

FAQ lactium®

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I/ General points on stress and stress symptoms

▪ What is stress?

Stress is the natural reaction of the body and of the mind to everyday tension and pressure. So, everybody can be concerned by stress. It is not a disease. Stressors can be internal (i.e. worry about a future event) or external (i.e. poor working conditions) and induce variable reactions in people. Stress may be harmful for individuals, depending on one's ability to cope with stress.

▪ How many people suffer from stress?

World Health Organization (WHO) estimates that 450 million of people in the world suffer from stress

▪ Where?

According to Eurostaf study (2004), the amount of stressed people has especially increased in developed countries. This number has indeed doubled for the past ten years. Figures also reveal that 30% of people from the developed countries would be stressed out at work. More than 60% of American executives would suffer from stress and 2/3 of the Japanese are facing stress at work. Among Europeans, 28% of working people declare that work is a source of stress.

▪ How does our body react to stress?

Stress induces specific physiological changes, in particular the increases of blood pressure and hormones such as adrenaline, ACTH (Adreno-corticotropin Hormon) and cortisol.

▪ When does stress become harmful for our body?

At the origin, stress has been at the origin described as the reaction to an immediate threat, also known as the "fight or flight" response or acute stress (Walter Cannon, 1929). It is followed by a relaxation state during which the level of stress hormones (cortisol) returns to normal.

However, modern life poses on-going stressful situations preventing physiological reactions to stress from slowing down. This induces physical and psychological troubles known as stress symptoms.

▪ What are stress symptoms?

Stress symptoms are troubles associated to chronic pressure. They can be of many kinds:

- **Physical symptoms:** muscular tensions, digestive disorders, sleep troubles, headaches, tiredness...
- **Mental & emotional symptoms:** irritability, anxiety, sadness, lack of libido, memory lapses, concentration troubles...
- **Behavioral symptoms:** relationship difficulties, detachment, increased consumption of tobacco, alcohol, chocolate, sweets, drugs, etc.

▪ What are the current remedies to treat stress?

Current remedies comprise of:

- **Pharmaceuticals** such as anxiolytics and antidepressants supported by scientific evidence but often blamed for negative side effects

- *Plants & vitamins* currently used for stress but with no clear evidence of efficiency.

II/ lactium®

1. lactium®: description

- What is lactium®?

lactium® is a natural milk protein hydrolysate containing a bioactive peptide with relaxing properties.

- What is the active anti-stress constituent?

The active compound is a decapeptide released from α_{S1} -casein (a major milk protein) after trypsin hydrolysis (a common digestive enzyme).

- What is its amino acid sequence?

YLGYLEQLLR or Tyr-Leu-Gly-Tyr-Leu-Glu-Gln-Leu-Leu-Arg

- What is its scientific name of the decapeptide?

The official name is α_{S1} -CN (f91-100).

2. General effects of lactium®

- What is lactium® used for?

lactium® is used to regulate stress symptoms and to help people coping with stress and overcoming stressful events.

- Can lactium® be used to fight depression or anxiety disorders?

lactium® can be associated to a behavioral therapy but it is not a drug. As a consequence, it can not replace any medical treatment prescribed to fight pathological anxiety or depressive illness.

3. The origin of lactium®

- Why did you search for a relaxing constituent in milk?

At the end of the 80's, searchers have wondered about the origin of the calm state of babies after drinking milk. They hypothesized that a milk constituent could explain this soothing effect. Through many years of research, they succeeded in identifying a bio-peptide present in milk protein with natural relaxing properties.

- How did you look for the peptide?

The research was focused on a tryptic hydrolysate of α_{S1} -casein because trypsin is a key enzyme in digestion of the neonate and because α_{S1} -casein is a major milk protein. This hydrolysate revealed anxiolytic-like activity in specific in vivo models in rats. The bioactive peptide was identified after in vitro receptorology experiments on GABA_A receptors.

4. Equivalence

- Does drinking milk have a similar effect as taking lactium®?

No. At first, the decapeptide is not present by itself in milk and is encrypted in α_{S1} -casein. It could be released in neonates but not in adults. The enzymatic system in adults is indeed much more complex and cannot allow the quantitative delivery of this specific sequence from the protein.

- Do you believe that milk casein products from other companies also have the same effect?

Our product is a unique hydrolysate from α_{S1} -casein. The process to obtain such a hydrolysate containing the bioactive decapeptide is very specific with defined parameters (substrate, enzymatic process conditions, etc...). For these reasons, other milk casein products should not develop the same effects as lactium®. Moreover, the effect of lactium® is patented.

III/ Scientific evidence

- Has the efficiency of lactium® been scientifically demonstrated?

lactium® efficiency has been validated in Elevated Plus-Maze rats and the Conditioned Defensive Burying test (CDB) rat models. Significant results were also obtained in humans through 5 human studies involving 190 volunteers, using physiological markers.

- What kind of clinical studies have you run?

We have run randomized, double-blind, placebo-controlled trials. Clinical studies were made according to Good Clinical Practice Guidelines with official ethical committees.

- What tests have you used in your clinical studies?

We have used tests which have already been validated by the scientific community (i.e. Stroop test, Cold pressor test, Spielberger questionnaires,...). They have not been developed specifically for our studies.

- Which physiological markers did you use in your clinical studies?

We used:

- blood pressure reactivity to stress (systolic and diastolic blood pressure)
- ACTH and cortisol which are stress-related hormones

They are validated physiological markers of stress.

- Does lactium® have any antihypertensive effect?

lactium® modulates blood pressure increase during a stressing situation but not the ambulatory blood pressure (hypertension). Thus, lactium® does not have an antihypertensive or hypotensive effect.

- Have you compared lactium® with other products like St. John's Wort, Kava Kava or Suntheanine®?

CDB tests have been run to compare lactium® with these ingredients and comparative documents are available upon request. Among these products, lactium® is, to date,

the only ingredient showing clear efficiency at modifying the hormonal response to stress.

IV/ Dosage and biodisponibility

1. Dosage

- **What dose of lactium® do you recommend?**

The usual recommendation is 150 mg per day. This dosage can be increased for special situations that induce an additional stress (exam, oral communication, important meeting etc...) or for overweighed people.

- **How have you established the 150mg dosage?**

The dosage has been defined through many steps. At first, different doses were tested on rats. 15mg dose was established to be the effective dosage in rats. Based on a FDA guidance document*, this dose was adapted to humans (150mg) and validated through human clinical studies.

* Guidance for Industry and Reviewers estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers, U.S. Department of Health and Human Services, Food and Drug Administration.

2. How to take lactium®?

- **When should lactium® be taken?**

During the different clinical studies in humans, lactium® intake was done either in the morning or in the evening. In both cases, the efficacy of lactium® was observed. In consequence, each person may adapt the intake, whether in the morning or in the evening, depending on his/her feelings and specific needs.

- **How long should I take lactium®?**

People should adopt their own way of taking lactium®. It can be taken in 2 ways:

- In a once off intake to cope with a specific immediate need (exam, important meeting...) or few days before a stressful event to anticipate.
- During difficult periods, lactium® can be taken as a daily diet for several days and can even be used for several weeks in case of chronic symptoms, depending on the person and the stress level.

- **Does the feeling of stress decrease if the dose or the duration increases?**

We do not have any precise data about this in humans but a dose effect response has been noticed in the CDB model in rats.

3. Biodisponibility

- **How many hours after consuming lactium® can we feel the effects?**

In animal studies, lactium® has shown to be physiologically effective about 1h after the intake. Nevertheless, a real sensation of well being frequently appears after one week to 15 days. Remember that lactium® helps to regulate stress and stress symptoms. The perceived effect may be different from one situation to another, depending on the level of stress and the individual.

- How long does the effect of lactium® last for?

In animal studies, lactium® effect begins to decrease about 6 hours after the intake. In humans, as we recommend lactium® to improve the ability to cope with stress, we have noticed that the feeling of well being could last after stopping the treatment.

- Can the peptide pass through the intestinal barrier to the brain?

Studies have been conducted on lactium® to determine the bioactive peptide structure (NMR, molecular dynamics simulation, circular dichroism). This peptide adopts an alpha-helix structure with both lipophilic and hydrophilic faces. Because of its amphipatic structure, the bioactive peptide is likely to pass through the intestinal barrier. Furthermore, the kind of effect induced by lactium® on stress requires the message to come to the brain. And the most plausible reason for this is that the peptide directly reaches the brain.

- What is the physiological mechanism behind the relaxation effect of lactium®?

The mechanism of action of lactium® is complex. Nevertheless, in vitro studies showed that the bioactive peptide contained in lactium® had an affinity for non peripheral-type GABA_A receptors. These receptors are commonly involved in anxiolysis. A sequence homology with DBI (diazepam binding inhibitor), an endogenous ligand of the GABA_A receptor, and a structural homology with benzodiazepines have been demonstrated, clarifying this fact.

4. Metabolism

- Is lactium® easily eliminated from our body?

lactium® is normally eliminated like any other protein derivatives.

V/ Side effects and toxicity

1. Side effects

- Can lactium® induce any side effects?

lactium® does not induce any side effect. In particular, lactium® does not induce addiction, tolerance, memory loss, disinhibition nor sedation. It does not cause arousal, reactivity or alertness decrease.

Furthermore, no physiological adverse effects were noticed. In particular, neither weight gain nor modification of the ambulatory blood pressure was observed.

- How did you study lactium® possible side effects?

Side effects were studied in rats (Irwin test) and validated in humans through the Hopkins checklist and Thayer questionnaire. Furthermore, for each of the human studies, open questions rated by the volunteers demonstrated that no significant side effect had affected them.

- If I take more than the recommended dosage, will I suffer from any side effect?

In humans, no side effects were evidenced after a single intake even 10 times higher than the recommended dose.

- Have you studied the long-term effect of the peptide in humans?

We studied the effect of a 30-day oral intake. Nevertheless, people who had been taking it for a longer time did not report any side effects.

2. Toxicity

- Is lactium[®] mutagenic?

lactium[®] does not induce any mutagenic effects in the mammalian cell line (L5178Y, Mouse Lymphoma Assay) when tested up to 5000 µg/mL, either with or without metabolic activation.

- Is lactium[®] teratogenic?

Daily oral intake of lactium[®] by female rats at a dosage of 150 mg/kg body weight/day (10 times the active dosage) during pregnancy does not alter pregnancy duration, maternal behavior or care of young rats. It does not induce any trouble in the physical, locomotive, behavioral or cognitive development in the neonates.

- Have you run toxicological studies?

We have studied possible overdose effects of lactium[®] in rats with behavioral, sub-acute, acute and immuno-toxicity studies. A 28-day sub-acute toxicity study in rats has shown that no toxic effect occurs when up to 1,000 mg/kg/day are administered. In the acute toxicity study, lactium[®] was determined to have a lethal dose (LD-50) greater than 2,000mg/kg.

VI/ Allergies, intolerance and contraindications

- Can lactose-intolerant people take lactium[®]?

Severe lactose intolerant people can ingest up to 5g/day of lactose. Knowing that lactose content in lactium[®] is typically 1% and that the daily intake is 150mg/day, there is no risk for these people.

We can even provide lactium[®] containing less than 0.5% of lactose.

- Have you studied the allergenic potential of lactium[®]?

What is considered as milk allergy is often lactose intolerance. If we consider milk-allergy stricto sensu, only 2-3% of infants are concerned in developed countries (HOST, 2002). Knowing that approximately 85-90% of children lose clinical reactivity to milk once they surpass 3 years of age, the adults are rarely concerned. Moreover, lactium[®] does not contain β-lactoglobulin which is one of the most allergenic protein. Furthermore, lactium[®] is a hydrolysate and therefore, can be considered as a hypoallergenic substance.

Host A. (2002) Frequency of cow's milk allergy in childhood. Ann Allergy Asthma Immunol;89(6 Suppl 1):33-7.

- Is there any contraindications for lactium[®] intake?

No specific contraindications concern lactium[®] but a basic warning regarding pregnant women and babies should appear on the package, according to local regulatory statement. This warning does not mean that the product is dangerous for these individuals; it only means that no such data is available today. Pregnant women and children could indeed not be included in the tested population during clinical studies, because of ethical guidelines.

- Is the bioactive peptide classified as a doping product?

According to our knowledge no milk extract is considered to be a doping product.

VII/ Quality

- How does INGREDIA guarantee the quality and homogeneity of lactium[®]?

Every manufactured batch is double-checked to guarantee the content in bio-active peptide and the activity of the product:

- 1) The concentration of the active peptide within the hydrolysate is controlled using a chromatographic analysis (HPLC, high performance liquid chromatography).
- 2) The relaxant activity is checked using a global anxiety score in rats with the CDB model: conditioned defensive burying.

- What does the CDB model consist of?

The Conditioned Defensive Burying (CDB) test used corresponds to Pinel and Treit's procedure (1978, 1981). After shock administration (stressing event), the behavior of the rats is recorded and a global anxiety score is attributed to this behavior. This score is the sum of the ranking of 3 parameters:

- duration of probe-burying
- number of head stretching towards the probe
- percentage of approaches towards the probe followed by retreats.

- Why do you use the CDB model?

The CDB model is based on the fact that burying is part of the rat's natural behavior when facing an aversive stimulation (source of stress).

Several investigators have shown that anxiolytics decreased or suppressed burying, suggesting that CDB may have advantages as a preclinical screening method for anxiolytic agents.

- Why do you use diazepam as a reference in some of your preclinical studies?

We use diazepam in some of the preclinical studies with lactium[®] to validate that the test is properly conducted.

VIII/ Regulation

- What is lactium[®] status?

lactium[®] is a food grade ingredient which does not need Novel Food status in Europe. lactium[®] is recognized by the Food and Drug Administration (FDA) and has obtained GRAS and NDI status (#rpt242). lactium[®] is a kosher and non GMO ingredient.

- What is lactium[®] labeling?

lactium[®] is clearly labeled as “milk protein hydrolysate” in Europe and as “casein hydrolysate” in the U.S.

- Is lactium[®] protected by any patent?

lactium[®] is patented by several international patents:

- U.S. patents # 5,846,939
- E.U. patent EP 0714910B1
- Japan # 8.268903

- Has lactium[®] obtained any claim authorization?

lactium[®] is one of the few ingredients in France which has received a claim authorization from the French Safety and Control Administration (DGCCRF*):

“... can moderate the tensional response to stress, in particular for hypertensive persons”.

*DGCCRF: Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes.

IX/ lactium[®] specifications and applications

1. Specifications

- What are the physical characteristics of lactium[®]?

lactium[®] is a white cream powder. Different bulk densities are available for lactium[®] regarding the application that you require, from a 0.2 density to a density greater than 0.5.

- How does lactium[®] taste?

lactium[®] has a slight bitter taste but has a neutral organoleptic profile, in comparison to typical protein hydrolysates.

- What is lactium[®]'s shelf life?

lactium[®] is stable for a three-year period at 25°C with a 70% relative humidity and this shelf-life has been validated by a CDB test.

- Is lactium[®] water or fat soluble?

lactium[®] has an amphipatic profile, which means that lactium[®] has affinity for both aqueous and lipid phases. At the recommended dosage, lactium[®] solubilizes correctly into water.

2. Applications in dietary supplements

- In what dietary supplements can we use lactium[®]?

lactium[®] characteristics have been optimized to comply with the different applicative demands. Its powder characteristics have been improved (density, compressibility,...) for use into capsules, sticks, tablets and chewing-gums. lactium[®] is also adapted to uses into soft-gels and drops.

- Is lactium® activity preserved in these applications?

The implementation of lactium® into the previous end-products do not alter lactium® efficiency. lactium® activity has been controlled on different products commercialized.

3. Applications in food

- In what functional food can we use lactium®?

lactium® incorporation into the following products has been validated by our technical team:

- Fresh, fermented and long life dairy products (acid dairy drink, yoghurts, UHT milk,..)
- beverages (fruit juices, teak drink, ...)
- Confectionery products (milk chocolate, dark chocolate, sugar free chocolate)
- Bakery products (biscuits, crisps, ...)

- Is lactium® active within these food products?

Our technical team has checked the activity of lactium® in the previous matrixes. lactium® remains active despite of drastic processes, corresponding to extreme heat tratements:

- baking-extrusion: 120° C/20s
- baking: 180° C/15min
- pasteurization: 95° C/5min
- UHT: 140° C/few seconds
- Freezing: -40° C

lactium® is also applicable in a wide pH-range: 2-8.

X/ Market segment opportunities

- What market segments do you target?

Our marketing team has thought of many market opportunities to which lactium® could respond, such as:

- Weight control (stress is often associated to appetite disorders),
- Tobacco-detoxification (stress-related periods),
- Performance optimization (by limiting sleep troubles).

Concepts with lactium® are available upon request.

XI/ INGREDIA's services

- How to develop a product with lactium®?

INGREDIA creates a real partnership with its customers and offers a wide range of technical services. Our application team is committed to teach you more about our ingredients functionalities. We also help you develop your own product, make our semi-industrial pilot tool available and help you optimize the process and recipe of

your end-product. INGREDIA also brings you analytical support, to guarantee the activity of lactium[®] within your application.

- How to communicate about lactium[®] in our end-products?

To bring credibility to your products, INGREDIA suggests a co-branding program consisting in the use of lactium[®] trademark and logo on your packaging. We also share our scientific documentation, to help you emit clear and consistent messages to consumers. We also bring you information on regulation and recommendations on labeling.

XII/ Conclusion: awards

- How do you guarantee lactium[®] success?

lactium[®] is an innovative and patented ingredient supported by clinical evidence, flexible in application and complying with regulations. As an acknowledgement by the profession, lactium[®] has received several awards. lactium[®] has notably won the 2004 HIE Bronze Award for “Best Innovation in Health Ingredients”.