RECUPERAT-ION® Hydration.

Muscle Function and Sport.

Clinical Research Summary

❖ OBJECTIVES.

Recuperat-ion Electrolitos s.l. designed in 2003 and is still developing today a clinical research program with the aim of evaluating the efficacy and safety of Recuperat-ion, a drink with a specific formulation based on mineral salts, in two approaches: athletes who sick to maintain their muscular performance along with its post-exercise recovery and in the reduction of fatigue and muscular pain associated with the general symptoms of Fibromyalgia patients. Since Recuperat-ion is conceptually defined as a solution for oral rehydration, this program collects current knowledge about hydric and ionic flows in the locomotor system of the human organism, and focuses its objectives in two aspects:

- Healthy male volunteers. Study of the effect of hydration on muscle fatigue of central or peripheral origin, and on post-exercise recovery in healthy male volunteers.

- Volunteers suffering from Fibromyalgia. Study of the effect of hydration on fatigue and muscle pain associated with the symptoms of Fibromyalgia.

In complicity with muscle health professionals, study and research projects are carried out with rigorous quality control and with a detailed external evaluation that ensures the transparency and fairness of the results.

Our maxim is the improvement of muscular performance and rehydration before, during and after exercise.

Hence, we encourage all muscle health professionals to join us in this quest to improve muscle performance and health naturally. We will be happy to consider any study or idea that helps us achieve these goals.

Below and after some considerations on rehydration, we present the studies, trials and tests already published.

CLINICAL RESEARCH.

Loss of fluid due to exercise can cause three possible situations. First, *dehydration*, which occurs when the loss of fluid as a result of exercise exceeds the fluid intake. Second, *hypo hydration*, which appears when they become dehydrated before the start of a competition due, for example, to a restriction of fluid intake or to the practice of a warm-up exercise, among others. Finally, *hyponatraemia* appears as a result of prolonged exercise with profuse sweating and excessive fluid intake, greater than loss through sweat and urine, or from the intake of fluids with low sodium content.

The consequences of the loss of liquid are basically two. On the one hand there is a *decrease in sports performance*. In the first place, it is conditioned by cardiovascular involvement, due to increased cardiac work. Second, due to the *functional deterioration of muscles and tendons*, due to the fact that the increase in muscle temperature alters the normal structure of contractile proteins and collagen, with the risk of musculoskeletal injuries. On the other hand, we have the risks of *dehydration*. Thus, the loss of 3% of body weight causes muscle contractures and cramps and an increased risk of lipothymia (body temperature: 38ºC); a 5% loss of body weight causes an increased risk of musculoskeletal injuries (body temperature: 39ºC); the 8% loss of body weight causes sustained muscle contraction without the possibility of relaxation (body temperature:> 39.5ºC); finally, losing 10% of body weight is life-threatening

.

Tap water is not the ideal drink for post-exercise rehydration when rapid and complete restoration of fluid balance is necessary and where all consumption is in liquid form.

The first studies of investigation of the mechanisms of post-exercise rehydration showed that the ingestion of large volumes of tap water after exercise-induced dehydration results in a rapid drop in plasma osmolality and sodium concentration, producing an immediate and marked diuresis. It soon became clear that the addition of sodium was absolutely necessary. Indeed, the addition of sodium chloride with water to get a saline solution with an effective concentration of 0.45% (77 mmol□L-1), the restoration of plasma volume was complete in less than 20 minutes.

Adding sodium to rehydration drinks can therefore be justified in two ways. The first, sodium stimulates the absorption of glucose at the level of the small intestine: the absorption of water from the intestinal lumen is purely a passive process that is largely determined by local osmotic gradients. The active transportation of glucose and sodium creates an osmotic gradient that acts to promote net water absorption, and the rate of rehydration is therefore higher when glucose solutions with sodium chloride are consumed than when drinking plain water. Second, the replacement of sweat losses with tap water leads, if the volume ingested is large enough, to hemodilution: the decrease in plasma osmolality and sodium concentration that occurs in this situation will reduce the urge to drink and will stimulate urine production and has potentially more serious consequences, such as hyponatremia.

It was originally proposed that beverages used for post-exercise rehydration should have a sodium concentration similar to that of sweat. However, the electrolyte content of sweat shows considerable variation between people and over time; it seems impossible to prescribe a single formulation for each person or for each situation. In a systematic investigation of the relationship between total body sweat sodium losses and the effectiveness of drinks with different sodium concentrations in restoring fluid balance, it was shown that, as long as sufficient volume of fluid is consumed, euhydration is achieved when sodium intake is greater than sodium loss through sweat (Fig. 1).

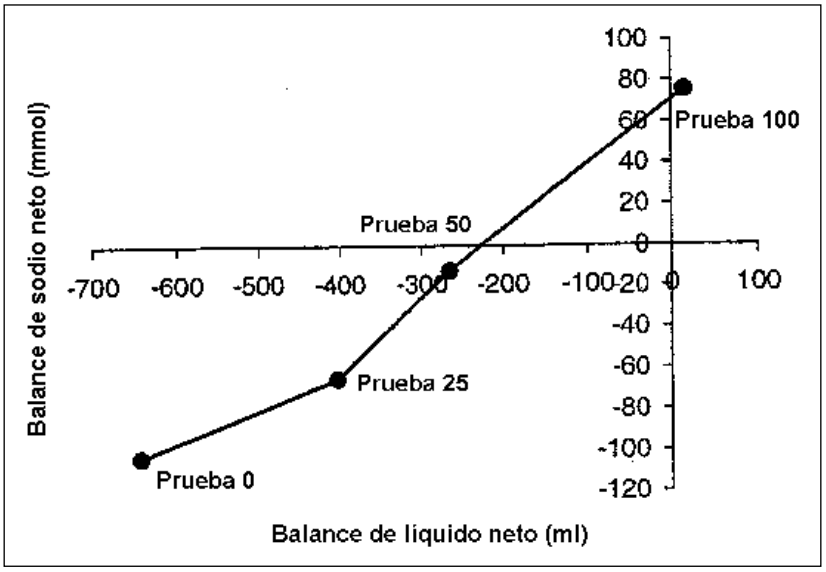

Figure 1. Net sodium balance vs. net fluid balance 6 hours after the end of the rehydration period. The beverages, with a volume equivalent to 150% of the loss through sweat, containing 0, 25, 50 and 100 mmol□L-1 of sodium (Tests 0, 25, 50 and 100 respectively) were consumed after exercise. Six hours after rehydration, fluid balance is maintained only when sodium intake has been greater than sodium loss from sweat.

However, this is not all. It is not enough to add sodium to drinking water. Other ions are also necessary. It is therefore necessary to analyse the concentration of sodium and potassium in the drinking water. Sodium is the largest ion in the extracellular fluid, but potassium is the largest ion in the intracellular fluid. Therefore, potassium may be important in achieving rehydration by helping to retain water in the intracellular space. Indeed, when a volume equivalent to the mass loss is ingested, it is observed that a smaller volume of urine is excreted after rehydration when a drink containing electrolytes is ingested compared to the drink lacking electrolytes. Furthermore, the volumes of urine excreted remain close to baseline levels and further significant reductions in urine excretion cannot take place when sodium and potassium are ingested. Therefore, potassium seems to be important in increasing rehydration favouring intracellular rehydration.

It is also advisable to analyse the amount of water ingested together with the sodium concentration to achieve correct post-exercise rehydration. Mandatory urine losses persist even in dehydration, due to the need to eliminate metabolic waste products. The volume of fluid consumed after secondary to exercise sweating or heat loss must therefore be greater than the volume lost through sweat if effective rehydration is carried out. This contradicts recommendations that athletes should match fluid intake exactly to the amount of body mass loss. When a volume of drink equal to 150% of the loss through sweat is ingested, subjects are slightly hypo hydrated 6 hours after drinking a drink with a low sodium concentration. With a high sodium drink, enough fluid is retained to maintain subjects in a state of hyper hydration 6 hours after ingestion of the drink when consuming 150% or 200% of their loss through sweat (Fig. 2). Therefore, the increase tends to be greater when a drink with a high concentration of sodium is ingested.

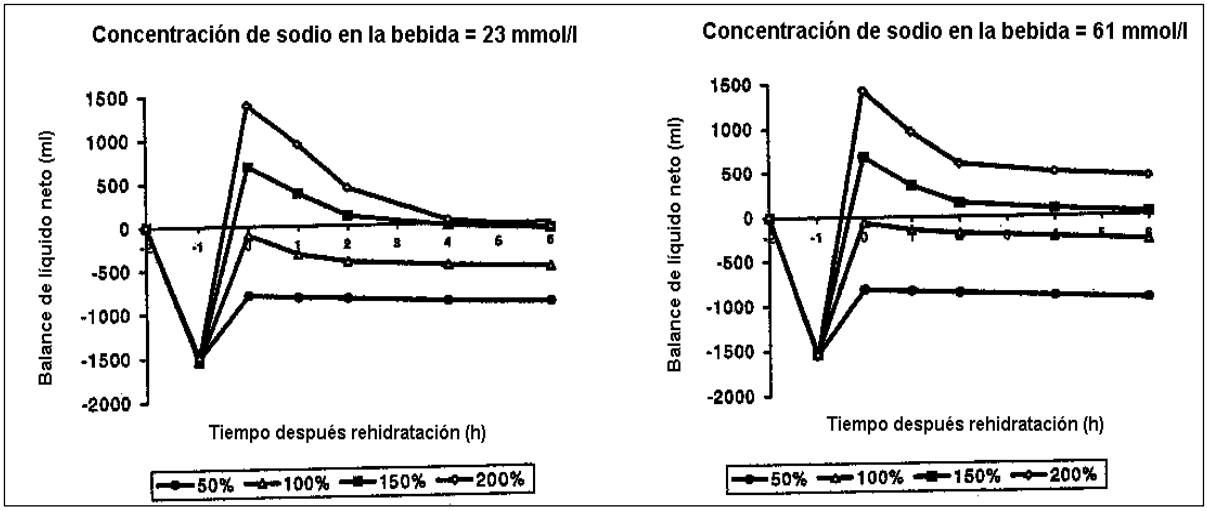

Figure 2. Net fluid balance versus time. The volume of drink consumed was half (50%), equal (100%), one and a half times (150%) and double (200%) of the loss due to sweat. The net zero fluid balance corresponds to the state of euhydration. Mild hypo hydration was present 6 hours after rehydration when a large volume of the low-sodium drink (23 mmol□L-1) was consumed, but with the same volume, hyper hydration was achieved with the high-sodium drink (61 mmol□L-1).

In a recent study at the CAR of San Cugat, Pujol et al. (2004) conducted a controlled, randomized and crossover study in 12 trained male athletes aged 27□4 years, with an aerobic capacity (VO2max) of 62.5□6.0 mL. .kg-1.min-1, in which they compared the efficacy - measured by plasma osmolality- of rehydration of a drink with a moderately high sodium content (0.15 g / 100 mL) (Recuperat-ion□) with a solution with a low sodium content (0.07 g / 100 mL) (Isostar Fast Hydration□), All volunteers ingested 150% of the post-exercise weight loss. With the drink with a relatively high sodium content, rehydration was faster than with the drink with a low sodium content (Fig. 3). Thus, the results of this and other studies indicate that rehydration can be achieved after exercise only if electrolyte losses through sweat as well as water are replaced with adequate sodium and water concentrations. Drinks containing approximately 50 mmol.L-1 sodium have been shown to be more effective than sodium concentrations in most sports drinks ranging from 10-25 mmol.L-1, and in some cases even lower. Therefore, drinks with low concentrations of sodium are ineffective to rehydrate and, in addition, they also decrease the stimulus to drink.

The addition of an energy source is not necessary for rehydration, although a small amount of carbohydrates can improve the intestinal absorption of sodium and water, and will also improve the taste. However, the addition of carbohydrates in intense and long-term exercises is advisable, because it normalizes hypoglycaemia, delays fatigue and accelerates the recovery of lost glycogen. Carbohydrates are usually added in the form of glucose, fructose, association of glucose and fructose, sucrose, among others.

When exercise causes ATP depletion, PCr resynthesis slows down, being independent of pH recovery. Thus, in the study by Pujol et al. (2004), the solution with a moderately high concentration of sodium, together with the addition of the monosaccharides glucose and fructose (Recuperat-ion□), achieved a more rapid recovery and marked post exercise Pi / Total, PCr / beta-ATP and Pi / PCr, compared to the drink with low sodium concentration (0.07 g / 100 mL) (Isostar Fast Hydration□), measured by nuclear magnetic resonance spectroscopy (31P -NMR) (Table 1). Rapid post-exercise rehydration likely contributed to this. The more pronounced restoration of intracellular energy metabolites by Recuperat-ion□ allows a more effective recovery from post-exercise muscle fatigue.

Therefore, for a correct and rapid post-exercise rehydration it is recommended to ingest at least 150% of the weight loss to cover the loss of fluid due to sweat plus the mandatory losses of urine, with a sodium concentration between 50 and 60 mmol.L-1, to maintain plasma osmolality and desire to drink. In addition, carbohydrates should be consumed as soon as possible after exercise, as muscle has a high affinity for glucose uptake immediately after exercise, and the greatest resynthesis of muscle glycogen occurs during the first 2 hours immediately after exercising. Finally, the rapid restoration of muscle glycogen stores is done at the expense of glucose, much faster than fructose, while it produces a resynthesis of hepatic glycogen, faster than glucose. It is recommended the intake of 0.7 g / kg of carbohydrates every 2 hours during the first 4 to 6 hours after exercise, to maximize glycogen resynthesis.

However, adding to water a drink containing only sodium and potassium, in addition to carbohydrates, is not enough. Indeed, it is known that powerful and prolonged muscle contraction leads to a well-known condition, muscle fatigue. Studies in athletes have shown that muscle fatigue increases almost in direct proportion to the rate of muscle glycogen depletion. Therefore, most of the fatigue probably results simply from the inability of the metabolic and contractile processes of the muscle fibre to continue providing resources to maintain muscle work at a high level. However, various experiments have shown that nerve transmission through the neuromuscular junction can decrease after prolonged and intense muscle exercise, with a consequent decrease in muscle contraction. Indeed, the contractile process of skeletal muscle is activated by calcium ions. Similarly, to achieve muscle relaxation, it is necessary to remove the calcium ions from the intracellular fluid that bathe the actin and myosin filaments, which is achieved by pumping these ions into the extracellular fluid or the sarcoplasmic reticulum. For its part, magnesium, called the "physiological calcium channel blocker", regulates its action. Indeed, since calcium plays an important role in the contraction of skeletal muscle and smooth muscle, magnesium depletion can cause muscle cramps. Furthermore, it is necessary for the generation of aerobic and anaerobic energy and for glycolysis, either indirectly as a Mg-ATP complex or directly as an activating enzyme. Finally, in conjunction with calcium, it is involved in neurotransmission at the level of the myoneural junction. Therefore, this is the reason why in Recuperat-ion® there are, in addition to sodium and potassium, calcium and magnesium that intervene in the maintenance of neuromuscular mechanics, so necessary for the athlete's performance.

On the other hand, it is a proven fact that vigorous physical exercise is generally associated with fatigue and pain. In well-trained subjects, exercise increases tolerance to pain and fatigue. Studies carried out in humans have shown that both maximum muscular effort and submaximal sustained effort lead to an increase in plasma concentrations of □-endorphins, which play an important role in regulating the perception of pain, the increase being proportional to effort accomplished. Individuals who show the best tolerance or resistance to pain may, therefore, delay the onset of fatigue and develop a better nociceptive inhibitory system. Serotonergic neurotransmission affects nociception through its effects on the enkephalin-endorphin system, and brain serotonin levels depend on the bioavailability of L-tryptophan. Thus, the theoretically most potent ergogenic effect of tryptophan resides in the formation of serotonin (5-HT). In this sense, some researchers suggest that this neurotransmitter can improve performance by increasing pain tolerance through the administration of L-tryptophan. Indeed, it has been seen that the administration of L-tryptophan before exercise prolongs the total time of exercise as well as the total workload due to an increase in pain tolerance.

Based on this hypothesis, at the Hospital de Bellvitge, Segura et al. (2005) conducted a randomized, controlled, double-blind crossover clinical trial in healthy white male volunteers, with a [V (dot) O2maxR] 40-50% and a HRR 55-65%, with a regular practice of physical exercise greater than 3 hours per week, with a VO2max greater than 45 ml.min-1, a BMI less

than 25 kg / m2 and with a score on the perception scale of Borg fatigue (RPE) 12-13 in which the maximum distance travelled in the last 20 'of the stress test was studied, power achieved in the last 20' of the stress test - also the comparison of power in the first 10 'and in the second 10'-, as well as performance variables with the Wingate test (maximum peak of laps, average of laps in 30 ”and fatigue index) and hormonal determinations and concentrations of amino acids and gases by means of a spirometry. 3 days prior to the test, 2 sachets of Recuperation + 600 mg of L-tryptophan or placebo daily were administered, and on the day of the test: 1 sachet of Recuperation® + 300 mg of active L-tryptophan or placebo for the morning and 1 sachet of Recuperation® + 300 mg of active L-tryptophan or placebo 2 hours before the exercise stress test.

In the variable *maximum peak of revolutions per minute*, after the ingestion of l-tryptophan, an improvement was observed in the maximum power developed, the difference being significant at 20 '(p = 0.046) and at 40' (p = 0.044) (Figure 4).

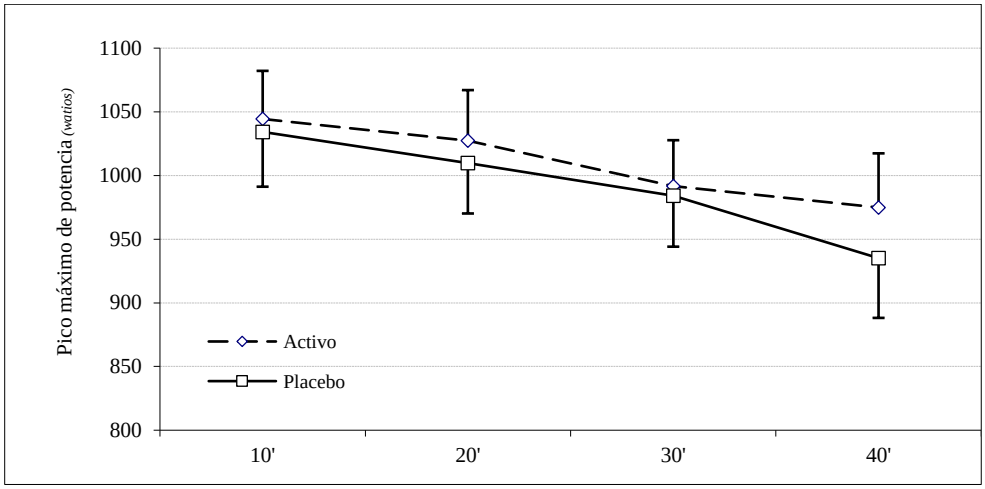

Figure 4. The maximum peak of revolutions per minute.

In the average variable of *revolutions per minute* (anaerobic power in 30 "), after the ingestion of l-tryptophan, an improvement in the maximum power developed was observed, with a significant difference at 10 '(p = 0.021) and at 40' (p = 0.016) (Figure 5).

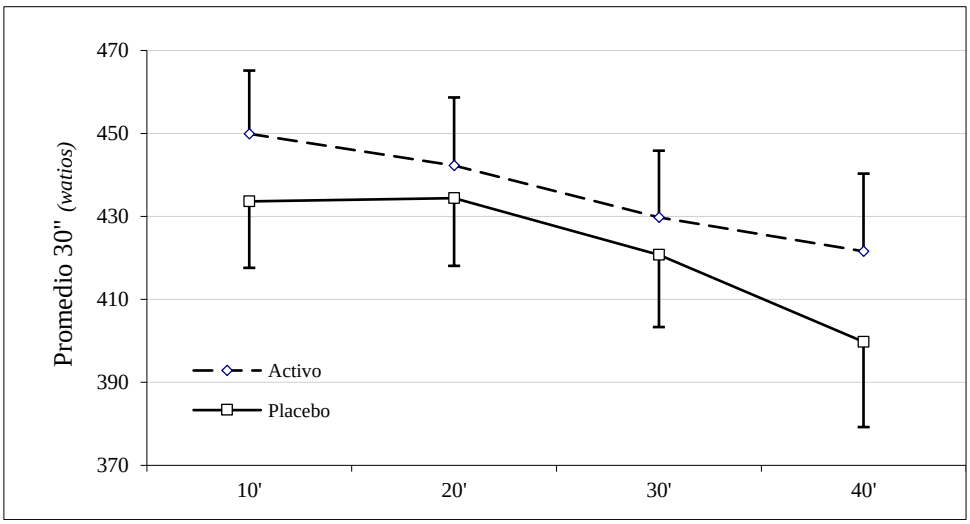

Figure 5. The average of revolutions per minute (anaerobic power in 30”).

In the variable *power developed at 50% of the maximum power load, during the last 20 minutes*, a statistically significant increase was observed in the meters travelled after supplementation with l-tryptophan, of 4.74%, going from a power of 2,306 watts with placebo to 2,411 watts with l-tryptophan (p = 0.026). During the first 10 minutes of this test, an increase of 3.3% was observed after the intake of Tryptophan and in the last 10 minutes the increase was 8.7%, an increase significantly higher than the values obtained with placebo (p = 0.029). The improvement in the average power made in these last 20 minutes was 4.6% greater compared to what was achieved with the placebo. In conclusion, the administration of an oral rehydration solution (Recuperat-ion®) together with an amino acid l-tryptophan (vitAmin-T®), can be useful to improve sports performance, especially in exercises of intermediate duration and high intensity, since together with the rapid restoration of muscle energy stores, a delay in the onset of muscle fatigue is achieved, mainly because of the increase in pain threshold probably due to l-tryptophan.

There are some syndromes that also cause muscle pain and fatigue, similar to what the athlete suffers, especially in fatigue due to overtraining. Fibromyalgia (FM) is a complex and chronic disease that causes widespread muscle pain and fatigue. The main symptom of fibromyalgia is chronic widespread pain. Compared to healthy controls, FM patients have a decreased pain tolerance and present two unexpected phenomena: (1) an increase in generalized dysesthesia (mainly a tingling or burning sensation) perceived both distally and proximally to the stimulated area, and (2) persistent dysesthesia around the stimulated area for 12 to 20 minutes after stimulation has ended. Researchers have also identified significant disturbances in the levels of certain key neurotransmitters in the brain and central nervous system (CNS). The level of the brain neurotransmitter substance P, an agent that sends pain signals to be recorded at the brain level, is 3 times higher in FM patients than in healthy controls. Also interesting is the discovery that the neurotransmitter serotonin, responsible for modifying the intensity of pain signals that reach the brain, appears to be deficient in patients with FM. As every time it is becoming more obvious that there is a pain processing disorder in FM, researchers are trying to determine whether the problem is an overreaction of the brain / body to normal stimulation (allodynia) or a magnified response to a real painful stimulus (hyperalgesia).

Based on the results obtained with Recuperat-ion® n the treatment of pain and muscle fatigue in athletes, as well as knowing that the use of the supplement with Recuperat-ion mineral ions and salts is wide in the group of patients affected by FM, and that manifest a positive effect that explains its use, Carbonell et al. (2006) conducted a multicentre, triple-blind, randomized, parallel, placebo-controlled pilot study to evaluate the efficacy of Recuperat-ion® in the treatment of FM, mainly of pain and fatigue, in patients of both sexes between 18 and 65 years of age, with FM diagnosed according to the official diagnostic criteria established in 1990 by the American College of Rheumatology (ACR), with a duration of the disease ≤ 10 years. The following parameters were evaluated at the initial visit and after 30, 60, 90, 120, 150 and 180 days and at the end of the post-treatment observation period: the severity of pain and fatigue, pain in tender points (using finger pressure), general state of health and quality of life (Nottingham health profile) and psychological well-being (Face Scale, Hospital anxiety and depression scale or HAD scale). At the end of the treatment period, a global assessment of the efficacy of the treatment was carried out by the investigator and the patient, evaluated using an ordinal scale. The results indicate that Recuperat-ion® significantly reduced the severity and intensity of pain throughout the treatment period, and even during the post-treatment period. In the remaining variables, none of the treatments reached statistically significant differences. These results probably indicate an enormous variability between subjects in terms of the perception of pain and fatigue.

Conclusions.

1.- Recuperat-ion® shows a slight effect on pain at 160 days and compared to placebo, in the intragroup comparison only a decrease in pain severity was detected in the group treated at 160 days. Its clinical relevance is slight.
2.- In the Recuperat-ion® group, a significant improvement in pain severity was observed.
3.- Treatment safety was good with few drug-related adverse reactions.
4.- The existence of responding patients makes it necessary to analyse their profile and plan new investigations with the type of potentially responding patient. Pending publication.

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COMPLETED AND PUBLISHED TRIALS.

A-1 Inter-individual differences in plasma cortisol concentrations after one hour of mixed aerobic-anaerobic exercise.

- Introduction and objectives. The objective of the study was to evaluate the differences in plasma cortisol concentration after performing a mixed aerobic-anaerobic effort in a group of physically active young people.

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Conclusion. Despite being evaluated a homogeneous group, interindividual differences were observed in plasma cortisol concentration after performing a mixed effort, which may have implications in the adaptation caused by exercise.

A-2. Effects of "Recuperat-ion" in the period of rest following an intense workout.

- The aim of the study was to evaluate the effect of the drink “Recuperat-ion” (Prec) both on sports performance and on different physiological parameters of a group of volunteers subjected to an intense exercise test. To carry out the study, a double-blind test with two more drinks was proposed, consisting respectively of salt solution at concentrations other than “Recuperat-ion” (Pas) and aqueous solution without salts (Pss), with the same sweeteners.

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Conclusions.
Based on the results obtained in this work, the beneficial effect of salts in treating muscle fatigue seems to be observed. In the specific case of "Recovered-ion", the variation observed in the concentration of lactate in the blood, suggests that this drink is able to decrease blood lactate.

A-3 Results of a Combined Rehabilitation Treatment

- Standard rehabilitative treatment comparing it with the same treatment in which the patients have also taken two daily sachets of Recuperat-ion Sugar Free, during the period of time of the treatment in each case.

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Conclusions
• A notable improvement has been noted in the combined treatments with Recuperat-ion in those cases where there is a severe muscle contracture.
• In cases of general muscle pain, an acceleration of the recovery process and activation of physiotherapeutic regeneration mechanisms has been observed.
• Similarly, in the case of the treatment of pain and inflammation in:
  • Osteoarthritis
  • Painful muscle contractions
  • Back pains (low back pain, cervical pain,...)
  • Tendinitis (shoulders, elbows, knees, etc.)
  • Capsulation and fibrosis (fibrillary tears)
  • Sprains

An improvement in general condition of patients was observed.

A-4 Peripheral origin fatigue. Athletes

- Rehydration in long-distance athletes. Effects of the administration of a drink with a relatively low content of sodium versus another drink with a moderately high content of sodium (Recuperat-ion□). Study of plasma osmolality and muscle bioenergetic effect by nuclear magnetic resonance spectroscopy (31 P-NMR). Controlled, randomized and crossover clinical trial. 2004..

  Pujol P[1] , Rosés JM[2], Drobnic F[1], Aliste L[2], González C[1] y Moreno A[3].
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Variables analysed:
Recovery of plasma concentration and muscle energy nutrients. Plasma osmolality. Nuclear Magnetic Resonance Spectroscopy.

Obtained results:
Statistically significant improvement of both variables with Recuperat-ion. Rehydration was more rapid and a faster and more marked post-exercise restoration of the phosphorylated energetic components ATP was achieved.

A-5 Fatigue of central origin. Athletes.

Randomized, controlled, double-blind, crossover clinical trial of the effect of an oral rehydration solution supplemented with L-tryptophan (vitAmin-T) on exercise in healthy male volunteers.

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Variables analysed. Exercise test on cycle ergometer.

Obtained results:
Statistically significant improvement with Recuperat-ion + vitAmin-T

B-1 Fatigue and muscle pain. Fibromyalgia patients.

- Multicentre, triple-blind, placebo-controlled and randomized clinical trial of the efficacy and safety of Recuperat-ion in the treatment of fibromyalgia (FM). 2006. Pending publication.

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Variables analysed.
American College of Rheumatology (ACR) diagnostic criteria.

Obtained results:

Statistically significant improvement with Recuperat-ion in pain severity.