

**ANALYSIS OF THE CHRONIC
PSYCHOPHYSIOLOGICAL EFFECTS
of ING 911**

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Summary

Objective: Study of the effects of a 150-mg daily dose of ING911, taken for 30 days by healthy subjects, on the baseline psychophysiological condition and psychophysiological reactions caused by a Stroop test of divided attention task.

Method: Psychophysiological effects of a single 150-mg daily dose of ING911 taken in the evening for 30 days were studied in 52 healthy subjects, 25 men and 27 women, randomly divided into two gender-stratified groups, ING911 and Placebo. Subjects were involved in 4 identical tests prior to the first nutriment consumption (D0), after 10 days and 30 days of nutriment consumption (D11 and D31, respectively) then after 12 wash-out days (D43). At every test, the baseline psychophysiological condition was assessed through measurements of heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure, urinary cortisol excretion, basic state-anxiety (trait-STAD), perceived stress (Cohen test) and behaviour (Vitaliano test). The reactivity to the laboratory stress was assessed during a 5-minute exposure to a cognitive conflict (Stroop test) following a relaxation phase and before a recovery phase of 5 minutes each one. At the end of each of these three phases, HR, SBP, DBP, state-anxiety (state-STAD), activation (Thayer test) and endocrine activation (salivary cortisol) were measured. Side effects were evaluated using the Hopkins checklist after 30 days of nutriment consumption.

Results: The ING911 nutriment calms the pressure stress response. No significant effect on baseline cardiovascular parameters or on psychological parameters was evidenced.

The analysis in low and high stress responders allowed to confirm the calming effect on the pressure stress reactivity and showed a contrasting effect on the heart rate reactivity which could reflect an action on the opiate system of cardiovascular stress response regulation.

In addition, ING911 seems to have a "stabilizing" effect on the sleep.

No side effect was observed after a 30-day consumption of the nutriment, or 12 days after having stopped the nutriment consumption.

Contents

INTRODUCTION	4
GENERAL INFORMATION	5
STRESS.....	5
MATERIAL AND METHODS	10
PURPOSES OF THE EXPERIMENT	11
STUDY PROTOCOL.....	11
SUBJECT SELECTION	11
SUBJECT RANDOMISATION	12
PRODUCTS USED	13
TEST PROCEDURE.....	14
STRESS METHOD USED.....	15
COLLECTION AND TREATMENT OF PHYSIOLOGICAL VARIABLES	16
COLLECTION AND TREATMENT OF PSYCHOLOGICAL VARIABLES	17
DATA PROCESSING	19
STATISTICAL ANALYSIS	19
RESULTS	21
RESULTS	22
CARDIOVASCULAR REACTIONS.....	23
ENDOCRINE REACTIONS.....	31
PSYCHOLOGICAL REACTIONS	34
SLEEP STUDY	40
SUBJECTIVE SIDE EFFECT ANALYSIS.....	43
FURTHER ANALYSIS IN HIGH AND LOW RESPONDERS.....	45
DISCUSSION-CONCLUSION	50
OVERALL QUALITY OF THE EXPERIMENT	51
COMPARABILITY OF THE EXPERIMENTAL GROUPS ON D0	51
STRESSOR EFFICACY	52
RESULTS	53
SIDE EFFECTS	55
RATIONALE OF THE ING911 DAILY DOSE	55
CONCLUSION.....	57
BIBLIOGRAPHY	58

Introduction

General information

The main objective of this study is to evaluate the effects of the 30-day consumption of a nutriment (ING911, milk hydrolysate) on the baseline psychophysiological condition and the reactions caused by a Stroop test of disturbed attention task. This study is based on an experimental model of laboratory stress defined according to the current state of scientific knowledge.

Stress

Definition

Nowadays, the word "stress" defines the body response to physiological or psychological stress factors (it will be used with this meaning hereafter) and not the stress factors themselves which are qualified as "stressors".

The stress concept appeared at the beginning of the XXth century. It was conceived by Walter Cannon who showed that physiological modifications accompanying fight or escape were due to adrenalin release by the adrenal medulla, and by Hans Selye who described in 1936 the "general adaptive syndrome" (Selye, 1973). This syndrome, not stressor-specific, includes three successive phases: alarm, resistance, exhaustion. The scientific developments have contributed to show the many mechanisms involved in the central nervous system (Chrousos & Gold, 1992) including various nuclei of the brain stem and base, the limbic system and the cortex as well as the neurovegetative system, the endocrine system, in particular the limbic-hypothalamic-pituitary-adrenal axis or HPA (Yehuda, 1997) and the immune system (Ader, 2000). Thanks to these contributions the stress neuropsychimmunology concept was created.

Stress has been an essential adaptive motor in the primitive living conditions as it allows the body to be ready for escape or fight in front of the attacker by immediately activating the sympathetic neurovegetative system (increased cardiac flow, distribution of the blood mass for muscles to the detriment of digestive areas, increased vigilance...). In the present living conditions, the stress is useful because it stimulates the central nervous system; it is then called *eustress*. However, it can become pathogenic inducing atherogenesis, immune depression and cancer. It is then called *distress*.

Biological expression of stress

Stress induction

Stress is the expression of very complex biological mechanisms. (Table No.1). Nevertheless, some appear particularly more significant either for historical (Cannon and Selye), methodological (easier exploration in human) and biological reasons (their worsening modifies the stress reaction quality). For instance, usually two main systems are distinguished: (i) the sympathetic nervous system which allows the central nervous system (vigilance increase) and the body (adaptation to fight or escape constraints) to react quickly, (ii) the hypothalamic-pituitary-adrenal axis, which can moderate the effects of the first one thanks to its delayed action mode. Both system activation at the central level involves a complex network which integrates emotional (*via* the limbic system), mnemonic (*via* the hippocampus), visceral (*via* the solitary tract nucleus), metabolic and vascular (*via* the circumventricular organs of the third ventricle), sensory and thermoalgesic (*via* the periaqueductal gray substance and the thalamus) information.

Peripheral consequences of both system activation are also complex. There are synergistic interactions between both systems at the secretion level (i.e., there is an adrenergic innervation of the adrenal cortex that increases the ACTH activating effect) and at effector level (i.e., the

adrenalin-induced glucose release by the liver will be all the more important that cortisol will have played a permissive role).

	Sympathetic nervous system	Hypothalamic-pituitary-adrenal axis
Structures and mediators	Locus Coeruleus: <i>noradrenalin</i> ----- Lateral hypothalamus Medulla sympathetic connections Sympathetic nerves: <i>noradrenalin</i> Adrenal medulla: <i>adrenalin</i>	Central CRF stimulating pathways Hypothalamic paraventricular: <i>CRF</i> Anterior pituitary: <i>ACTH</i> Adrenal cortex: <i>cortisol</i>
Effector	Cardiovascular system Metabolism Ventilation	Metabolism Central nervous system
Time of intervention	About one second	Superior at 3 minutes
Length of activation (exceptional stressor)	Some tens seconds	Several tens minutes

Table 1
Summary table of biological activations during the stress

Biological consequences of stress

Prolonged stress can prove to be noxious for the body which is experiencing it. The whole body is then affected.

In the central field, if the stress improves short-term performances, it involves sleep (Buguet, Bourdon, Canini, Cespuglio, & Radomski, 1999), memory (de Quervain, Roosendaal, & McGaugh, 1998) and food (Hotta, Tamotsu, Arai, & Demura, 1999) disturbances. In the long term, dysfunctions and even lesions of the central nervous system were described in subjects presenting long term high serum concentrations of glucocorticoids (Sapolsky, 1996)

In the cardiovascular field (Hjemdahl, 2000) long term activation of the sympathetic system could lead to many pathologies. Angor and myocardial ischemia are representing the first risk (Glassman & Shapiro, 1998). Sympathetic stimulation activates atherogenesis appearance, facilitates platelet aggregation, induces arrhythmia and can lead to myocardial ischemia onset by increasing the needs of the myocardium for oxygen and metabolites and by reducing its contribution. So, the angor incidence is significantly higher in "type A" individuals whose ambitious character, competitive spirit in permanent fight with the associates are representing a true risk factor (Shaw & Dimsdale, 2000; Sparagon, *et al.*, 2001). In addition, many studies have highlighted the role of the professional or psychosocial stress in some hypertension (Vrijkotte, van Doomen, & de Geus, 2000).

In the digestive field, the stress modifies digestive secretions and motility (Murison, 2000b), causes biliary dyskinesia, lead to gastro-duodenal ulcer and often underlies evolutionary recurrence of ulcerative colitis or Crohn's disease (Murison, 2000a).

In the endocrine field, the stress upsets all the balances: the gonadotrophic function is inhibited, explaining the classically observed anomalies of the menstrual cycle, the adrenocortical system is activated (Sapolsky, 1997) decompensating or starting type-1 diabetes or Cushing's diseases, the calcium/magnesium balance is disturbed deteriorating the neuromuscular excitability resulting in spasmophilia signs (Boulenger, 1985), etc.

The immune system is also affected by the stress. Many interactions between stress-released mediators and immune factors were described (Cacioppo, Malarkey, & Kiecolt-Glaser, 1995).

Psychological expression of stress

General information

Stress is the physiological reaction of the body towards a situation perceived as threatening. This threatening connotation results from an emotional and cognitive evaluation of the situation which depends on individuals. This leads to define personal adaptive strategies towards stressor and the cognitive constraint evaluation.

Schachter and Singer work showed, in 1962, that the Cannon and Selye physiological model was insufficient. The stress would not be only responding to a catastrophic event, but would also relate to the whole perceptions of inability and malaise which overcome the individual when he is confronted with an event that he does not control. So, there are two aspects that must be separated: (i) Lindsay and Norman (1980) mentions that "*in a stressing situation, the subjective evaluation of the situation is more important than the objective facts*". Thus, it is more the interaction between the agent stressor and the individual which is important than the stressing event or the individual himself. This interaction was modelised by Cohen *et al.* under the name of "perceived stress" (Cohen, Kamarck, & Mermelstein, 1983). For them, the stress "... *is not a survival reaction proceeding in a stereotyped way whatever the stressor agent, but it is a compromise which first implies the perception and the interpretation of the situation*" (Dantzer, 1989). (ii) In addition, we nowadays traditionally admit that the noxious psychophysiological stress reactions are related to a constraint that we have not been able to control (Laborit, 1976; Laborit, 1986). Thus, the research about the professional stress has focussed on the congruity between the individual and his environment, the possibility of environmental control and the individual's liberty of action. The stress effect is then measured in term of environmental control quality. On a physiological level, Frankenhauser *et al.* had already made a difference between the neuroendocrin consequences resulting from a control attempt (concomitant with catecholamine release) and from a control loss (concomitant with plasma cortisol level increase) when facing the same stressing situation. On a psychological level, Rodin and Salovey, 1989, have showed that the feeling of controlling the situation was significantly associated with a favourable conclusion and the feeling of control loss with an unfavourable outcome.

Psychological and physiological conditions are thus proving to be interdependent: an attempt to keep the control would be associated with an activation of the sympathetic and adrenal medulla system, whereas a resignation would be associated with an activation of the hypophysis-adrenal cortex system (Laborit, 1986). In the same spirit, Dantzer has distinguished the notion of *effort* when we face up associated with a catecholamine increase, and that of *distress* (dissatisfaction, impatience, irritation, trouble), associated with an increase in the cortisol production (Dantzer, 1989).

Coping theory

This stress analysis thus led to define a new psychological theory corresponding to the facing methods of the individuals confronted with an aggression. English speakers name it *coping* or *personal coping mechanism* which defines "all the processes that a subject interposes between himself and the threatening-perceived event to control, tolerate or decrease its impact on his own physical and psychological wellbeing" (Paulhan & Bourgeois, 1998)

The coping theory gathers all the work relating to the various cognitive filters used by the individual for facing up to the stressor and trying to control it. This relation is fundamental because it implies that the stress can be explained neither through the stressor, nor through the psychophysiological reaction, but through their relation materialised in intermediate processes ("filters") that the body interposes between the aggression and itself. These factors are studied using questionnaires such as Vitaliano questionnaire which questions the subject about control methods he adopted towards a difficulty met in the last week (Paulhan, Nuissier, Quintard, Cousson, & Bourgeois, 1994; Vitaliano, Russo, Carr, Maiuro, & Becker, 1985).

Low and high stress responders

Recent studies showed that individuals could be classified according to their stress response profile which appears reproducible for the same individual confronted with stressors of the same intensity (Negrao, Deuster, Gold, Singh, & Chrousos, 2000). Subjects can be classified as low and high responders depending on the intensity of the HPA axis activation (Kirschbaum, *et al.*, 1995; Petrides, *et al.*, 1997; Roy, Kirschbaum, & Steptoe, 2001) or the cardiovascular response to the stressor, both responses being of the same type (Cacioppo, Malarkey, & Kiecolt-Glaser, 1995; Gerra, *et al.*, 2001). The highly responsive subjects seem to be more liable to develop stress-related pathologies in particular via immune system modifications (Cacioppo, Malarkey, & Kiecolt-Glaser, 1995).

Laboratory stress effects

General information

Laboratory stress study is particularly difficult owing to the importance of the cognitive and environmental factors involved in the regulation of the emotive reactions. Thus, the various "space station" tests during which some volunteers are locked for a few months in a space simulator, are failing simply because the subjects being aware that they are on the ground cannot believe in the experiment reality.

Stressors

In the everyday life, stressors are physical (noise, temperature, excessive effort, surgery) or psychic (relational stress, inadequacy of the individual to his environment, perception of a physical risk). They are due to professional life (employment insecurity, conflicts, insufficient organisation, bad definition of the task, urgency, excessive or insufficient workload ...), social life (emigration, change of social status.) or personal life (financial embarrassment, bereavement of a close relation, adversarial relationship...).

In laboratory, it is usual to try to reproduce these stressors if possible in a reproducible and calibrated way. Among those, we can use following physical stressors:

- noise (from 80 dB)
- climatic environment, in particular the room temperature (Buguet, Bourdon, Canini, Cespuglio, & Radomski, 1999).
- intense and/or prolonged physical exercise (Buguet, Bourdon, Canini, Cespuglio, & Radomski, 1999; Negrao, Deuster, Gold, Singh, & Chrousos, 2000; Singh, Petrides, Gold, Chrousos, & Deuster, 1999)
- exposure to a situation perceived as dangerous like the parachute jump.

Any mental stressors are also used in laboratory. They can be mental calculations (Stoney & Hughes, 2001), oral questionnaires or examinations (Ennis, Kelly, Wingo, & Lambert, 2001) audiovisual tests of attention like the "Stroop Color-Word Interference test " (Gerra, *et al.*, 2001), casino game simulation (Meyer, *et al.*, 2000) or finally tests of public conversations which are generating stressing social situation such as to speech before a live audience, to introduce oneself to a critical public, to expose oneself to stressing relational situations (Larson, Ader, & Moynihan, 2001). The psychic pressure generated by these stressors is accentuated by using various means: by requiring prompt answers, by blaming for the erroneous answers systematically (making feel guilty), by associating every error with a painful sensory stimulation, by not taking into account the too slow answers (frustration), or by quantifying the results (competition).

Laboratory stress measurement

Various tools are used to characterise the different components of stress reaction: questionnaires of psychology, endocrine assay and physiological measurements.

Psychological measurements are based on questionnaires whose information can be crossed and selected according to the adopted psychological theory. For instance, within the scope of the coping theory, we use a perceived stress scale (Cohen questionnaire), a coping strategies questionnaire (Vitaliano questionnaire), and an internal-state questionnaire (anxiety with the Spielberger questionnaire, mental activation with the Thayer questionnaire, etc).

Endocrine response measurement is based on the cortisol, adrenalin, urine noradrenalin, plasma or saliva assay. Otherwise, prolactin or 1β interleukin can also be assayed (Icardo, *et al.*, 2001). But they are always in the research scope.

Physiological effects of stress are usually measured on cardiovascular and respiratory systems. The cardiovascular consequences of stress are related to the sympathetic activation which causes a rise in the blood pressure with an increase in peripheral vascular resistances and an acceleration of the heart rate (Larson, Ader, & Moynihan, 2001). They are studied by recording the heart rate, by measuring the systolic and diastolic blood pressures, the mean or "useful" pressure and differential blood pressure which expresses the artery wall effort, likely to induce a vascular pathology. The spectral analysis of the instantaneous heart rate variability was recently suggested to study cardiac regulatory factors such as the sympathovagal balance corresponding to the ratio of activating sympathetic /slowing down parasympathetic activities (Dishman, *et al.*, 2000; Porges, 1995). The respiratory effects of anxiety and stress can also be analysed (amplitude, frequency, abdominal ventilation part in total ventilation) (Boiten, Frijda, & Wientjes, 1994).

Other physiological effects were described but must be cautiously used such as the increase in sweat secretion of palm and sole resulting in a modification of cutaneous electric conductivity (Turpin & Harrison, 2000).

Methodology difficulties

Laboratory stress exploration comes up against methodological difficulties, some that can be avoided and others that cannot be resolved. Thus, we can point out:

- Interference of measurement in stressor perception.
- Stressor standardisation
- Stressor credibility. Beyond the subject investment in the experimentation, most of the studies try to put him in realistic situations making the stressor credible: request for performance (Moradi, Tagha, Neshat Doost, Yule, & Dalgleish, 1999; Stuss, Floden, Alexander, Levine, & Katz, 2001), examination (Spangler, 1997), casino game (Meyer, *et al.*, 2000), etc
- Separation of the anticipatory stress emotion of a first test. This effect of anxious anticipation is visible during the first exposure to the stressor and decreases as the number of stressor exposure increases. Analysis of the nutriment effects must take into account this distortion. In this study, the resting values, used as reference to measure the cardiovascular and psychological reactivity to the stressor, were measured at the end of the relaxation period following the stress period, allowing to limit the distortion due to anticipation.
- The uniqueness of the individual. This feature leads to work more on variations within a same individual than on differences of population of which the statistical homogeneity poorly hides the high interindividual variance. This variability is accentuated if the notion of preferential channel is considered, expressing the fact for example that an individual responds mainly either by a blood pressure or a heart rate increase.

Material and methods

Purposes of the experiment

The purpose of the experiment is to study the effect of prolonged consumption of ING911 on the cardiovascular reactivity during an acute stress. The primary criterion is the importance of the variation of cardiovascular variables at the beginning of stress exposure.

The secondary purposes of the experiment are, in order of consideration: (i) to evaluate the effect of prolonged consumption of ING911 on cardiovascular, endocrine and psychologic variables after exposure to the constraints of the everyday life, (ii) to evaluate the effect of prolonged consumption of ING911 on psychic and endocrine reactivity during an acute stress. The secondary criteria are resulting from these objectives.

Study protocol

The study is a comparative randomised trial of a nutriment, the ING911, versus a Placebo on two groups of unpaired subjects, gender-stratified, with a double blind procedure for nutriment attribution.

It is carried out over 45 days and comprises four identical psychophysiological tests separated by an exposure to the everyday life of 10, 20 and 12 days. On D0, the subjects are submitted to a first test evaluating their stress reactivity before nutriment consumption. After D0 they take nutriment for 30 consecutive days during which they are submitted to other two tests on D11 and D31, i.e. the day after both the 10th and 30th days of nutriment consumption. On D43, i.e. twelve days after the stop of nutriment consumption, the subjects are submitted to a last test evaluating their final reactivity beyond the pharmacological effect of nutriment consumption. At the end of this final test, the experimentation is completed and a 14-day regulatory exclusion period begins. During the inter-test periods, the subjects have to report in a diary their nutriment consumption and the significant events in order not to forget taking their dose and to assess their intercurrent events.

Subject selection

Subjects were recruited by way of advertisements in public places (Faculties of Medicine and Pharmacy, UFRAPS,...). Experimentation was detailed to voluntary subjects and they were shown the devices used for the experiments before obtaining their express informed consent. The informed consents were filed in the personal file of every subject. The subjects had never been confronted with this type of experiment. Their eligibility to the experiment was decided after a complete medical examination and an ECG.

Inclusion criteria

- volunteers aged 18-40 who have signed informed consent for their participation
- normal clinical examination;
- normal electrocardiogram;
- resting heart rate between 50 et 80 bpm ;
- absence of blood pressure: SBP < 140 mm Hg and DBP ≤ 80 mm Hg;
- absence of overweight: body mass index < 25

Non-inclusion criteria

- minor subjects, protected subjects over 18 and pregnant women in accordance with article L-209 of the amended law of December 20, 1988;
- dairy product allergy;
- significant consumption of fermented dairy products (> 2 yoghurts per day);
- known Raynaud's syndrome;

- coronary artery disease;
- current medical treatments, including analgesic-type treatment;
- usual practice of martial arts and relaxation techniques (yoga, sophrology, etc.)
- night-work;
- weekly alcohol consumption over 150 g;
- tabagism over 1 packet daily;
- positive HIV or HCV or HBV serology;
- current participation in another clinical study.

Psychophysiological profile of subjects

At the end of the inclusion visit, subjects have completed a series of inclusion questionnaires comprising:

- a Bortner questionnaire allowing to judge subject A/B classification;
- a Home and Osberg morningness and eveningness questionnaire allowing to know if the subject is rather a "morning subject" or an "evening subject".
- a questionnaire for estimating the sport practice level of subjects

Subject randomisation

At the end of medical visit, 58 subjects were eligible of which 53 have really taken part in the experiment, 27 women and 26 men. Subjects were randomised in two groups of 8 subjects, ING911 versus placebo. A gender-stratification was performed within every group. The randomisation led to the distribution shown in Table 2

	Man	Woman	Total
Placebo	12	14	26
ING911	14	13	27
Total	26	27	53

Table 2
Inclusion distribution of subjects

A subject from placebo group was excluded before D31 due to anxiety symptoms with somatisation. This exclusion was performed on medical decision without code opening by the investigators. The opening of the subject code was made by the pharmacist in charge of the randomisation management and was communicated to the subject. This exclusion led to a population of 27 women and 25 men divided as mentioned in Table 3

	Man	Woman	Total
Placebo	11	14	25
ING911	14	13	27
Total	25	27	52

Table 3
Subject distribution in the experiment

No voluntary withdrawal from the study was noted. The analysed population was defined according to the technique of the given treatment: all the subjects included in the experiment which have completed it were taken into account in the analysis.

It was a double blind experiment in order not to create distortion for interest variables.

Time distribution of subjects

Ten experimental series of six subjects were defined. Experimental slots of every test day were defined on the basis of 3 subjects tested in the morning (8 am, 9.30 am, 11 am) and 3 subjects tested in the afternoon (1 pm, 2.30 pm, 4 pm). Experimental slots were proposed to the subjects as follows:

1. experimental series were chosen by the subjects according to their availability so that they can certainly be present in all the tests of the series without changing the date.
2. subjects of a same experimental series in the course of the day were distributed depending on their possibility as well as according to the outcome of the Horne and Osberg questionnaire. The clearly morning or clearly evening subjects were rather tested at the end of the morning or at the beginning of the afternoon respectively so that their reactivity could be comparable to that of the intermediate subjects.

Products used

General information

Products were available in bottles containing 30 capsules coded by a letter representative of the sex (F, M) and a number (1 to 28). The correspondence between the codes and the capsule contents was kept by the pharmacist, independent investigator in charge of the nutriment randomisation. The products were stored in a closed cupboard of the laboratory at a temperature lower than 25°C and a relative humidity lower than 70%. The products were used within 12 months after the manufacturing date. The unused products were returned to INGREDIA when the experiment was completed.

Products (ING911 and Placebo) were given for 30 consecutive days on the basis of one capsule per evening at bedtime. The whole products were delivered to the subject after the first test, i.e. 30 capsules corresponding to the 30 days of nutriment consumption. Subjects were requested to keep these capsules in a dry and fresh place.

Subjects received their pill box at the end of D0 and signed the diary proving that they personally received the product. They checked that the pill box number was corresponding to their randomisation number. Every subject controlled his consumption every day by ticking off on a diary the exact number of capsule taken.

Placebo product

Placebo is presented as identical 150-mg capsules containing skimmed milk powder.

ING911 product

ING911 product is a 150-mg capsule containing powdered alpha S1 casein hydrolysate (batch No.: 067101). It is manufactured by the INGREDIA Company in Arras (62). This is a nutriment whose main characteristics are as follows:

Physicochemical analyses:

- Humidity:	5.3 %
- Fat content:	< 0.1 %
- Nitrogen content:	74.9 %
- Mineral content:	19 %
- Lactose (by difference):	0.8 %

Bacteriological analyses:

- Total germs: 100/g.
- Coliform bacteria: Abs/g.
- E. Coli: Abs/g.
- Spores of clostridium SR: Abs/g (24 hours at 46°C).
- Staphylococcus aureus: Abs/g.
- Salmonellas: Abs/25 g.
- Yeasts and Moulds: Abs/g.

Test procedure

All test procedures of a same experimental series are identical (Table 4). The day before, subjects had a "normal" activity, avoiding in particular during the afternoon to practise any sport, to take coffee, alcohol and tobacco, they were going to bed at the usual time after having emptied their bladder.

On the morning of the test, subjects collected their urines in a bottle placed at their disposal for this purpose as soon as they are rising from bed. If necessary during the night, urines were also collected so that urinary volume corresponds to urines of the night. Then, they informed the questionnaire on sleep (Buguet) and took their breakfast normally before going to the laboratory.

Arrived at the laboratory, the subjects give the experimenters the duly informed questionnaire on sleep, which is filed in their file, as well as the urine bottle which is treated immediately. They also present the diary on which they confirmed the nutriment consumption. Then, the subjects are led to a quiet room to inform initial questionnaires: the Vitaliano coping questionnaire, the Cohen perceived stress scale, the Spielberger Trait Anxiety Questionnaire and finally, the Hopkins symptom checklist (only on D0, D31 and D43). The subjects are then sitting down comfortably, in thermal neutrality, for approximately half an hour during which they are proposed to look at a video with neutral affective value.

At the end of this period, they are going to the test room. The armband to measure blood pressure is installed. After having equipped subjects with thoracic electrodes, an electrocardiogram is recorded continuously until the end of the experiment using a Temec™ -type recorder

1) "Ambulatory" parameter measurement: The first measurement of blood pressure and heart rate is taken, to assess "Ambulatory" values under the current living conditions. Then the subjects remain quiet for 10 minutes again and look at a video with neutral affective value (documentary on Zen civilisation, series "Grandes civilisations").

2) Initial relaxation period R: a first relaxation period (period R) of 5 minutes is performed. During the sixth minute of relaxation, blood pressure and heart rate are measured (R6). Then the subjects inform the questionnaires (Thayer activation questionnaire, Spielberger state anxiety questionnaire) while a saliva sampling is taken using a Salivette.

3) Period of stress S: At the end of this phase, video is stopped, the armchair of subjects is then turned towards the computer on which the Stroop test is implemented and applied for 5 minutes. During this period, like during the immediately following 3 minutes, blood pressure and heart rate are measured every minute (S1 to S5 and P1 to P3 measurements). During the three minutes following the Stroop, subjects inform the questionnaires (Thayer activation questionnaire, Spielberger state anxiety questionnaire) while a saliva sampling is taken. The recording of heart rate and blood pressure during the first three minutes after the end of the test (P1 to P3) called "period of initial recovery", allows to analyse the kinetics of return to baseline state of these cardiovascular variables.

4) Period of recovery Z: After this period, the subject remains quiet for 10 minutes under the same conditions as during the phase R in order to relax. Then, a second relaxation period of 5 minutes (period Z) is performed. During the sixth minute of relaxation, a final measurement of blood pressure and heart rate (Z6) is taken. Lastly, subjects inform questionnaires (Thayer

activation questionnaire, Spielberger state anxiety questionnaire) while a saliva sampling is taken using a Salivette.

Blood pressure and heart rate values measured in Z6 were regarded as reference values at rest (to avoid a possible distortion due to anticipation regarding values measured at the initial period R). Similarly, the Thayer activation scores and state-anxiety scores obtained at this period Z were used as reference scores.

At the end of the test, subjects equipment is withdrawn and they can return to their usual occupation without particular instruction or recommendation, the submitted tests are not affecting their medico-psychological state.

Duration of recording phases		(R)	(S)	(Z)
		# 50 min 5 minutes	# 10 min. 8 minutes	# 20 min. 5 minutes
Measurements	Ambulatory	Reference (R)	During test (S)	Recovery (Z)
Questionnaires	- Sleep - Coping of Vitaliano - Perceived Stress of Cohen - Checklist of Hopkins	- Activation of Thayer - Anxiety " state " of Spielberger	- Activation of Thayer - Anxiety " state " of Spielberger	- Activation of Thayer - Anxiety " state " of Spielberger
Cortisol	urinary	salivary	salivary	salivary
BP	yes	yes	yes	yes
ECG	no	continuous	continuous	continuous

Table 4
Summary table of biological measurements taken during experiment

Stress method used

The selected activation method is the Stroop test of mental conflict (Stroop, 1935). This test involves significant mental effects with cognitive sensory conflict generating a moderate well reproducible stress. Consequently, it was used in many studies concerning the psychophysiological reactivity (Gabr, Birkle, & Azzaro, 1995; Gerra, *et al.*, 2001; Linde, Hjemdahl, Freyschuss, & Juhlin-Dannfelt, 1989; Moradi, Tagha, Neshat Doost, Yule, & Dalgleish, 1999; Naesh, Haedersdal, Hindberg, & Trap-Jensen, 1993).

Subjects must type with a finger of the dominant hand on the coloured keys of the keyboard (blue, green, yellow or red) as series of four colours written with words of different colours are scrolling on the computer. Every good answer and every error are recorded and indicated by two different ringings. The tape speed, initially fixed at "8", varies in the course of time depending on the performance of subjects: many errors lead to reduce the tape speed whereas a few errors are accompanied by the opposite phenomenon.

Performance is summarised by the total number of answers. This number is proportional to the number of right answers because the speed of presentation is automatically reduced in the event of error.

Collection and treatment of physiological variables

Cardiovascular variables

Collection of cardiovascular variables

Systolic and diastolic blood pressures as well as heart rate are measured by a Dinamap apparatus (Critikon, Tampa, Florida) whose automatic armband is placed on the non-dominating arm (Larson, Ader, & Moynihan, 2001; Lovallo & Al'Absi, 1998). Measurement is taken before the phase R (Ambulatory), at the end of the phase R (R6), every minute during Stroop test and during the following three minutes (S1 to S5 and P1 to P3) as at the end of phase Z (Z6). The measured variables are diastolic (DBP) and systolic (SBP) blood pressures. They are expressed as mm Hg.

Derived cardiovascular variables

The first-order calculated variables are differential ($P_{diff}=SBP-DBP$) and mean ($MBP=DBP+1/3(SBP-DBP)$) blood pressures.

Vascular stress pressures are assessed by the mean of pressures measured during the first three minutes of stress. Vascular pressures of relaxation and recovery retained to be analysed are those measured during the sixth minute of relaxation of the periods 'R' ('R6' measurement) and 'Z' ('Z6' measurement). Vascular parameters of initial recovery were assessed by the mean of measurements taken during the first three minutes after the end of the stress.

The cardiovascular stress reactivity was calculated as the difference between the stress value and the reference value measured at the end of the second relaxation period in Z6.

Endocrin variables

Urinary cortisol

Urinary cortisol is measured on the urine collection of the night preceding the test. The 3 urine sampling are kept at -20°C . They are used to assay creatinine and cortisol content, the third sampling being set aside. Cortisol is assayed according to a radioimmunological solid phase technique (Kit RIA cortisol ref. TKC050, Dade Behring, Paris La Défense, France) using cortisol solubilising (ref. 25COZ, batch No. 37A, expiry date 31/12/2001, Dade Behring, Paris La Défense, France) and immunoassay control serums (ref. CONO, batch 17, expiry date 31/08/2001, Dade Behring, Paris La Défense, France). Creatinine is assayed by mean of an automat.

Total cortisol excretion is expressed as the product of the urine cortisol content ($[\text{Cort}]_U$, nmol/l) by the volume emitted during the night (l). The results are expressed as absolute values (nmol/night). The aberrant values were removed on a criterion of inadequate emitted urine volume (partial omission of the subject, etc).

Salivary cortisol

Salivary cortisol content is measured on the saliva samples obtained after relaxation, stress and recovery phases. The saliva necessary for assays is collected by subjects themselves. They keep in the mouth an absorbent plug for the time necessary to be well soaked with saliva, in general between 2 and 3 minutes. The device used ("salivette", Starstedt S.A., Orsay, France) allows the saliva to be collected and then directly centrifuged without contamination. The salivettes used by a subject are kept at 4°C for the test duration. They are centrifuged together and saliva samples are kept at -20°C . The cortisol is assayed according to the above mentioned radioimmunological technique.

Salivary cortisol production is assessed from the salivary cortisol content ($[\text{Cort}]_S$, nmol/l).

Subjects were tested at the same time for 4 test days in order to control the effects of the circadian variability of cortisol.

Salivary cortisol measured at the end of the first relaxation period was considered as resting value of salivary cortisol. Salivary cortisol measured 20 minutes after the beginning of the stress test i.e. at the end of the second relaxation period was considered as stress value of salivary cortisol, the salivary cortisol reactivity being the difference between this measurement and the resting measurement.

Collection and treatment of psychological variables

Bortner questionnaire

The Bortner questionnaire assesses the capacity of the subject to act like an "aggressive rival" or like a "quiet subject", corresponding respectively to classification A and B of Rosenman and Friedman (Bouvard & Cottraux, 1998). The questionnaire was validated in French-speaking population for an mean total score going from 169 to 184. The subject is declared of type A if his score exceeds 190. The derived variable is the total score. The missing answers are replaced by the mean score of the subject.

Sport questionnaire

The sport questionnaire evaluates the predicted maximal aerobic speed from data of biometrics and sport practice intensity (Melin, *et al.*, 1998). Validated in the French population, it allows a reliable assessment of the physical quality of subjects. The derived variable is the predicted maximal aerobic speed.

Home and Osberg questionnaire

The Home and Osberg questionnaire allows qualifying the morning or evening character of subjects (Home & Östberg, 1976). It is self-administrated and was validated in the French-speaking population (Billiard, 1994). When the questionnaire is completed, the subjects are classified in 5 categories: clearly early riser (score from 70 to 86), early riser (score from 59 to 69), indifferent (score from 42 to 58), night owl (score from 31 to 41) and clearly night owl (score from 16 to 30). The derived variables are thus the mean score and mean typology.

Vitaliano coping questionnaire

The Vitaliano coping questionnaire (Vitaliano, Russo, Carr, Maiuro, & Becker, 1985) allows to qualify how the subject thinks of facing a difficulty. This questionnaire is divided into two parts:

1- the first part relates to the subjective scoring on a three-level scale (low, moderate, high) of the everyday life constraints.

2- the second part is a 42-item questionnaire which was validated in the French-speaking population (Paulhan & Bourgeois, 1998; Paulhan, Nuissier, Quintard, Cousson, & Bourgeois, 1994). The derived variables are the scores of each of the 5 axes. Coping method axes are defined as follows:

- + Coping directed towards problem solving: items No. 1, 4, 6, 13, 16, 18, 24, 27
- + Coping directed towards avoiding reaction with positive thought: items No. 7, 8, 11, 17, 19, 22, 25
- + Coping directed towards quest for social support: items No. 3, 10, 15 (ci), 21, 23
- + Coping directed towards positive reevaluation: items No. 2, 5, 9, 12, 28
- + Coping directed towards self-accusation: items No. 14, 20, 26, 29

Missing values are replaced by the rounded off mean score of the subject in the axis in which the missing value is noted.

Cohen and Williamson perceived stress scale

The Cohen and Williamson perceived stress scale or PSS (Cohen, Kamarck, & Mermelstein, 1983) allows to qualify the importance with which the life situations are generally perceived as threatening. This questionnaire was validated in the French-speaking population (Bruchon-Schweitzer & Dantzer, 1998). It was used in its 14-item form.

The derived variable is the sum of scores obtained for every item. The missing values are replaced by the mean score of the subject calculated from the items filled in during the same test and rounded off to the nearest scoring unit.

Spielberger state anxiety and trait questionnaires

Spielberger state and trait-anxiety questionnaires assess the anxiety level of a subject either in general (trait-Spielberger or trait-STAI), or at a particular moment (state-Spielberger or state-STAI). These tests were validated in the French-speaking population. They are distributed by the "Editions du centre de psychologie appliquée" to which they were bought.

The derived variable is the sum of scores obtained for each of the 20 items. In the trait-STAI, missing values are replaced by the mean score of the subject calculated from the items filled in during the same test and rounded off at the nearest scoring unit. On the other hand, no missing values was replaced in the state-STAI owing to the minimal rate of missing values (11 of 12,480, i.e. 0.08%).

Thayer activation questionnaire

Thayer activation-deactivation questionnaire evaluates the vigilance and reactivity level of a subject (Thayer, 1986). The derived variables correspond to the axes of activation and deactivation.

The initial analysis is done on 4 axes defined by the following items:

- Interior tension: uncomfortable, anxious, irritated, tensed, contracted
- Interior relaxation: indifferent, calm, relaxed, quiet, silent
- Overall activation: active, energetic, dynamic, « top form », good form
- Overall deactivation: half-asleep, tired, attentive (reverse scoring), sleepy, alert (reverse scoring).

The secondary analysis is performed by grouping these initial axes into two axes:

- Tension axis: Interior tension / Interior relaxation (scoring TA)
- Energy axis: Overall activation / Overall deactivation (scoring EA)

Sleep questionnaire

The sleep questionnaire was developed by A. Buguet *et al.* (Buguet, 1994) and validated in the French-speaking population (Buguet, Bumblebee, Canini, Cespuglio, & Radomski, 1999; Buguet, Lonsdorfer, Bogui, Yapi, & Eboulé, 1992). It is used every morning of the test days.

It consists of 10 quantified items exploring the overall subjective sleep quality (Q1), the facility and subjective duration of falling asleep (Q2 and Q3), awakening number during the night (Q4), effects on working desire (Q5), physical condition (Q6, reverse scoring), moral (Q7) and mood (Q8). The objective characteristics of the night are described by bedtime (Q9) and rising time (Q10).

The questionnaire is withdrawn from the subjects after every scoring in order to avoid scoring contamination from a test to another.

Hopkins symptom checklist

This checklist allows to assess the overall psychological and somatic effects of a nutritional or medicinal product or a situation (Derogatis, Lipman, Rickels, Uhlenhuth, & Covi,

1974). The derived variables are the total score and the total scores obtained for every axis defined as follows:

- + Axis No. 1, Somatisation: Items No. 1, 4, 12, 14, 27, 42, 48, 49, 52, 53, 56, 58
- + Axis No. 2, Obsession-compulsion: Items No. 9, 10, 28, 38, 45, 46, 51, 55
- + Axis No. 3, Interpersonal sensitivity: Items No. 6, 11, 24, 34, 36, 37, 41
- + Axis No. 4, Depression: Items No. 5, 15, 19, 20, 22, 26, 29, 30, 31, 32, 54
- + Axis No. 5, Anxiety: Items No. 2, 17, 23, 33, 39, 50

Missing values were not replaced because they corresponded primarily to a single subject not having filled in the test on D43.

Data processing

Data entry procedures

Data were manually entered by an operator and checked at various levels of the entry. They were checked according to following procedures:

- cardiovascular data: graphic checking under Excel of all the data for location of the aberrant values and entry faults
- psychological data: checking during entry. The entry was performed on a different Excel sorter from that of data storage. Transfer from the one to the other was made by automatic copy. The entry sorter contained a sheet per test and the operator has to enter only the value "1" in the adequate column. Scorings were automatically deduced while taking into account of possible reverse scorings and automatically carrying out the sums of scoring. Calculation lines/columns were generated allowing to check the absence of a double scoring on the same line or the same column. Some subject data were checked *a posteriori* before validating the entry.
- Endocrine data: values generated by measurement automats were entered manually and checked before calculation.

Data freeze and decoding procedures

Entered values were stored in Excel sorters whose date of last modification is earlier than October 19, date of breaking the blind.

Decoding was carried out after 19 October by opening the envelope containing the codes. The opened envelope remains classified in the experiment file. The intermediate statistical analysis was carried out blindly (nutriment A *vs* nutriment B). Code adequacy to the subjects was checked on personal files on which subject code and pillbox N° appear and adequacy between nutriment type and pill box N° was checked twice by two different people.

Statistical analysis

Statistical analysis regarding qualitative variables such as nutriment distribution throughout series, time windows and parts of the day, involves the Fisher exact probability test (2x2 data) or the panel-corrected Chi2 test.

Comparability of experiment groups on D0 was checked by T tests regarding general characteristics, baseline condition and stress reactivity parameters.

Parameter stability in Placebo subjects during the study was tested by an ANOVA with repeated measurements. When they were positive, a Tuckey HSD test was used for inter-day comparisons.

Similarly, evolution over the time of Stroop test performances was analysed by an ANOVA with repeated measurements (4 repetitions) with two factors (nutriment and *gender*)

The nutriment impact evaluation on the general population was statistically analysed by an ANCOVA with repeated measurements with nutriment effect (ING911 and Placebo) while regarding the D0 value of the analysed parameter as covariant. Secondarily, an ANCOVA with

both nutriment and sex factors was carried out. The result considered is the "nutriment" effect adjusted for "gender" effect. This analysis allows to be free from differences related to genders it is usually observed for cardiovascular and psychological variables (Kudielka, Hellhammer, & Kirschbaum, 2000)

In the case of a significant nutriment /sex interaction on the main criteria of cardiovascular reactivity, a contrast study was carried out and nutriment effect was tested using an F test in every sub-group.

In the case of a significant nutriment /sex interaction on the secondary criteria, the nutriment effect was tested in the sub-groups using a Tuckey post-hoc test.

A residual nutriment effect on D43 was searched by an ANCOVA while regarding the D0 value of the analysed parameter as covariant.

A complementary analysis was carried out to study the ING911 effects in high stress responders (HR) and in low responders (LR). Subjects were classified as HR and LR on the basis of the SBP stress reactivity and the trait-anxiety on D0 according to the k-mean method (MacQueen, 1967), after data standardisation.

In every class of subjects, the nutriment effect was analysed by an ANCOVA with repeated measurements, ANCOVA with both nutriment and stress sensitivity factors with the D0 value of the analysed parameter as covariant.

In the case of a significant nutriment x stress sensitivity interaction on the main criteria of cardiovascular reactivity, a contrast study was carried out and nutriment effect was tested by an F test in every sub-group.

Statistical results were expressed as means (standard deviation), m(SD). Result significance is accepted at $p < 0.05$ threshold and trend at $p < 0.10$ threshold. Significance is expressed according to the code: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; t: $p < 0.10$.

NB: Mean values (SD) of results obtained on D0, D11, D31 and D43 are presented in tabular format, where only inter-group values significantly different on D0 by test t are indicated by initials (t: $p < 0.10$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

Results from the ANCOVA with repeated measurements on D11 and D31 and possible interactions between factors are summarised in a second table.

Results

Results

Subject distribution and biometrics at inclusion

Taking into account the subject excluded for medical reason, 52 subjects were included in the experiment, distributed as reported in Table 3

No difference between nutriments and sex is observed in the subject distribution (i) in experimental series, (ii) in experimental schedules within the same day, whether this distribution is considered as schedule or morningness/eveningness. Most of the subjects (43/52) were of median type, remaining subjects being mainly early riser (morning: 8 and evening: 1).

No significant difference is observed between nutriments for the following morphological variables: age (years), weight (kg), size (cm), body mass index (BMI, kg/m²).

No significant physical difference is observed between nutriments in men and women. Subjects have the same expected maximum aerobic speed (km/h) which corresponds to rather sportive subjects.

No significant difference in total score for the Bortner test is observed between nutriments in men and women. Values are corresponding to non-type A subjects representative of the French population.

Physiological variables are not different between Placebo and ING911 groups except for the ambulatory HR higher in ING911 men than in Placebo men and for urinary cortisol higher in ING911 subjects than in Placebo subjects.

	Placebo group	Ing911 group	Women		Men	
			Placebo	ING911	Placebo	ING911
Population	25	27	14	13	11	14
Age	29.7 (8.1)	29.1 (5.8)	31.4 (8.1)	28.5 (7.0)	27.6 (7.8)	29.6 (4.6)
Weight	63.6 (11.7)	65.3 (11.6)	56.4 (8.7)	56.7 (8.6)	72.7 (8.0)	73.2 (7.7)
Size	169.9 (9.6)	170.7 (11.0)	163.6 (5.8)	162.5 (8.3)	178.0 (7.0)	178.3 (6.9)
BMI	21.9 (2.3)	22.3 (2.3)	21.0 (2.4)	21.4 (2.5)	22.9 (1.8)	23.0 (1.8)
HR	64.7 (12.3)	67.0 (11.7)	71.2 (13.1)	67.3 (14.5)	57.7 (6.4)	66.6 (9.3)*
SBP	118.5 (11.6)	119.4 (15.5)	114.1 (10.4)	108.6 (8.8)	123.3 (11.3)	128.6 (14.1)
DBP	71.5 (6.5)	73.4 (7.0)	71.3 (5.3)	71.5 (7.1)	71.8 (7.9)	75.0 (6.8)
Urin. cortisol	32.1 (22.3)	46.5 (22.9)*	22.6 (10.0)	39.6 (24.4)	44.2 (28.0)	53.0 (20.2)
Bortner	183.1 (21.8)	182.3 (24.8)	185.5 (16.6)	179.6 (27.9)	180.0 (27.6)	184.9 (22.2)
Trait-STAI	35.9 (8.9)	38.6 (9.7)	35.0 (8.3)	40.3 (10.1)	37.1 (9.9)	37.1 (9.4)
Cohen	31.6 (5.5)	32.6 (5.2)	31.1 (5.5)	32.8 (4.5)	32.3 (5.7)	32.5 (5.9)

Table 5 Characteristics of experimental groups

Age (years), weight (kg), size (cm), body mass index (BMI, kg.m⁻²), ambulatory heart rate (HR, bpm), ambulatory systolic blood pressure (SBP, mmHg), ambulatory diastolic blood pressure (DBP, mmHg), urinary cortisol (nMol/night), Comparisons between placebo and ING911 by t test, t: p<0.10; *: p<0.05; **: p<0.01; ***: p<0.001

Stroop test results

For the 4 experimental days, no significant difference in the number of answers is noted between nutriment groups in men and women, during the 5-minute exposure to the stressor. The result increases significantly ($p < 0.001$) as exposures recur. No difference between nutriment groups was evidenced regarding the result improvement.

	Placebo group	Ing911 group	Women		Men	
			Placebo	ING911	Placebo	ING911
D0	275 (45)	270 (37)	288 (47)	256 (40)	261 (41)	284 (29)
D11	316 (46)	306 (43)	319 (41)	296 (44)	314 (54)	316 (41)
D31	338 (44)	331 (45)	345 (38)	322 (59)	329 (51)	340 (26)
D43	354 (45)	345 (46)	355 (41)	329 (55)	351 (50)	360 (33)

Table 6. Total score with Stroop test

Test duration: 5 minutes. Mean (SD). Comparisons between placebo and ING911 on D0 by t test.

Cardiovascular reactions

Cardiovascular reactions during D0

The Stroop test exposure results in a significant modification of cardiovascular parameters ($p < 0.001$).

On D0, ambulatory heart rate (HR) and HR variation under stress are not different between nutriment groups in the general population or in the women sub-group but they are higher in ING911 men than in placebo men (Table 7).

Ambulatory systolic (SBP) and diastolic (DBP) blood pressures and SBP and DBP variations under stress are not different between nutriment groups in the general population or in women and men sub-groups (Table 9 and Table 11).

Heart rate

The ANCOVA with repeated measurements does not show, in the total population, nutrient effect on the ambulatory HR or on the HR variation under stress, on D11 and D31, (Table 8, Figure 1 and Figure 2). The sex-factor consideration does not modify this result.

On D43, no significant difference is observed between nutrients, in the total population, regarding neither the resting HR nor the HR variation under stress.

	Placebo group	ING911 group	Women		Men	
			Placebo	ING911	Placebo	ING911
D0						
Ambulatory (bpm)	64.7 (12.3)	67.0 (11.7)	71.2 (13.1)	67.3 (14.5)	57.7 (6.4)	66.6 (9.3)*
Rest (bpm)	64.3 (8.6)	69.2 (12.4)	68.9 (7.6)	68.2 (13.4)	58.4 (5.8)	70.1 (11.7)**
Stress (bpm)	73.7 (9.6)	82.1 (14.7)*	78.7 (7.8)	79.4 (14.3)	67.7 (8.3)	84.4 (15.2)**
Variation (bpm)	11.5 (6.9)	15.8 (11.8)	13.2 (6.6)	13.1 (12.4)	9.6 (7.0)	18.1 (11.2)*
Recovery (bpm)	62.6 (7.1)	66.6 (12.3)	66.2 (6.9)	66.8 (15.5)	58.1 (4.1)	66.3 (9.0)**
D11						
Ambulatory (bpm)	64.5 (7.4)	67.1 (10.8)	66.9 (6.3)	69.8 (13.6)	61.5 (7.9)	64.7 (7.1)
Rest (bpm)	65.5 (6.3)	66.5 (10.5)	67.7 (6.0)	68.1 (12.5)	62.7 (5.7)	65.0 (8.5)
Stress (bpm)	76.9 (13.3)	82.9 (11.2)	79.2 (9.2)	81.1 (9.6)	74.0 (17.3)	84.6 (12.7)
Variation (bpm)	13.4 (12.4)	17.2 (9.2)	14.2 (7.5)	14.9 (8.3)	12.5 (17.2)	19.4 (9.7)
Recovery (bpm)	63.5 (6.9)	65.7 (9.3)	65.1 (6.5)	66.2 (12.5)	61.5 (7.1)	65.2 (5.3)
D31						
Ambulatory (bpm)	63.6 (5.8)	66.5 (10.0)	65.7 (5.8)	65.8 (12.1)	61.0 (4.8)	67.2 (7.9)
Rest (bpm)	64.8 (6.4)	66.1 (10.8)	67.0 (6.5)	66.0 (13.4)	62.0 (5.1)	66.1 (8.2)
Stress (bpm)	77.6 (12.7)	81.6 (14.0)	79.7 (11.9)	78.3 (11.9)	74.8 (13.7)	84.6 (15.5)
Variation (bpm)	13.7 (12.0)	16.1 (9.8)	15.0 (9.3)	13.8 (8.4)	12.1 (15.0)	18.2 (10.8)
Recovery (bpm)	63.8 (6.6)	65.5 (9.9)	64.7 (6.2)	64.5 (10.9)	62.7 (7.2)	66.4 (9.3)
D43						
Ambulatory (bpm)	63.5 (7.7)	67.5 (9.8)	63.4 (7.1)	67.7 (12.4)	63.6 (8.7)	67.3 (7.2)
Rest (bpm)	64.8 (7.5)	68.3 (9.5)	65.8 (6.9)	67.5 (10.8)	63.5 (8.3)	69.1 (8.5)
Stress (bpm)	74.6 (9.2)	82.5 (10.5)	77.0 (8.8)	79.3 (10.1)	71.6 (9.2)	85.4 (10.3)
Variation (bpm)	10.9 (7.6)	14.6 (9.9)	12.4 (5.2)	13.7 (9.9)	8.9 (9.7)	15.4 (10.2)
Recovery (bpm)	63.7 (6.6)	67.9 (10.6)	64.6 (6.2)	65.5 (12.1)	62.6 (7.1)	70.0 (9.0)

Table 7. Heart rate

Absolute values in bpm, and *variation compared to the reference value at the end of final relaxation.*

Mean (SD).

Comparisons between placebo and ING911 on D0 by t test,

t : p<0.10; * : p<0.05; ** : p<0.01; *** : p<0.001

		1	2	3		1x2	1x3
		Nutrimet	Sex	D11	D31		
Ambulatory HR	ANCOVA 1	0.181		Session			0.972
	ANCOVA 2	0.193	0.765	0.566	0.456	0.123	0.916
HR variation	ANCOVA 1	0.516		Session			0.557
	ANCOVA 2	0.539	0.656	0.455	0.455	0.834	0.560

Table 8 Heart rate

Results (P-values) of ANCOVA with repeated measurements (D11-D31 session) with the D0 measured value as covariant.

ANCOVA 1: 1-factor (Nutrimet) ANCOVA with repeated measurements.
 ANCOVA 2: 2-factors (Nutrimet and Sex) ANCOVA with repeated measurements.
 1x2: interaction between nutriment and sex effects
 1x3: interaction between nutriment and D11-D31 session effects

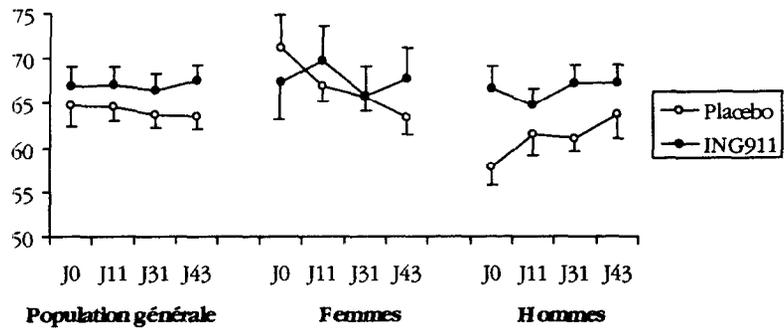


Figure 1. Ambulatory heart rate (bpm)

Mean ± mean standard deviation, ● ING911, ○ Placebo

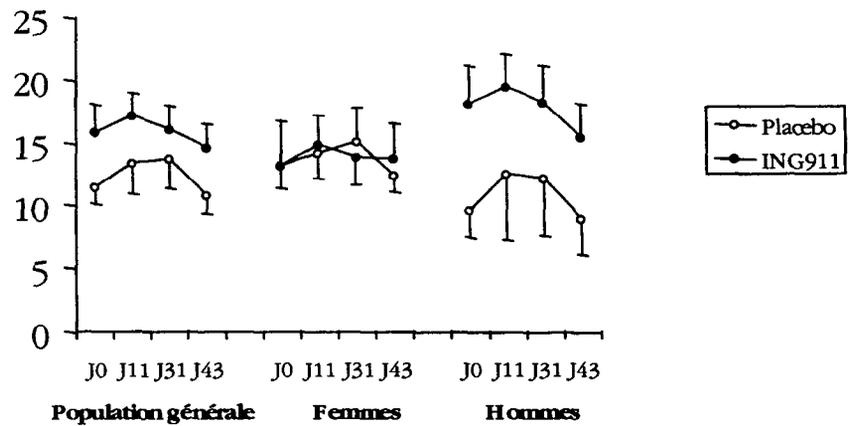


Figure 2. HR stress reactivity in absolute value

Mean ± mean standard deviation, ● ING911, ○ Placebo

Systolic blood pressure

No nutriment effect is observed on the ambulatory SBP (Table 10 and Figure 3). There is an expected sex-effect (ambulatory SBP is higher in men than in women) as well as a significant nutriment /sex interaction, but the Tuckey test does not show a nutriment effect in men ($p = 0.99$) and women ($p = 0.83$) sub-groups.

SBP reactivity tends to decrease on D11 and D31 in ING911 group compared to Placebo group (Table 10 and Figure 4). This trend persists after sex adjustment, without nutriment /sex interaction.

On D43, no significant difference is observed in the total population between nutriment groups regarding neither the resting SBP nor the SBP variation under stress.

	Placebo group	ING911 group	Women		Men	
			Placebo	ING911	Placebo	ING911
D0						
Ambulatory (mmHg)	118.5 (11.6)	119.4 (15.5)	114.1 (10.4)	108.6 (8.8)	123.3 (11.3)	128.6 (14.1)
Rest (mmHg)	111.2 (10.6)	114.4 (12.9)	108.0 (10.3)	105.4 (8.7)	115.2 (10.0)	122.8 (10.2)
Stress (mmHg)	129.3 (13.4)	134.3 (18.4)	125.7 (12.3)	121.6 (15.0)	133.6 (13.9)	146.1 (12.7)*
<i>Variation</i> (mmHg)	19.0 (9.2)	20.0 (11.2)	19.1 (9.9)	15.7 (11.9)	18.9 (8.8)	24.1 (9.2)
Recovery (mmHg)	111.2 (9.7)	114.3 (12.8)	108.4 (10.4)	105.9 (9.6)	114.7 (7.7)	122.1 (10.5)
D11						
Ambulatory (mmHg)	112.7 (12.4)	115.3 (12.5)	105.8 (11.2)	110.6 (11.7)	121.5 (7.2)	119.6 (11.9)
Rest (mmHg)	107.6 (11.1)	110.4 (9.2)	100.4 (7.2)	105.5 (7.2)	116.9 (7.7)	114.9 (8.7)
Stress (mmHg)	126.7 (15.7)	129.2 (15.9)	120.8 (14.3)	118.9 (13.2)	134.2 (14.6)	138.8 (11.7)
<i>Variation</i> (mmHg)	17.1 (12.4)	15.2 (11.4)	18.0 (12.7)	11.9 (11.6)	16.0 (12.5)	18.3 (10.7)
Recovery (mmHg)	109.6 (10.4)	114.0 (11.4)	102.8 (6.6)	107.0 (9.4)	118.2 (7.4)	120.5 (9.3)
D31						
Ambulatory (mmHg)	114.0 (13.6)	113.5 (12.4)	107.3 (11.9)	106.8 (11.5)	122.5 (10.9)	119.7 (9.9)
Rest (mmHg)	108.6 (12.3)	108.2 (9.5)	100.6 (6.9)	102.1 (7.7)	118.7 (10.0)	113.9 (7.2)
Stress (mmHg)	127.1 (15.7)	124.6 (15.7)	120.3 (13.6)	114.7 (12.8)	135.7 (14.5)	133.8 (12.4)
<i>Variation</i> (mmHg)	17.9 (9.8)	12.3 (11.2)	19.1 (10.1)	9.5 (8.3)	16.4 (9.6)	15.0 (13.1)
Recovery (mmHg)	109.2 (12.1)	112.3 (12.6)	101.2 (6.0)	105.2 (11.6)	119.3 (10.1)	118.9 (9.8)
D43						
Ambulatory (mmHg)	110.8 (14.2)	112.8 (14.0)	102.8 (9.8)	104.0 (10.5)	121.0 (12.3)	120.9 (11.8)
Rest (mmHg)	107.9 (14.8)	110.2 (12.6)	98.1 (8.1)	102.2 (8.1)	120.3 (11.7)	117.7 (11.6)
Stress (mmHg)	124.3 (13.9)	125.6 (18.3)	117.4 (10.5)	114.2 (14.7)	133.1 (12.9)	136.3 (14.6)
<i>Variation</i> (mmHg)	13.8 (10.4)	13.5 (10.5)	15.1 (10.4)	9.9 (10.9)	12.0 (10.7)	16.9 (9.3)
Recovery (mmHg)	110.5 (13.2)	112.1 (13.5)	102.2 (6.4)	104.2 (10.2)	121.1 (12.0)	119.4 (12.2)

Table 9 Systolic blood pressure

Absolute values in mmHg, *variation compared to the reference value at the end of final relaxation.*
Mean (SD).

Comparisons between placebo and ING911 on D0 by t test,
t : $p < 0.10$; * : $p < 0.05$; ** : $p < 0.01$; *** : $p < 0.001$

		1	2	3		
		Nutrimet	Sex	D11 D31	1x2	1x3
				Session		
Ambulatory SBP	ANCOVA 1	0.690		0.754		0.096
	ANCOVA 2	0.585	0.015 *	0.820	0.041*	0.091
SBP variation	ANCOVA 1	0.081 t		0.568		0.216
	ANCOVA 2	0.091 t	0.997	0.570	0.627	0.233

Table 10. Systolic blood pressure

Results (P-values) of ANCOVA with repeated measurements (D11-D31 session) with the D0 measured value as covariant.

ANCOVA 1 1-factor (Nutrimet) ANCOVA with repeated measurements.
 ANCOVA 2. 2-factors (Nutrimet and Sex) ANCOVA with repeated measurements.
 1x2: interaction between nutriment and sex effects
 1x3: interaction between nutriment and D11-D31 session effects

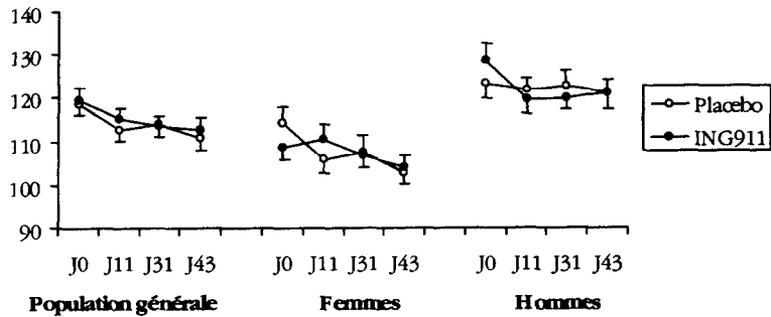


Figure 3. SBP ambulatory

Mean ± mean standard deviation ●ING911, ○Placebo

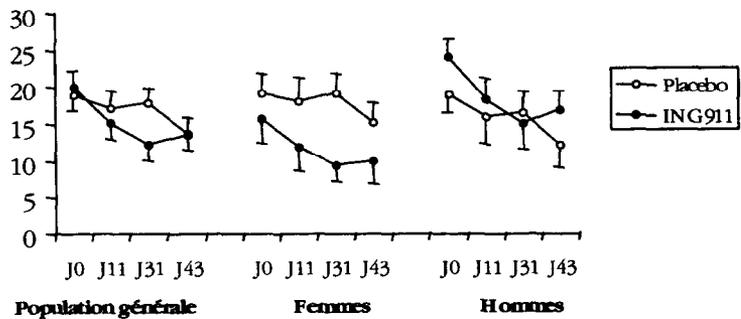


Figure 4. SBP Stress reactivity in absolute value

Mean ± mean standard deviation, ● ING911, ○ Placebo

Diastolic blood pressure

No nutriment effect is observed on the ambulatory DBP (Table 12 and Figure 5). There is an expected sex effect (ambulatory DBP is higher in men than in women) without nutriment /sex interaction.

There is a significant nutriment effect on the DBP reactivity, which is reduced on D11, and D31 in ING911 group compared to Placebo group. (Table 12 and Figure 6). It persists a trend after sex adjustment, without nutriment /sex interaction.

On D43, no difference is observed between nutriment regarding neither the resting DBP nor the DBP variation under stress.

	Placebo group	ING911 group	Women		Men	
			Placebo	ING911	Placebo	ING911
D0						
Ambulatory (mmHg)	71.5 (6.5)	73.4 (7.0)	71.3 (5.3)	71.5 (7.1)	71.8 (7.9)	75.0 (6.8)
Rest (mmHg)	68.4 (6.6)	70.1 (6.3)	69.4 (6.2)	67.8 (5.0)	67.3 (7.2)	72.2 (6.8)
Stress (mmHg)	78.9 (7.5)	82.0 (8.2)	79.3 (8.4)	79.3 (9.1)	78.3 (6.7)	84.2 (6.8)
<i>Variation</i> (mmHg)	8.9 (5.9)	10.4 (6.7)	10.1 (5.7)	10.5 (8.3)	7.4 (6.1)	10.4 (5.3)
Recovery (mmHg)	69.8 (6.4)	71.4 (6.2)	68.9 (6.5)	68.8 (4.9)	70.9 (6.3)	73.9 (6.4)
D11						
Ambulatory (mmHg)	67.8 (4.6)	69.6 (7.1)	67.1 (4.3)	69.5 (6.1)	68.6 (5.1)	69.6 (8.2)
Rest (mmHg)	66.4 (5.4)	68.3 (5.7)	65.7 (6.1)	67.4 (5.9)	67.4 (4.5)	69.1 (5.6)
Stress (mmHg)	77.3 (6.0)	78.3 (7.2)	77.6 (7.0)	76.1 (7.5)	76.9 (4.7)	80.4 (6.4)
<i>Variation</i> (mmHg)	10.8 (3.9)	8.5 (5.4)	11.5 (4.8)	8.6 (6.1)	9.8 (2.1)	8.5 (4.8)
Recovery (mmHg)	66.5 (5.8)	69.8 (6.8)	66.1 (6.4)	67.5 (7.1)	67.1 (5.3)	71.9 (5.9)
D31						
Ambulatory (mmHg)	66.8 (6.3)	66.5 (6.5)	68.9 (6.5)	64.8 (7.7)	64.3 (5.3)	68.1 (5.0)
Rest (mmHg)	65.4 (3.9)	66.3 (6.3)	64.9 (4.3)	64.2 (6.6)	66.1 (3.5)	68.3 (5.4)
Stress (mmHg)	77.3 (7.2)	76.8 (6.4)	77.4 (8.3)	73.9 (6.3)	77.1 (5.8)	79.4 (5.3)
<i>Variation</i> (mmHg)	11.3 (5.6)	9.8 (5.3)	11.5 (6.0)	9.1 (4.5)	11.2 (5.3)	10.5 (6.0)
Recovery (mmHg)	65.9 (4.6)	66.9 (5.4)	65.9 (4.6)	64.8 (5.4)	65.9 (4.9)	68.9 (4.7)
D43						
Ambulatory (mmHg)	66.1 (6.5)	67.9 (8.2)	65.7 (6.3)	65.3 (7.8)	66.5 (6.9)	70.3 (8.1)
Rest (mmHg)	64.7 (5.0)	68.0 (7.0)	64.9 (3.7)	65.8 (5.5)	64.5 (6.5)	70.0 (7.9)
Stress (mmHg)	76.1 (6.8)	77.0 (7.5)	76.5 (7.8)	74.4 (8.0)	75.5 (5.7)	79.4 (6.3)
<i>Variation</i> (mmHg)	10.3 (6.6)	8.2 (6.1)	11.1 (7.8)	7.1 (8.2)	9.4 (4.9)	9.4 (3.3)
Recovery (mmHg)	65.7 (6.1)	68.7 (6.7)	65.4 (5.8)	67.4 (6.2)	66.1 (6.7)	70.0 (7.2)

Table 11 Diastolic blood pressure

Absolute values in mmHg, and *variation compared to the reference value at the end of final relaxation.*

Mean (SD).

Comparisons between placebo and ING911 on D0 by t test,

t : p<0.10; * : p<0.05; ** : p<0.01; *** : p<0.001

		1	2	3		
		Nutrimet	Sex	D11 D31	1x2	1x3
				Session		
Ambulatory DBP	ANCOVA 1	0.910		0.043*		0.29
	ANCOVA 2	0.584	0.024*	0.449	0.266	0.58
DBP variation	ANCOVA 1	0.049 *		0.296		0.77
	ANCOVA 2	0.057 t	0.940	0.308	0.600	0.81

Table 12 Diastolic blood pressure

Results (P-values) of ANCOVA with repeated measurements (D11-D31 session) with the D0 measured value as covariant.

ANCOVA 1: 1-factor (Nutriment) ANCOVA with repeated measurements.
 ANCOVA 2: 2-factors (Nutriment and Sex) ANCOVA with repeated measurements.
 1x2: interaction between nutriment and sex effects
 1x3: interaction between nutriment and D11-D31 session effects.

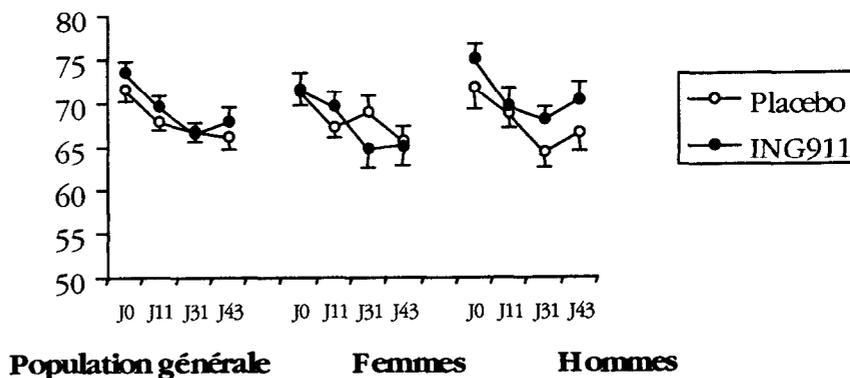


Figure 5. Ambulatory DBP (mm Hg)

Mean ± mean standard deviation ● ING911, ○ Placebo

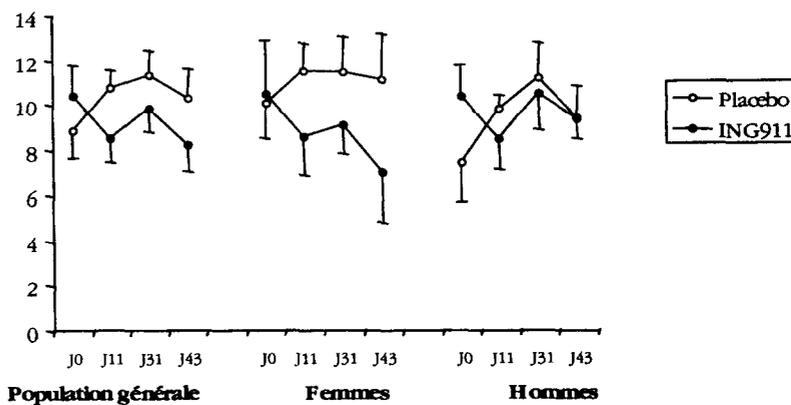


Figure 6. DBP stress reactivity in absolute value

Mean ± mean standard deviation ● ING911, ○ Placebo

Mean blood pressure

No nutriment effect is observed, in the total population, regarding ambulatory MBP.

There is a significant nutriment effect on the MBP reactivity which is reduced on D11 and D31 in ING911 group compared to Placebo group (Table 13 and Figure 7). This effect persists after sex adjustment, without nutriment /sex interaction.

On D43, no significant difference is observed between nutriments regarding neither the resting MBP nor the MBP variation under stress.

		1	2	3		
		Nutriment	Sex	D11 D31	1x2	1x3
				Session		
Ambulatory DBP	ANCOVA 1	0.724		0.201		0.145
	ANCOVA 2	0.696	0.117	0.143	0.517	0.127
DBP variation	ANCOVA 1	0.028 *		0.702		0.623
	ANCOVA 2	0.030 *	0.724	0.715	0.540	0.617

Table 13 Mean blood pressure

Results (P-values) of ANCOVA with repeated measurements (D11-D31 session) with the D0 measured value as covariant.

ANCOVA 1: 1-factor (Nutriment) ANCOVA with repeated measurements.

ANCOVA 2: 2-factors (Nutriment and Sex) ANCOVA with repeated measurements.

1x2: interaction between nutriment and sex effects

1x3: interaction between nutriment and D11-D31 session effects

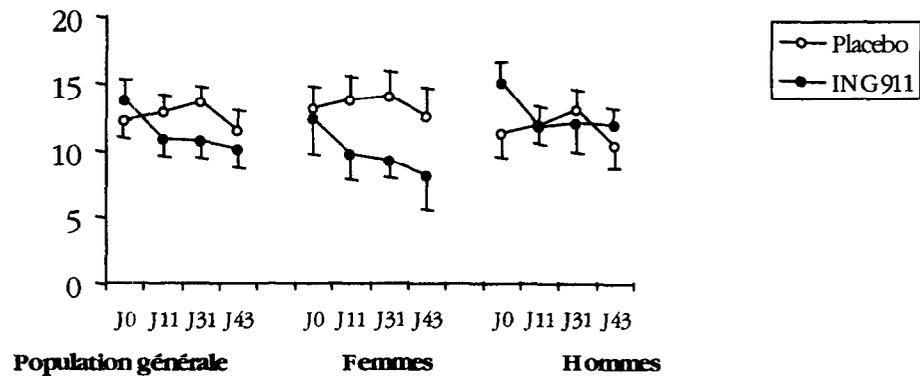


Figure 7. MBP stress reactivity in absolute value
Mean ± mean standard deviation, ● ING911, ○ Placebo