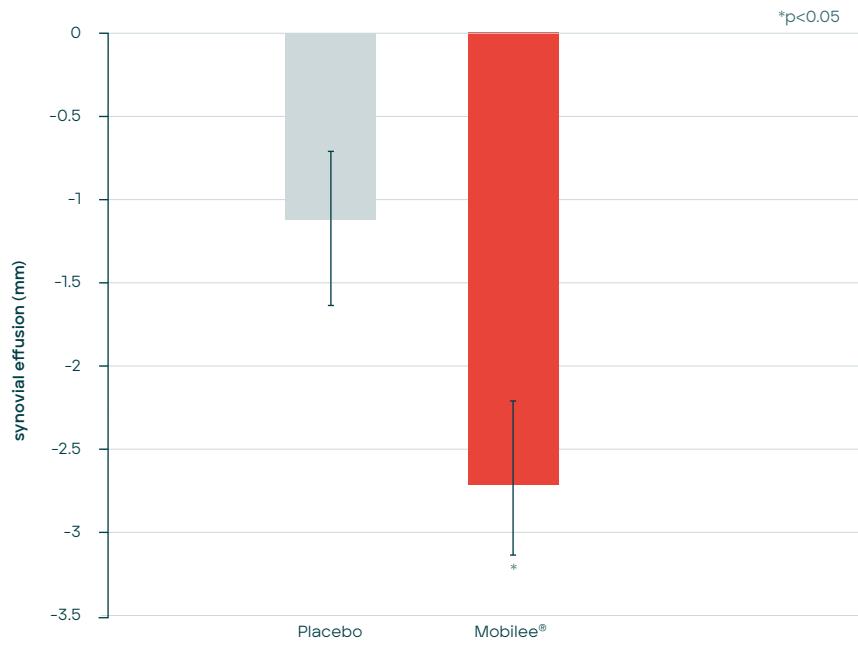


Figure 6: Degree of synovial effusion after three months compared to basal values.

Conclusion:

Three months of Mobilee® supplementation in healthy individuals with knee discomfort reduced pain and joint effusion, and provided improvements in muscle strength in individuals without joint effusion. The expression of some genes after Mobilee® supplementation correlate with indicators of articular pain and muscular strength.

4. Clinical study: Prospective, randomised double-blind, placebo-controlled study investigating the benefits of Mobilee® on muscle strength and quality of life⁹

Objective

Determine the efficacy of a yoghurt supplemented with Mobilee® in healthy individuals with mild joint discomfort.

Methods

The study included 40 healthy individuals with mild joint discomfort (VAS <4). The subjects were divided into two groups (n=20). One group was offered the yoghurt supplemented with Mobilee® daily for a period of 90 days and one group ate the same yoghurt without Mobilee®. Efficacy was evaluated by assessing functional and quality of life parameters. An isokinetic dynamometer was used to measure maximum muscle strength, total work and mean power of knee flexors and extensors at two different angular velocities.

Results

The increase in maximum muscle strength of the knee extensors was 7.6 ± 7.6 Nm compared to baseline values for the supplemented group, and 2.5 ± 4.7 Nm for the control group at $180^\circ/\text{s}$ ($P=0.0582$). At $240^\circ/\text{s}$, the values were 6.5 ± 5.8 Nm in the supplemented group and -1.0 ± 7.1 Nm in the control group ($P<0.05$). The same pattern of response was observed in total work and mean power ($p<0.05$), however, differences were less pronounced in the knee flexors.

There was also a significant difference in the quality of life social functioning subscale of the supplemented group at the one month follow up.

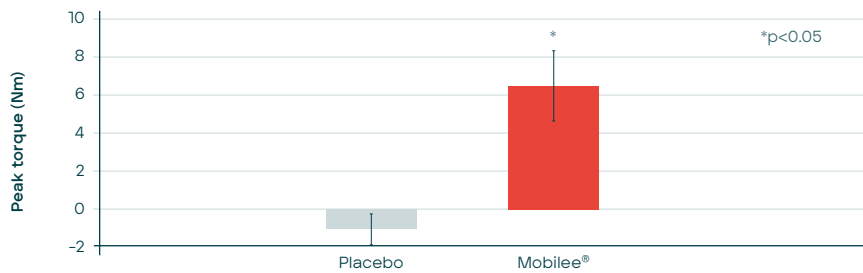


Figure 7: Increase in maximum peak torque compared to baseline values (Nm) for knee extension at $240^\circ/\text{s}$

Conclusion:

The results indicate that oral Mobilee® supplementation improves joint mechanics and muscle function, as determined via isokinetic testing, in healthy individuals with joint discomfort.

5. In vivo: Effect of Mobilee® in dogs with cranial ligament injury¹²

Objective

Evaluate the effects of Mobilee® on synovial fluid HA concentrations, plus several selected biomarkers, in dogs with cranial cruciate ligament (CCL) injury.

Methods

This prospective, randomised, double-blind clinical study included 55 dogs, which were randomly assigned to receive either a placebo (group A; n=25) or oral Mobilee® supplementation (group B; n=30) for 10 weeks post-operation.

Dogs with CCL injury and operated on using the tibial tuberosity advancement (TTA) technique were included. Clinical examination, and radiographic and ultrasound evaluations, were performed before surgery, and on week two, four and ten postoperatively. Synovial fluid samples were obtained before surgery and ten weeks postoperatively to measure HA concentration, and haptoglobin (HAP), nitric oxide (NO) and paraoxonase-1 (PON-1) levels.

Results

Both groups showed a significant decrease in lameness and pain values over time. Synovial fluid concentrations of HA, HAP, NO and PON-1 showed no significant differences between the two groups at baseline.

After ten weeks of treatment, the Mobilee® group showed a significant increase in HA concentration compared to baseline ($p=0.0016$), while HA decreased in the placebo group over time. Similarly, a significant decrease of PON-1 concentrations was found in Mobilee® supplemented dogs ($p=0.011$), unlike the control group ($p=0.055$).

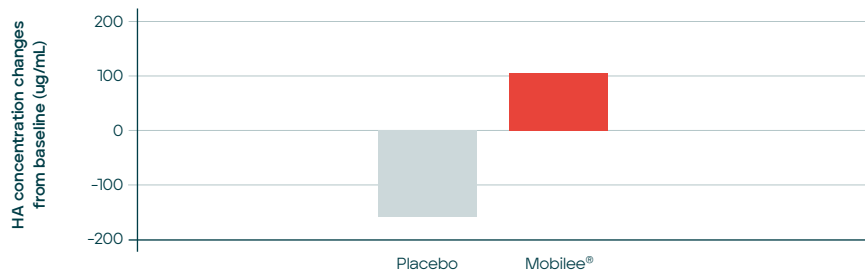


Figure 8: HA concentration in synovial fluid vs baseline

Conclusion:

Postoperative oral administration of Mobilee® in dogs with OA secondary to CCL injury leads to improvements in OA biomarkers, specifically HA and PON-1 concentrations.

6. In vivo: Effect of Mobilee® in rats developing type II collagen arthritis¹³

Objective

Determine the effect of Mobilee® – administered orally twice daily – on inflammation, cartilage destruction and bone resorption in rats with developing type II collagen arthritis.

Methods

A total of 24 female rats, weighing 136-154 g, were used in this research. Arthritis was induced with subcutaneous collagen injections (300 µL) on days zero and six (n=20). Non-immunised rats (n=4) were used as controls. Rats with developing type II collagen arthritis (n=20) were given Mobilee® orally, twice daily on days 0-16 (7.5 mg, BID; n=10) or a placebo (H₂O, disease control; n=10). Animals were terminated on day 17 of the study.

Livers, spleen and thymus were collected, trimmed of extraneous tissue and weighed. Efficacy evaluation was based on animal body weight, ankle diameter expressed as area under the curve (AUC), and there was histopathologic evaluation of ankles and knees.

Results

Body weight gain was significantly increased toward normal in the Mobilee® group (24% increase) compared to disease controls. Ankle diameter AUC was not significantly reduced (14%; $P=0.15$). Relative liver, thymus and spleen weights in the Mobilee® group were similar to the normal controls, confirming the solution's safety. Summed histopathology scores were significantly reduced in ankle (17%, $P<0.05$) and strongly reduced in knee (51%, $P<0.01$) toward normal for the Mobilee® group compared to disease controls.

Collagen-induced arthritis led to a significant increase in knee synovial fluid volume in both vehicle and Mobilee® treated rats, being less pronounced in the latter. HA concentration decreased 33% in the disease controls, but maintained similar to normal values in the Mobilee® group.

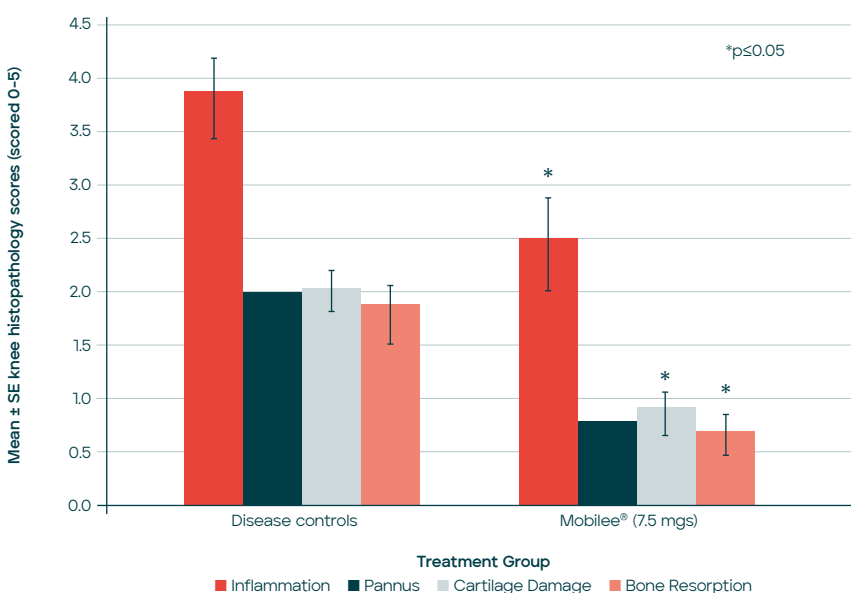


Figure 9: Individual knee histopathology scores of rats treated with Mobilee® compared to disease control

Conclusion:

The findings suggest that Mobilee® supplementation for the management of developing type II collagen arthritis is safe and effective, with beneficial effects on the histopathology parameters in ankles and knees.

7. **In vitro:** Effects on muscle health¹⁴

Objective

Evaluate the effect of Mobilee® on muscle health, using *in vitro* models of health and sarcopenia.

Methods

80 mg of Mobilee® was digested in an *in vitro* simulated gastrointestinal digestion model. L6-myocytes were then treated with 6 µg/mL Mobilee® for 24 hours. Physiological conditions – such as cell proliferation, myocyte size and cell regeneration – were measured before and after the treatment. Sarcopenic conditions (induced by exposure to 15 ng/mL TNFα) were also observed, including myogenic differentiation, muscular atrophy (waste), muscle damage and apoptosis.

Results

In myocyte cells, digested Mobilee® significantly promoted myocyte proliferation (27%), increased myocyte size (with a 6.6% rise in area and 4.9% growth in perimeter) and promoted myocyte hyperplasia (7% increase). The ingredient did not support myocyte regeneration. No changes from healthy baseline were observed for myogenic differentiation, atrophy or damage. In the sarcopenia model, promising effects were detected. Here, Mobilee® significantly increased myogenic differentiation (Foxo1 gene expression upregulated 80%). It also showed an effect against muscle atrophy (decreased expression of Murf1 by 40%) and muscle damage (reduced activity of creatinine kinase activity by 21%). Additionally, Mobilee® exhibited anti-apoptotic properties, highlighted by a significant decrease in LDH release (22%) compared to basal conditions.

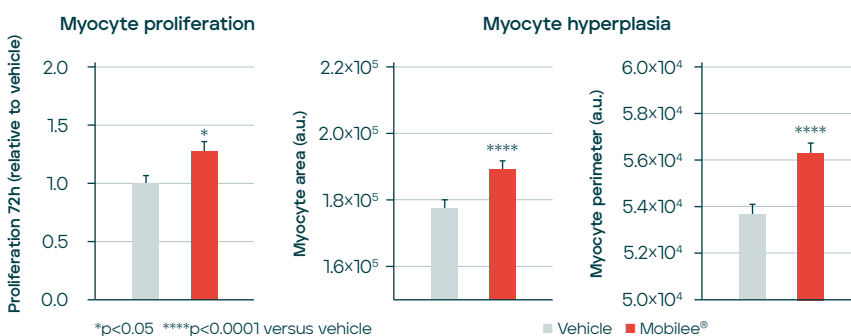


Figure 10: Effect of Mobilee® on myocyte proliferation and hyperplasia.

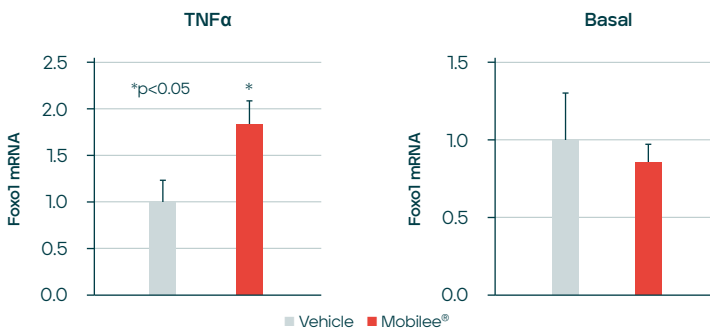


Figure 11: Myogenic differentiation in a sarcopenic model following Mobilee® treatment.

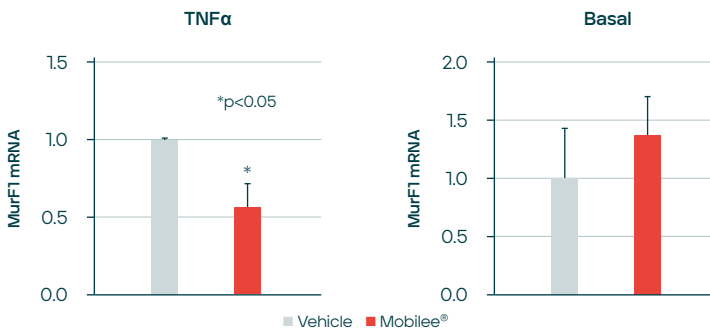


Figure 12: Effect of Mobilee® on muscle atrophy in sarcopenic model.

Conclusion:

Mobilee® is effective for balancing muscle cell turnover and has an effect against myocyte apoptosis, suggesting it may therefore aid sarcopenia management.

8. **In vitro:** Effect of Mobilee® and whey protein on muscle regeneration¹⁵

Objective

Explore the effect of a combination including Mobilee® and whey protein on muscle regeneration using *in vitro* models of myocytes.

Methods

80 mg of Mobilee® and 15 g whey protein isolate (Lacprodan® DI-9213; Arla) were digested in an *in vitro* simulated gastrointestinal digestion model. L6-myocytes were then treated with the formulation (6 µg/mL Mobilee® plus 19 µg/mL whey protein) for 24 hours. The effect of the combination on physiological conditions – like myocyte size and cell regeneration – was evaluated. Sarcopenic characteristics – such as myogenesis and muscle damage – were also investigated in a sarcopenic model (induced by exposure to 15 ng/mL TNFα).

Results

A significant increase was observed in the area (6%) and perimeter (4%) of myocytes after treatment with Mobilee® and whey protein, confirming myocyte hyperplasia. The formulation also reduced wound size (by 47.7%), due to the promotion of myocyte regeneration. In the sarcopenic model, a significant decrease (250%) in the expression of MyoD (a gene that regulates muscle cell differentiation and is involved in muscle regeneration) was observed in myocytes treated with the formulation. This confirms that the combination of Mobilee® and whey protein had a positive effect on myogenesis (muscle regeneration) under sarcopenic conditions. No significant changes in muscle damage were revealed.

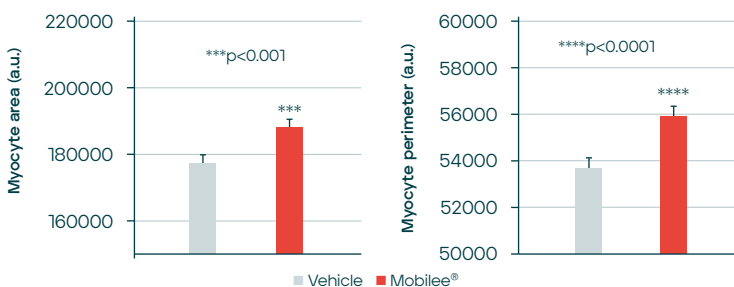


Figure 13: Effect of Mobilee® and whey protein combination on myocyte hyperplasia (myocyte size).

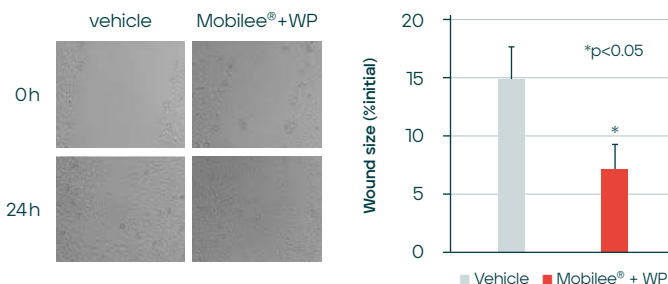


Figure 14: Impact of Mobilee® and whey protein on wound size due to myocyte regeneration promotion.

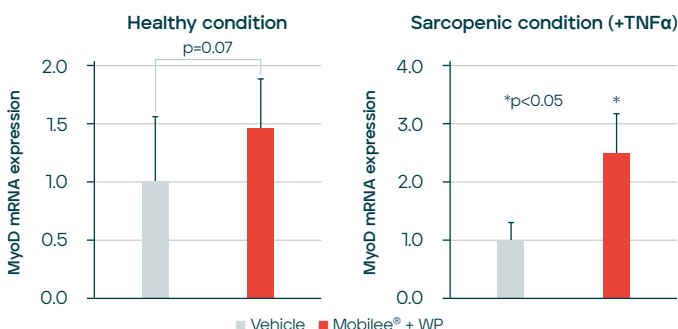


Figure 15: Effect of Mobilee® and whey protein formulation on myogenic differentiation.

Conclusion:

A combination including Mobilee® and whey protein is effective for balancing muscle cell turnover and injury recovery, and could therefore be beneficial in addressing muscle degeneration and supporting muscle health.

9. In vitro: Maintenance and treatment of muscle atrophy⁵

Objective

Investigate the therapeutic potential of Mobilee® in combatting muscle atrophy.

Methods

Mobilee®'s potential to stimulate proliferation of murine C2C12 muscle cells under two different conditions was evaluated – cells cultured with growth media (10% FBS) and cells cultured with low-serum media (2% FBS). Its effect on myoblast proliferation in the presence of the cytokine IL-6 was also tested. In another test, Mobilee®'s potential at preventing muscle atrophy (i.e. myotube thickness) during serum starvation conditions was evaluated.

Results

Mobilee® exerted little effect on cell proliferation in 10% FBS growth serum, but it significantly stimulated proliferation in low-serum media after 48 hours treatment (131% increase). The myoblast proliferation reduction produced by IL-6 was partially counteracted by the presence of Mobilee®. In addition, Mobilee® treatment was able to counteract myotube atrophy compared with the 0% control, leading to a 20% reduction in myotube width.

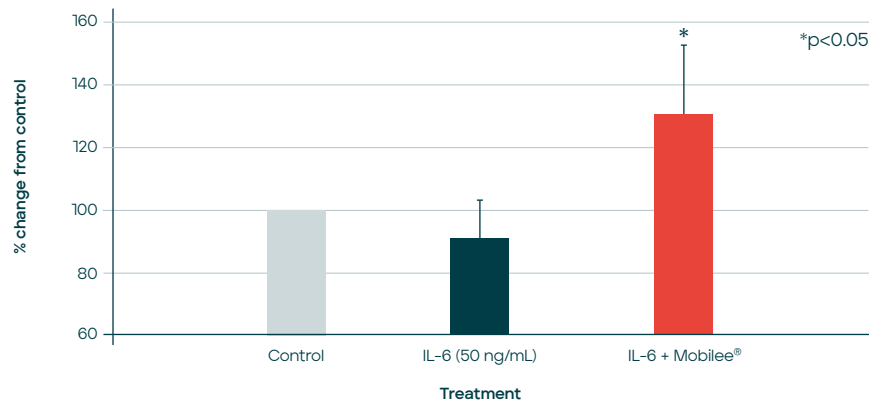


Figure 16: Effect of Mobilee® on myoblast proliferation under inflammatory conditions

Conclusion:

Mobilee® has potential anabolic effects that could promote myogenesis and anti-catabolic effects on muscle that could counteract atrophy under adverse conditions. Therefore, Mobilee® may help to prevent atrophy in muscle wasting conditions, such as disuse atrophy, sarcopenia, and other disorders.

10. **In vitro:** Anti-inflammatory effects of Mobilee®⁸

Objective

To study the effects of Mobilee® on the synthesis of cell-catabolism mediators, prostaglandin E2 (PGE2) and metalloproteinase 1 (MMP-1) in cases of inflammation (Interleukin-1 β , IL-1 β).

Methods

The effect on inflammation was determined by using human dermal fibroblasts stimulated with IL-1 β and co-treated with 3 concentrations of Mobilee® (5, 50 and 500 μ g/ml). Levels of Prostaglandin E2 (PGE2) and Metalloprotease-1 (MMP-1) were determined by EIA. The compound NS 398 at 1 μ M was used as a positive control of cyclooxygenase-2 (Cox-2) inhibition in this assay.

Results

The study showed that Mobilee® reduced inflammation by significantly lowering PGE2 levels in fibroblast cells cultured under conditions similar to those of inflammation. Furthermore, it showed a tendency to lower MMP-1 levels.

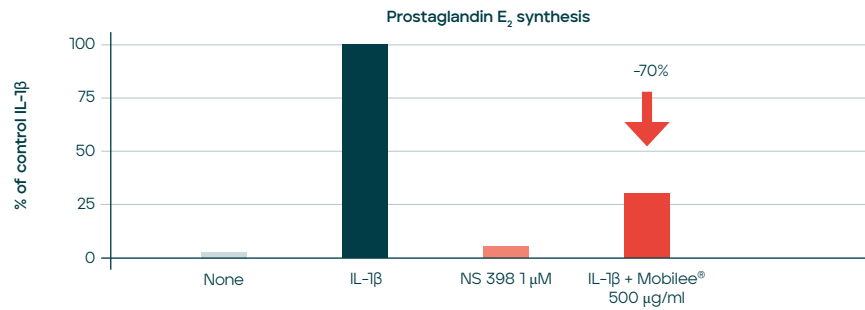


Figure 17: Effects of Mobilee® on PGE-2 synthesis induced by IL-1β in human dermal fibroblasts

Conclusion:

These results indicate that Mobilee® may have anti-inflammatory effects.

11. In vitro: Intestinal absorption of Mobilee®⁸

Objective

To determine the intestinal absorption of Mobilee® using an everted gut sac model.

Methods

Male OFA-strain rats weighing approximately 200 g were used. Three parts of the intestine were studied. The duodenum: first part of the small intestine, located between the stomach and the jejunum. Food is combined with stomach acids and then enters the duodenum, where it is mixed with bile and digestive juices from the pancreas. The jejunum: a portion of the intestine that extends from the duodenum to the ileum to form the small intestine (although there is no morphological line of distinction between the jejunum and the ileum). The ileum: the last part of the small intestine, located between the jejunum and the large intestine.

The amount of absorbed Mobilee® was analysed following the technique described by Farndale et al. (1982) for glycosaminoglycan determination. This technique is based on measuring the absorbance of a glycosaminoglycan and dye complex at 535 Nm.

Importantly, the absorption-assay model measured intestinal absorption under conditions that are more similar to human physiological conditions, and therefore closer to real conditions than *in vitro* cell models.

Results

Absolute absorption values were obtained for each portion of intestine. Mobilee® was absorbed in the intestine – mainly in the duodenum, where the highest percentage of absorption was observed. The jejunum and ileum also showed absorption activity.

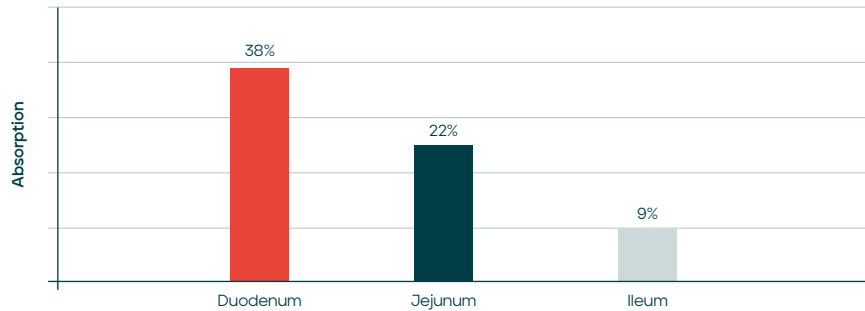


Figure 18: Absolute absorption values
NB: These absolute values are approximate and should not be understood to be exact due to the variability of the method.

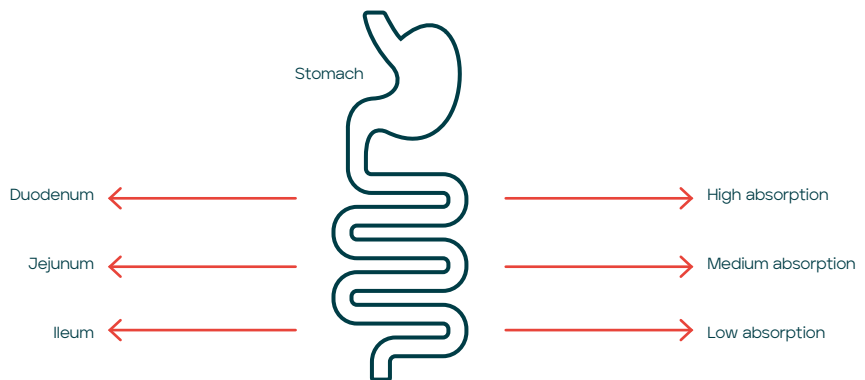


Figure 19: Levels of absorption throughout the intestine

Conclusion:

The study confirmed that Mobilee® is absorbed through the intestinal mucous membrane, with the highest levels of absorption occurring in the duodenum.

12. In vitro: Difference in endogenous HA synthesis via Mobilee® compared to fermented HA⁴

Objective

Determine the differences between Mobilee® and HA produced by bacterial fermentation in the stimulation of endogenous HA (eHA) synthesis by human synoviocytes.

Methods

A culture of human osteoarthritic synoviocytes was stimulated with Mobilee® and HA from bacterial fermentation at different concentrations. After incubating the samples for 12 and 24 hours, the concentration of eHA in the cell cultures was measured.

Torrent A et al. Comparative efficacy of IB0004 extracted hyaluronic acid (HA) and fermented HA on the synthesis of endogenous HA by human synoviocytes. *Osteoarthritis and Cartilage*, 2009, vol. 17, pg. S278-S279.

Results

Both molecules had a dose-dependent effect, with the most efficacious dosages being 100 and 200 µg/mL. After 12 and 24 hours of incubation, Mobilee® stimulated higher levels of eHA than HA from bacterial fermentation.

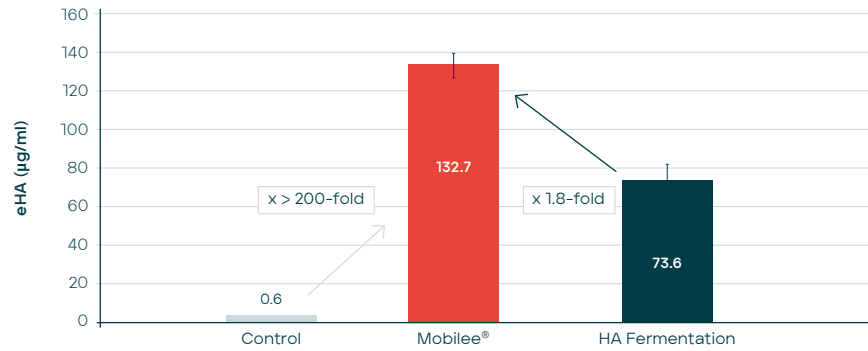


Figure 20: Effect of Mobilee® and fermented HA on the endogenous synthesis of HA in human osteoarthritic synoviocytes – results at a concentration of 200 µg/mL and 24-hour incubation

Conclusion:

At the same concentration levels of each product, the highest levels of eHA were measured in the cells stimulated with Mobilee®. It was therefore concluded that there are differences between the action of Mobilee® and HA from bacterial fermentation in the synovial-fluid cells.

References

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