CLINICAL STUDIES

EXTRACT OF FERTILIZED PARTIALLY INCUBATED HEN EGGS

IMPORTANT CONFIDENTIALITY AND OTHER NOTICES

The recipient of this document acknowledges that the information contained in this document, other than information that was already in the public domain, is confidential and the property of LifePharm Global Network.

In accepting delivery of this document, the reader agrees not to use, or disclose any information contained herein to any other parties without the consent and/or written permission of LifePharm Global Network.

Likewise, no part of this document may be reproduced, stored in a retrieval system, or transcribed in any form or by any means without the express written permission of LifePharm Global Network.

The material contained in this document is the sole and exclusive property of LifePharm Global Network and must be returned upon demand.

SUMMARY

The avian egg is an important source of nutrients, containing all the proteins, lipids, vitamins, minerals and growth factors required by the developing embryo, as well as a number of defense factors to protect against bacterial and viral infections. Moreover, eggs are now understood to contain substances with biological functions beyond basic nutrition, and extensive research has been undertaken to identify and characterize these biologically active components (Kovacs-Nolan J, Philips M, Mine Y: Advances in the value of eggs and egg components for human health. J. Agra Food Chem. 2005; 53:8421-31)

YTE™ is a patented extract from fertilized, partially incubated hen eggs, obtained through separation of oligopeptides from the total mass. These have molecular weights of 0.5 – 1.8 kD and are able to pass freely through the digestive barrier. These embryonic peptides work via elevation of 17-ketosteroid levels in the adrenal glands which improve anabolism through increased synthesis of androgens and a decrease in the catabolic hormone cortisol, which offers multiple benefits.

Initially, a less processed powder using the whole content of the egg (excluding shell) was used. Administration was in powder form in sachets. The taste of the product was not considered as acceptable to the public.

Later (from 1999) separation of inactive parts were performed before further processing, hence providing a more concentrated powder. This was first planned for use in tablets, but putting it in tablet form proved difficult due to hygroscopic properties.

The present product in hard-gel capsules has been on the market since 2001. The difference between the original powder (as used in clinical work prior to 1999) and the current product equates to 1:1.79, i.e. that 1.79 g of old product corresponds to 1 g of the currently available YTE™.

Initial trials with the original product were of experimental character and have not been reported. These gave, however, grounds for expectations of libido enhancing properties, improvement of stamina and muscle strength, mood enhancement and also other properties.

The first structured studies were performed during 1992 *(ref 1)* in order to investigate the effects on sexual desire with a follow-up during 1992/93 *(ref 2)* with the same purpose. Both of these studies were double-blind, placebo-controlled studies. These two studies were published in an article

in the Journal of International Medical Research in 1997 *(ref 4)* together with the conclusions of an open post-marketing study performed in Sweden *(ref. 3).* This last study supports the findings of the two placebo-controlled studies, but cannot be used as documentation alone.

As it is known that anti-depressant medication often leads to decreased sexual lust and performance, a small pilot-study in patients on anti-depressant medication (SRI) for a minimum of 6 weeks were performed in Denver, Colorado during 1996/97. Although a small sample size, this study gave statistically significant results. (*Ref 5)*

At the same time an animal study was performed in Japan in order to study the effects on sexual behavior of YTE™ in rats. The results of this study is consistent with the findings in the human studies – further documenting the findings that YTE™ has a stimulating effect on the sexual desire and behavior.*(ref. Pre-clinical 1)*

In order to study the effects on physical performance, muscle size etc a study was performed during the same period – also in Denver, Colorado. As creatine is widely used among body builders, this substance was used as placebo treatment, while a combination of YTE™ and creatine was used as active treatment. As the effects of creatine are known, this study can be regarded as a test against creatine (as placebo) and no treatment (given the documented effects of creatine).

The result of this showed that the addition of YTE™ gave a significant increase in motivation (mental effect) as well as a non-significant improvement in strength and muscle size. On this basis we can conclude that YTE™ has a tendency to increase the effects of creatine in bodybuilding, i.e. a positive effect in strength and muscle size. (*Ref. 6)*

A hypothesis was during this period established that the clinical effects seen were due to hormonal effects. A study into the hormonal effects was subsequently performed in Oslo, Norway, into the hormonal effects of a single dose of YTE™.

This study was designed solely with the aim of testing potential hormonal changes as a result of a large, single dose of YTE™ and showed that YTE™ may significantly reduce cortisol levels and improve the cellular uptake of testosterone – thus supporting the hypothesis that these hormonal effects may help explain the clinical effects seen. *(Ref. 7)*

Another study was performed during the same period on 14 elite soldiers of the Norwegian Armed Forces into muscular strength,

restitution time and quality of life, but the details of this study are missing. (At the time, Med-Eq was only partially involved in the process.)

Further studies

Med-Eq has sponsored two studies into the mood enhancing properties of YTE™. These studies use the present recommended dosage of four YTE capsules per day of 420 mg each. The results of these studies have been submitted for publication and are also attached in this document.

Several studies have also been performed into the effects in memory enhancement, and the results of these studies are under processing with the intention of submitting for publications.

Conclusions

The two studies on YTE for stress and anti depressant concluded that YTE selectively improves adaptation to acute stress by normalizing the endocrine and the subjective stress response and that YTE might have a place in the treatment of mood disorders as a supplement to drug treatments used today.

It can also be stated that YTE™ strengthens the effect of creatine and this may be of value to people exercising and building muscles. (The Norwegian Army study also indicated that YTE™ alone gave improvements in muscle strength and restitution time.) T

The Academy of Sports study shows that YTE™ has an effect on the cortisol levels in the blood and the cellular uptake of testosterone.

Clinical and animal studies provide sufficient documentation that YTE™ has a stimulatory effect on sexual lust and behavior – in the population in general as well as in patients experiencing lack of sexual lust due to anti-depressant medication.

Clinical Studies

A. STUDY ON YTE ON STRESS

1. Effects of powdered fertilized eggs on the stress response

Background: Effects of nutritional supplements on psychological wellbeing receive increasing attention. This double-blind placebo-controlled study investigated effects of a four week intake of powder of fertilized eggs (YTE) in a laboratory protocol (Trier Social Stress Test; TSST).

Methods: Aside the laboratory stress test, we examined differential effects on subjects with high and low levels of chronic stress. Thus, subjects were further divided into two subgroups with scores for chronic stress scores below and above average, respectively.

Results: Beneficial effects of YTE were observed in subjects with enhanced levels of chronic stress. When compared to placebo these subjects showed an improvement of both the psychological and endocrine stress response.

Conclusions: Group differences suggest that YTE selectively improves adaptation to acute stress by normalizing the endocrine and the subjective stress response. Subjects with less chronic stress also reported less subjective stress but did not show beneficial effects on the endocrine stress response.

B. STUDY ON YTE FOR ANTI DEPRESSANT

2. A placebo controlled, double-blind study on the efficacy of a nutritive supplement containing embryonic peptides in treatment of mild to moderate depressive mood.

Background: Embryonic oligopeptides might have a therapeutic effect in the treatment of depressed mood.

Objectives: To investigate if a powder from fertilized eggs have a clinical effect on patients with mild to moderate depressed mood

Methods: In the present comparative, randomized, placebo controlled, double–blind, parallel group study we have investigated the effect of a nutritive supplement containing embryonic peptides (YTE™) in the

treatment of mood disorders. Patients with mild to moderate mood disorders according to Hamilton rating scale (Ham-D) and Beck Depression Inventory (BDI) were included in the study according to the protocol.

The patients were randomly assigned to receive placebo, Deprevent™ (YTE) or Deprevent™ Forte (YTE plus lemon balm extract) for 12 weeks. The main outcome was change in the Ham-D total score from baseline to 12 weeks as well change in BDI from baseline to controls after 3, 6 and 12 weeks.

Results: 57 patients concluded the study. It was a significant effect in favor of the two active groups in the primary outcome measures as compared to placebo. Between the two active groups, however, there was no significant difference in the outcome measures, even if it was a weak tendency in favor of the Deprevent™ forte group. There were no reports of adverse effects in any of the groups during the study.

Conclusion: Based on the results from this study we conclude that these nutritive supplements might have a place in the treatment of mood disorders. With its excellent tolerability it might be an attractive supplement to the drug treatments used today.

C. YTE FOR STRENGTH INCREASE & HORMONE PRODUCTION

3. Effects of nutritional Supplements designed to promote lean tissue accretion on body composition and strength performance.

Investigator: Dr. Kjell Törnblom, University of Denver, Colorado

Participants: Originally 39 volunteers (mixed gender) of which 26 healthy volunteers completed the study. Of these 7 were advanced bodybuilders and 19 actively training at a local gym.

Dose: 5 g of unprocessed material corresponding to a daily dose of 2.8 g of YTE™

Design: 17 participants took a combination of YTE + creatine as “active” and 9 took creatine as placebo. Single blind placebo-controlled study over 6 weeks. The difference in change between the two groups is regarded as due to YTE.

Parameters: Measurement of muscle mass, strength.

Results: Significant improvement in training motivation along with significant change in strength and physical change.

4. Effects of Protein Supplements on the natural Production of Hormones subsequent to hard Training. 1996/97

Investigator: Bjødne Eskeland PhD in cooperation with researchers at the Norwegian Academy of Sports. Oslo, Norway.

Participants: 9 advanced (male) bodybuilders of 20 – 30 years of age.
Dose: Single dose: 200 g of unprocessed powder corresponding to 111 g of YTE™

Design: Single dose double-blind cross-over study with 7 days wash-out period between in order to test the immediate effects of the active substance on hormone production.
Parameters: Laboratory tests of testosterone, insulin, cortisol and growth hormone in blood samples

Results: Participants receiving active substance had a significant reduction of kortisol compared to placebo as well as increased cellular uptake of testosterone and higher insulin levels.

D. YTE FOR SEXUAL DESIRES

5.”A randomized placebo-controlled study of”Libido” (YTE) on sexual lust in middle-aged, healthy men” 1992

Investigator: Erling Thom PhD. Oslo, Norway Participants: n= 16 healthy male volunteers between 47 and 60 years (average 52.5) with reduced sexual lust. Average weight 84 kg, height 181 cm, BMI 25.6

Dose: 2 x 3 g of unprocessed material. Corresponds to a daily dose of 3.35 g of YTE™

Design: Group A (n=8) obtained active for 3 weeks, and subsequently placebo for 3 weeks.

Group B (n=8) obtained placebo for 3 weeks, and subsequently active for 3 weeks. Parameters: Visual analogue score of 10 cm every week at end of week self-assessment of improvement in sexual lust.

Results: Participants had a significantly higher score on VAS scale at the end of 2nd and 3rd week during Active period vs Placebo period.

Comments: Study reported as study 1 in published article (4)

6. "A randomized placebo-controlled study of "Libido" (YTE) on sexual desire in healthy middle-aged men" 1993

Investigators: Dr. KO Svendsen, Dr. Einar Christiansen. Statistics: Erling Thom PhD. Oslo, Norway

Participants: n=31 healthy male volunteers between 38 and 65 years (average 50.9)

Dose: 2 x 3 g of unprocessed material. Corresponds to a daily dose of 3.35 g of YTE™

Design: Two groups receiving alternating active-placebo or placebo-active for 6 periods of 2 weeks (3x2 weeks of active and 3x2 weeks of placebo = 12 weeks)
Parameters: Visual analogue score self-assessment at end of every week of improvement of sexual
lust.

Lab tests of testosterone levels in n=11.

Results: There is a tendency of higher scores in active periods than in placebo periods, but the difference is not significant. 40 – 50 % of the study subjects have no effect. Testosterone levels increased by on average 25 % during the study period, but were well within normal testosterone levels in a healthy population.

Comments: Study reported as study 2 in published article (4)

7. "The Swedish Study"

Investigator: Bjødne Eskeland PhD. Statistics: Erling Thom PhD.

Participants: n=31 male volunteers. No data on age available.

Dose: 2x3 g of unprocessed material. Corresponds to a daily dose of 3.5 g of YTE™.

Design: This was an open, uncontrolled marketing study in healthy volunteers recruited through ads throughout Sweden. The study was performed without medical supervision. All participants took

active substance over 3 weeks.

Parameters: 5 point scale of increase in sexual desire -self assessment.

Results: 54.9 % reported definite and very pronounced increase, while 16.1% reported no increase.

Comments: Study reported as Swedish study in published article (4)

8. Sexual Desire in Men: Effects of Oral Ingestion of a Product Derived from Fertilized Eggs.

Authors: B Eskeland, E Thom, KOB Svendsen. Published in the J. of International Med. Research

This is an article summarizing the 3 studies referred to above.

9. The Effect of "Libido" (YTE) on decreased sexual Desire associated with Anti-Depressant Medication. 1996/97

Investigator: Dr. Kjell Törnblom, University of Denver, Colorado Participants: 11 persons taking anti-depressant medication and having experienced reduced sexual lust in connection with this. 8 persons -5 male and 3 female – completed the study. Age of male participants are 29 – 67 years, females 26 – 39 years.

Dose: 2 x 6 tablets of 420 mg unprocessed material (5 g) corresponding to a daily dose of 2.8 g of YTE™.

Design: Single blind, placebo-controlled study over 6 weeks. Initially, 3 weeks of active -followed by 3 weeks of placebo.

Parameters: a. Derogatis Affects Balance Score (DABS) self assessment at start-up, after 3 weeks and after 6 weeks. b. 9-point Likert scale assessing sexual lust and behavior at same times + after 2 and 5 weeks.

Results: Statistically significant improvements in Intensity of sexual desire, Frequency of sexual desire, energy, and confidence and self esteem.

Pre-Clinical Studies

10. Effects of Fertilized incubated Shell Eggs on sexual Behavior of Male Rats 1996

Investigator: S. Kawashima et al, Zenyaku Kogyo Co, Ltd, Japan Test subjects: 3 groups of 10 (n=30) rats – one group received YTE, one group non-incubated eggs and the third group the solvent vehicle.

Dose: 2x 583mg of unprocessed material per kg bodyweight over 3 weeks. (corresponding to 6501 mg/kg of YTE™

Design: 3 string placebo-controlled behavioural study during 3 weeks.

Parameters: mounting frequency, intromission frequency, ejaculation frequency, mount-intromission-ejaculation latencies, reflexive erection test (after 3 weeks), blood samples and autopsy.

Results: Mounting frequency significantly higher in active group vs control groups. Statistically significant difference in ejaculation frequency. Improvement of intromission frequency not statistically significant. Mounting-intromission-ejaculation latencies not statistically significant difference. No statistical difference in reflexive erection. No statistically significance in development of testosterone levels.

YTE ON STRESS

Clinical Nutrition xxx (2009) 1–6

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original Article

Effects of powdered fertilized eggs on the stress response

Johannes Schult [a], Torsten Hero [a,b], Juliane Hellhammer [a,*]

[a] DAaCRO, Diagnostic Assessment and Clinical Research Organization, Science Park, D-54296 Trier, Germany
[b] FPP, Center for Psychobiology and Psychosomatic Research, University of Trier, D-54290 Trier, Germany

ARTICLE INFO

Article history:
Received 15 May 2009
Accepted 16 September 2009

Keywords:
Nutritional supplement
Stress
TSST
YTE™

SUMMARY

Background & aims: Effects of nutritional supplements on psychological wellbeing receive increasing attention. This double-blind placebo-controlled study investigated effects of a four week intake of powder of fertilized eggs (Young Tissue Extract; YTE™) in a laboratory protocol (Trier Social Stress Test; TSST).
Methods: Aside the laboratory stress test, we examined differential effects on subjects with high and low levels of chronic stress. Thus, subjects were further divided into two subgroups with scores for chronic stress scores below and above average, respectively.
Results: Compared to placebo, a four week intake of YTE™ did not result in superior effects on general wellbeing. However, beneficial effects of YTE™ were observed in subjects with enhanced levels of chronic stress. When compared to placebo these subjects showed an improvement of both the psychological and endocrine stress response.
Conclusions: Group differences suggest that YTE™ selectively improves adaptation to acute stress by normalizing the endocrine and the subjective stress response. Subjects with less chronic stress also reported less subjective stress but did not show beneficial effects on the endocrine stress response.

1. Introduction

The avian egg contains a multitude of the proteins, lipids, vitamins, minerals, and growth factors. There are also additional defense factors contained to protect against bacterial and viral infection, and biologically active components, making it more than just a source of nutrients. Proteins and peptides can be derived from the whole egg. Intake of egg components has been reported to be associated with novel antimicrobial activities, antiadhesive properties, immunomodulatory, anticancer, and antihypertensive activities, antioxidant properties, protease inhibitors, and nutrient bioavailability.[1,2]

The egg powder used in the present study, YTE™ (Young Tissue Extract; Med-Eq as, Tønsberg, Norway), is extracted from fertilized, partially incubated hen eggs. It is obtained through separation of oligopeptides from the total mass and contains proteins and peptides from freeze-dried egg powder. These can pass freely through the digestive barrier. The embryonic peptides work via elevation of 17-ketosteroid levels in the adrenal glands, which improves anabolism through increased synthesis of androgens and a decrease in cortisol. Double-blind, placebo-controlled studies showed that the substance has a positive effect on libido in healthy humans as well as in patients on anti-depressant medication.[3,4] It has also shown to improve cellular testosterone uptake in addition to its effect on cortisol levels.[3] These findings raise the question whether the substance can help dampen the physiological stress reaction and the perceived psychological stress in an acute stress situation.

Although stress has been described as a non-specific response of the body, it is possible to discern specific endocrine stress responses caused by specific emotional reactions to novel, ambivalent or uncontrollable situations and stimuli. Social stress induces elevated cortisol levels, particularly if the stressor is perceived as uncontrollable, unpredictable, and constitutes a social-evaluative threat due to the judgment of others.[5] The hypothalamic-pituitary-adrenal (HPA) axis plays a major role in the response to this kind of stressors with a robust increase of adrenocorticotropin hormone (ACTH) and cortisol.

An analysis of Dickerson and Kemeny[6] showed that the Trier Social Stress Test[7] (TSST) is the best standardized and most efficient psychological stress protocol in humans which is currently available. With respect to psychological parameters, the TSST leads to a moderate rise in fear. The biological response comprehends an increase of ACTH, cortisol, prolactin, growth hormone, norepinephrine, epinephrine, heart rate and blood pressure.[7] Cortisol is

* Corresponding author. DAaCRO, Science Park, Max-Planck-Str. 22, D-54296 Trier, Germany. Tel.: +49 0 651 9120 494; fax: +49 0 651 9120 564.
E-mail address: hellhammer@daacro.com (J. Hellhammer).

0261-5614/$ – see front matter © 2009 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
doi:10.1016/j.clnu.2009.09.004

involved in development, metabolism, cognitive and emotional processes, and the immune system. It also exerts influence on the HPA axis itself.[8]

The primary objective of the present study was to determine the efficacy of egg powder YTE™ in dampening stress reactivity in an acute stressful situation by assessment of cortisol, heartrate and perceived stress in the TSST. The secondary objective of this study was to discriminate effects between subjects which scored relatively high or low on a measure of perceived chronic stress, respectively.

2. Methods

2.1. Subjects

Forty (mean age 23 (range 20–29) years) out of 44 screened healthy non-smoking young men completed the study. Two were excluded because they were already familiar with the TSST. One was excluded because of lactose intolerance. One aborted the study after the screening visit and before substance intake started.

Only healthy, non-diabetic, and non-smoking subjects were included to this study. Neither the two treatment groups, nor the four subgroups (experimental group membership and high stress/low stress group) differed in terms of body mass index (BMI), weight, height, age and baseline heart rate (all $p > 0.44$).

2.2. Study procedure

Subjects were informed about the study procedure and gave their informed consent for study participation during the initial screening visit. Their health status was then examined with a medical questionnaire. Subjects were randomly assigned to one of the two treatment groups. Both, subjects and the investigator were blind to the groups' identity.

The following questionnaires were administered during the screening visit: Trier Inventory of Chronic Stress (TICS), Perceived Stress Scale (PSS), Mood Questionnaire (''Mehrdimensionaler Befindlichkeitsfragebogen'', MDBF, long form), State Trait Anxiety Inventory (trait version: STAI- X2), and Short Form 12 Health Survey Questionnaire (SF-12).

Subjects then received a container filled with capsules containing either the test product or the placebo. The containers were locked with MEMS (Medication Event Monitoring System) TrackCaps. During the four weeks leading up to the second visit (including the day of the second visit), subjects ingested a daily dose of four capsules (i.e., 1680 mg YTE™/placebo per day): two capsules with breakfast and two with the principal meal.

After 28 days of substance intake, subjects returned to the study site and performed the TSST protocol. The TSST consisted of a resting and anticipation period (45 min), a test period (15 min), and a subsequent resting period (60 min).

During the first half of the test period subjects had to deliver a free speech. In the second half they had to perform mental arithmetic in front of an audience.

Subjects also had to fill out a number of questionnaires during the TSST visit: PSS and SF-12 before the stress test; MDBF short version A (pre-TSST) and short version B (post-TSST), respectively; the State Trait Anxiety Inventory (STAI-X1, state anxiety) pre- and post-TSST; ratings of perceived stress, anxiety, and insecurity on visual analogue scales (VAS) three times (pre-TSST, in the middle of the TSST, and post-TSST).

Heart rate was recorded from −20 min to +20 min in relation to TSST timing by Polar Vantage NV heart rate measurement devices. 10 min after the beginning of heart rate measuring subjects had to stand up. This served to avoid confounding orthostatic effects during the TSST measurement. Once a subject returned from the TSST, he remained standing for further 10 min after the end of the TSST. The protocol included measures of saliva cortisol (at −2 min, +1 min, +10 min, +20 min, +30 min, and +60 min, respectively) relative to the TSST. After the last saliva sample was collected, subjects were debriefed and received their reimbursement. The time line of the TSST protocol is summarized in Fig. 1.

2.3. Treatment

The test substance YTE™ is an egg powder extracted from fertilized, partially incubated hen eggs. It is obtained through separation of oligopeptides from the total mass. The resultant oligopeptide product contains 80–85% protein fractions (peptides), approximately 8% lipids, 5.5% carbohydrates and 3.5% ash in addition to some moisture. The YTE™ used in the present study was produced by Med-Eq as, Tønsberg, Norway and encapsulated by the Laboratoire GEFA, ZA Bas-Rocomps Route de Noyal-sur-Vilaine, Chateaugiron, France.

The placebo product contained rice starch, hydroxypropylmethylcellulose (HPMC), magnesium stearat, colouring agent (yellow iron oxide, black iron oxide, red iron oxide on a lactose carrier) and was produced by the Laboratoire GEFA, ZA Bas-Rocomps Route de Noyal-sur-Vilaine, Chateaugiron, France.

2.4. Measures

The TICS[9] assesses the subjective perception of stress load during the last three months. It comprehends 57 items out of which

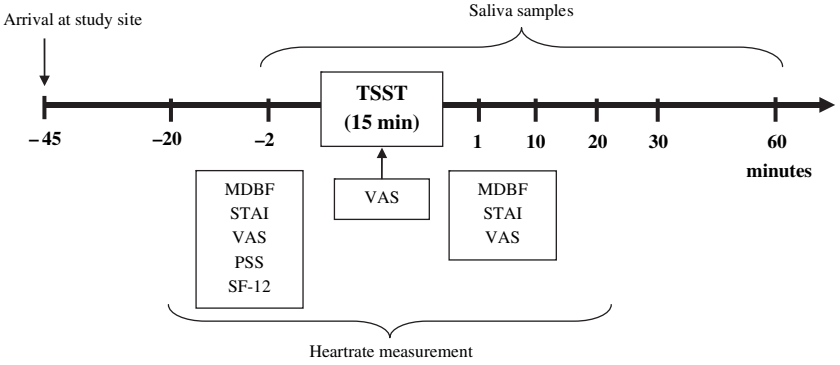


Fig. 1. Time line of the TSST protocol.

Please cite this article in press as: Schult J, et al., Effects of powdered fertilized eggs on the stress response, Clinical Nutrition (2009), doi:10.1016/j.clnu.2009.09.004

12 form a screening scale for chronic stress. The STAI[10] has two scales with 20 items each, which assess state and trait anxiety, respectively. The PSS[11] is a psychological instrument for measuring the perception of stress in the past month. A German translation was used. It consists of 14 items. The MDBF[12] uses three bipolar dimensions of present psychological wellbeing: good-bad mood, wakefulness-tiredness, and calmness-agitation. There are two short forms (A and B), which together form the long form. The SF-12[13] is a health-related measure of quality of life, discriminating physical and psychological components. Subjects further rated their subjective perception of the stress test three times on VAS of 10 cm length, on which they marked on bipolar dimension (“low” to “high”) the perceived stress load, anxiety and insecurity.

YTE™ capsules were provided in containers locked with MEMS TrackCaps (AARDEX Ltd., Zug, Switzerland), which record the time and date of each opening. This system was implemented to enforce and to check for subjects’ compliance.

Heart rate assessment took place with a Polar watch device (S610i and S710i, Polar Electro GmbH, Büttelborn, Germany) that recorded data every 5 s. Data were aggregated to mean values for 7 time phases: sitting (10 min), standing (10 min), introduction to the TSST and preparation (5 min), interview (5 min), mental arithmetic (5 min), standing (10 min), and sitting (10 min).

2.5. Laboratory analyses

For saliva sample collection cotton swaps in suspenders (Salivette®, Sarstedt AG & Co., Nümbrecht, Germany) were used. Saliva samples were stored at 4 °C and frozen at –20 °C before they were transferred to the laboratory. After thawing, saliva samples were centrifuged at 3000 rounds per minute for 5 min, which resulted in a clear supernatant of low viscosity. Free saliva cortisol levels were determined using an optical density immunoassay (Coated Well EIA, Salimetrics, State College, PA, USA) based on the competition principle. Intra-assay variation of this assay ranges between 3.88 and 7.12%, inter-assay variation between 6.69 and 6.88%.

2.6. Data analysis

Analyses of data took place after data collection and laboratory analyses of biological parameters. Interim analyses were not conducted.

If values of biological parameters were implausible, respective sample analyses were repeated before the statistical analysis. Cases with missing data were case wise excluded of analyses. Data analysis was carried out using SPSS 15.0.1.

Analyses of endocrine, cardiovascular and psychological parameters for both groups were performed with analyses of variance (ANOVAs) for repeated measurements. In case of significant results in the Mauchly test for sphericity, a Greenhouse-Geisser correction of degrees of freedom was conducted. Change parameters were compared with *t*-tests.

2.7. Ethics approval

The study was performed in accordance with the principles of the declaration of Helsinki and was approved by the International Medical & Dental Ethics Commission (IMDEC), Freiburg, Germany.

3. Results

3.1. Development during the intake period

Comparing the groups’ PSS scores at baseline with those at the TSST day yielded no significant results (effect of time: $F_{(1,38)} = 0.40$, $p = 0.53$, effect of group: $F_{(1,38)} = 0.71$, $p = 0.41$, effect time x group: $F_{(1,38)} = 0.26$, $p = 0.61$). The scores of the two SF-12 scales didn’t differ significantly between groups, between baseline and TSST day, and between groups across time (physiological well-being: effect of time: $F_{(1,35)} = 1.17$, $p = 0.29$, effect of group: $F_{(1,35)} = 0.23$, $p = 0.64$, effect time x group: $F_{(1,35)} = 0.41$, $p = 0.53$; psychological well-being: effect of time: $F_{(1,35)} = 0.02$, $p = 0.89$, effect of group: $F_{(1,35)} = 0.00$, $p = 0.95$, effect time x group: $F_{(1,35)} = 0.03$, $p = 0.86$).

Food intake of participants was not recorded during the study. When asked about their experience during the intake period, subjects did not report on changes in food consumption. Subjects accepted and tolerated the supplement well.

3.1.1. Free cortisol in saliva

The stress test induced a significant increase in cortisol levels in saliva (effect of time: $F_{(1.7,65.1)} = 35.80$, $p = 0.00$). The two groups did not differ in the overall saliva cortisol levels (effect of group: $F_{(1,38)} = 0.11$, $p = 0.74$), nor in the course of saliva cortisol secretion (effect of time x group: $F_{(1.7,65.1)} = 0.89$, $p = 0.40$).

In order to explore differential treatment effects on cortisol levels, similar repeated measurement ANOVAs were run for half of the sample with TICS screening scale scores above and below the median, respectively. In our study, the sample median of the screening scale for chronic stress was 16 which corresponds to the 52nd percentile of the norm sample distribution.[9]

As expected, the change across time remains significant (effect of time for high stress: $F_{(1.7,30.1)} = 22.46$, $p = 0.00$; effect of time for low stress: $F_{(1.7,30.3)} = 15.48$, $p = 0.00$). For the high stress group, there is a trend for the overall saliva cortisol levels to be higher in the egg powder group (effect of group: $F_{(1,18)} = 3.27$, $p = 0.09$). For the low stress group, there is no significant treatment effect (effect of group: $F_{(1,18)} = 0.82$, $p = 0.38$). The interaction term is not significant in both subgroup analyses (effect of time x group for high stress: $F_{(1.7,30.1)} = 2.61$, $p = 0.10$; effect of time x group for low stress: $F_{(1.7,30.3)} = 0.40$, $p = 0.64$). Differential cortisol levels are shown in Fig. 2.

Splitting the sample into more than two groups depending on their chronic stress leads to very small (e.g., $n = 3$) and unbalanced groups. As a consequence, these results are not pursued.

3.1.2. Heart rate

The TSST induced a significant increase of heart rate (effect of time: $F_{(2.3,73.5)} = 48.25$, $p = 0.00$). However, the two experimental

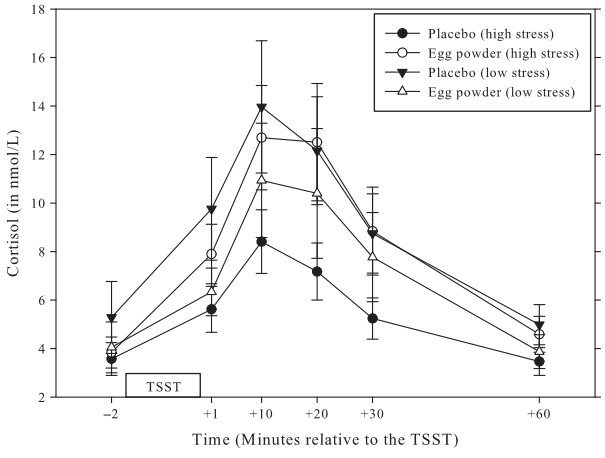


Fig. 2. Time course of saliva cortisol secretion in response to the TSST separately for the high stress half and the low stress half of the sample ($n = 40$). The graph shows group means with standard error bars.

Please cite this article in press as: Schult J, et al., Effects of powdered fertilized eggs on the stress response, Clinical Nutrition (2009), doi:10.1016/j.clnu.2009.09.004

groups showed no overall differences in heart rate (effect of group: $F_{(1,32)} = 0.07$, $p = 0.80$), nor differences in the time course of heart rate changes (effect time x group: $F_{(2.3,73.5)} = 0.24$, $p = 0.82$).

Additional heart rate analyses were run for the half of the sample with TICS screening scale scores above and below the median, respectively. The change across time remains significant (effect of time for high stress: $F_{(2.4,40.0)} = 20.23$, $p = 0.00$; effect of time for low stress: $F_{(2.0,25.8)} = 31.28$, $p = 0.00$). There is no significant group effect in either subsample (effect of group for high stress: $F_{(1,17)} = 1.10$, $p = 0.31$; effect of group for low stress: $F_{(1,13)} = 0.43$, $p = 0.53$), and also no significant interaction term (effect of time x group for high stress: $F_{(2.4,40.0)} = 0.76$, $p = 0.50$; effect of time x group for low stress: $F_{(2.0,25.8)} = 0.23$, $p = 0.80$). Fig. 3 shows the change of heart rates across time.

3.2. State anxiety

Subjects had significantly higher post-test STAI-state anxiety scores (effect of time: $F_{(1,37)} = 15.95$, $p = 0.00$). There was no significant main effect of experimental group and no interaction effect (effect of group: $F_{(1,37)} = 0.13$, $p = 0.72$; effect time x group: $F_{(1,37)} = 1.41$, $p = 0.24$).

The increase of state anxiety was also tested for group differences. There was no significant group effect for the whole sample ($F_{(1,37)} = 1.41$, $p = 0.24$). The high stress subsample shows an average increase of 2.4 (egg powder) and 12.5 (placebo) scale points, respectively ($F_{(1,18)} = 3.98$, $p = 0.06$), with a one-sided t-test achieving significance ($t_{(18)} = 2.00$, $p = 0.03$). This result is illustrated in Fig. 4. For the low stress subsample, there was no significant effect of group ($F_{(1,17)} = 0.18$, $p = 0.68$).

3.3. Mood, wakefulness, calmness

The effect of the TSST was similar for all three MDBF subscales. Mood ratings changed significantly towards bad mood (effect of time: $F_{(1,37)} = 25.56$, $p = 0.00$), but there were no significant group or interaction effects (effect of group: $F_{(1,37)} = 0.00$, $p = 0.98$; effect time x group: $F_{(1,37)} = 0.53$, $p = 0.47$). Tiredness ratings increased significantly (effect of time: $F_{(1,37)} = 6.57$, $p = 0.02$), but again there were no significant group or interaction effects (effect of group: $F_{(1,37)} = 0.05$, $p = 0.83$; effect time x group: $F_{(1,37)} = 0.02$, $p = 0.89$). Agitation ratings also increased significantly (effect of time: $F_{(1,37)} = 12.18$, $p = 0.00$), but there were also no significant group or interaction effects (effect of group: $F_{(1,37)} = 0.21$, $p = 0.65$; effect time x group: $F_{(1,37)} = 0.30$, $p = 0.59$).

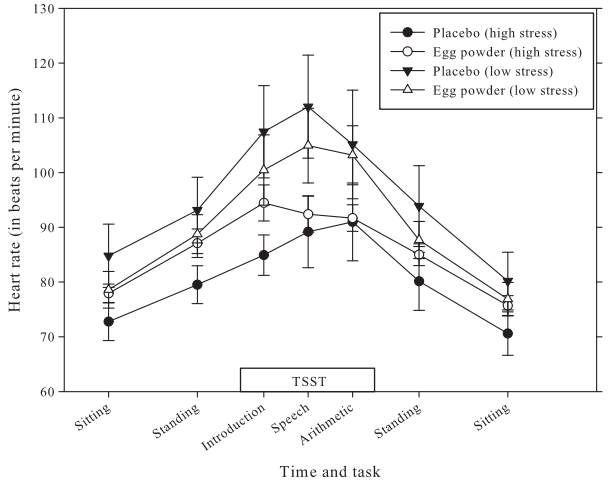


Fig. 3. Time course of heart rates separately for the highly stressed and low stressed subjects ($n = 34$). The graph shows group means with standard error bars.

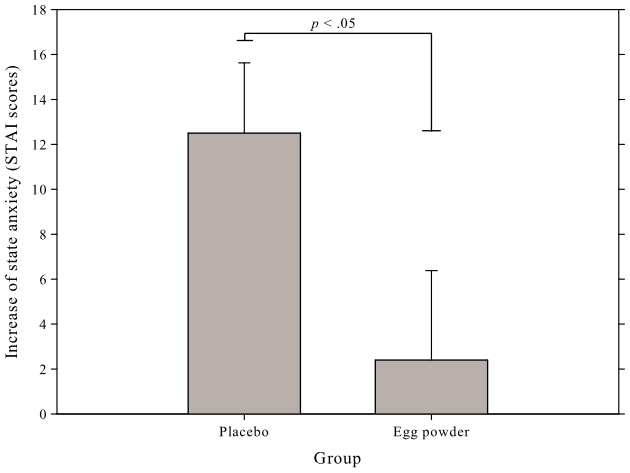


Fig. 4. Increase of state anxiety (STAI) in response to the TSST for the high stress subsample ($n = 20$). The graph shows group means with standard error bars and one-sided t-test.

3.4. Visual analogue scales

There were no initial differences in the VAS ratings between the two experimental groups before the test; effect of group for stress: $F_{(1,38)} = 1.77$, $p = 0.19$, effect of group for anxiety: $F_{(1,38)} = 1.18$, $p = 0.29$, effect of group for insecurity: $F_{(1,38)} = 0.24$, $p = 0.63$.

The TSST induced a significant increase in stress experience in both groups (effect of time: $F_{(1.7,65.9)} = 25.56$, $p = 0.00$). The time x group effect did not achieve significance ($F_{(1.7,65.9)} = 1.84$, $p = 0.17$). There was no significant difference between the two experimental groups (effect of group: $F_{(1,38)} = 0.02$, $p = 0.90$). Ratings on the other two visual analogue scales, anxiety and uncertainty, also increased significantly in both groups (effect of time for anxiety: $F_{(1.6,58.9)} = 17.21$, $p = 0.00$; effect of time for insecurity: $F_{(1.6,60.8)} = 33.34$, $p = 0.00$). However, there were neither time x group, nor group effects approaching or achieving significance (effect time x group for anxiety: $F_{(1.6,58.9)} = 0.24$, $p = 0.73$; effect time x group for insecurity: $F_{(1.6,60.8)} = 0.83$, $p = 0.42$; effect of group for anxiety: $F_{(1,38)} = 0.00$, $p = 0.95$; effect of group for insecurity: $F_{(1.,38)} = 0.28$, $p = 0.60$).

Additionally, maximum and mean increase for each scale were also tested for group differences. The comparison of maximum increase in reported subjective stress showed a trend towards significance (effect of group: $F_{(1,38)} = 3.330$, $p = 0.08$). A one-sided t-test of this effect is significant ($t_{(38)} = 1.83$, $p = 0.04$). Fig. 5 shows the differential increase in VAS stress ratings. The differences for anxiety and insecurity were less pronounced (effect of group for anxiety: $F_{(1,38)} = 0.12$, $p = 0.73$, effect of group for insecurity: $F_{(1,38)} = 0.58$, $p = 0.45$).

4. Discussion

The present study investigated whether the intake of YTE™, an extract from fertilized, partially incubated hen eggs, dampens the stress response to an acute stressful situation.

The TSST, a standardized psychosocial stress test, successfully induced significant changes in cortisol and heart rate, as well as in several psychological variables such as perceived stress, state anxiety, mood, and calmness.

Please cite this article in press as: Schult J, et al., Effects of powdered fertilized eggs on the stress response, Clinical Nutrition (2009), doi:10.1016/j.clnu.2009.09.004

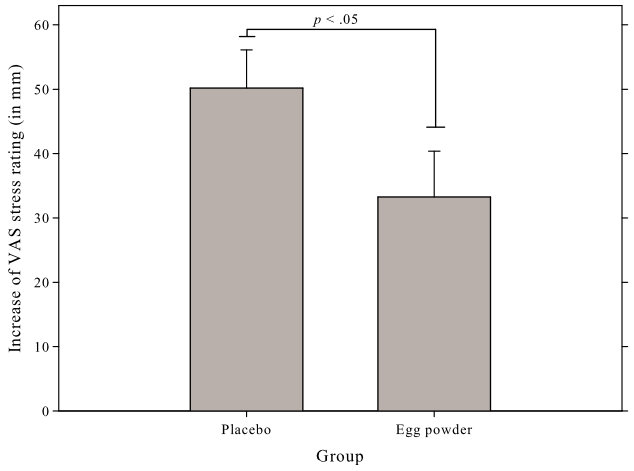


Fig. 5. Maximum increase of stress rating (VAS) in response to the TSST for the whole sample ($n = 40$). The graph shows group means with standard error bars and one-sided *t*-test.

An overall group comparison of cortisol levels during the TSST did not yield significant results. Interestingly, however, differences became visible once groups were divided into subjects with rather high levels of chronic stress and rather low levels of chronic stress (median split of TICS screening scale at baseline). Chronic stress permanently mobilizes the stress response network in the brain and results in a compensatory down regulation at the respective receptor sites. For example, chronic work stress has been reported to be associated with a dampening of the HPA axis response to the TSST.[14] In a recent review, Hellhammer et al. [15] summarized the current knowledge on the differential role of parvocellular arginine vasopressin (AVP) and corticotrophin releasing factor (CRF) in adaptation to chronic stress. They refer to the interplay between CRF and CRF/AVP neurons, which seem to differentially respond to chronic stress: "In animal studies, chronic stress results in a shift towards an increased AVP/CRF ratio in parvocellular neurons (…). Findings in humans are currently not consistent but a certain HPAA hyporeactivity in chronically stressed individuals seems to be likely" (p. 165). CRF/AVP neurons activate ACTH and other peptides, as well as noradrenergic and autonomic responses, which all vary with chronic stress. This complex interplay has been considered to be one reason behind the missing covariance between psychological measures of stress and salivary cortisol levels.[15] It seems that a permanent activation of CRF/AVP neurons desensitizes postsynaptic receptor sides on both corticotropic cells of the anterior pituitary and noradrenergic neurons of the locus caeruleus, thus resulting in a blunted endocrine and autonomic response to acute stress. Interestingly, several studies observed an elevation of previously low cortisol levels after stress management in patients with posttraumatic stress disorders,[16] fibromyalgia,[17] burnout,[18] foster care children,[19] and children with conduct disorders.[20]

As expected, high stress subjects of the placebo group showed the tendency towards a blunted cortisol response in the TSST whereas low stress subjects showed a normal increase to this challenge test. Cortisol means of subjects with a high impact of chronic stress almost reached levels of low stressed subjects indicating that they benefit in terms of YTE™ raising their cortisol levels up to a normal range in an acute stressful situation. Group differences suggest that the egg powder actively improves adaptation to acute stress by enhancing the endocrine and reducing the subjective stress response, thus counteracting effects of chronic stress. Subjects with less chronic stress do not show any beneficial effects.

Heart rate as an indicator of the autonomic nervous system showed less pronounced results but points into a similar direction as the endocrine data: whereas an overall treatment group comparison showed no significant group differences, a similar pattern emerged when looking at the high stress subgroup. Again, YTE™ raises subjects' heart rate in the TSST when compared to the high stress placebo-treated group.

In sum, these findings suggest that YTE™ restores the ability of chronically stressed subjects to adapt to acute stress. Since the brain has no own energy stores, it organizes its own glucose supply via the endocrine and the autonomic stress response. Particularly under enhanced demands (e.g., stress conditions), these mechanisms serve the brain by enhancing the synthesis and release of glucose and to support the allocation of glucose from the muscles to the brain.[21]

Notably, these effects could only be observed under stimulated conditions, whereas the circadian levels (CAR) remained unaffected. In addition, the findings from Eskeland[3] suggest that such effects cannot be observed under physical stress (e.g., muscle activity). Rather, cortisol levels seem to drop under these conditions after intake of YTE™. This supports the view that YTE™ has no unspecific effects on the pituitary-adrenal axis but rather differentially improves adaptation to mental and physical stress, depending on the nature of the stressor.

This hypothesis lends further support from the observation that YTE™ dampened subjects' perceived stress assessed by VAS scales. The maximum increase during the stress test protocol was smaller for the egg powder group compared to the placebo. Analyzing the data set separately for subjects with high and low stress chronic stress ratings, this result remains similar for both subgroups. This suggests that all subjects may benefit from YTE™ with respect to their perceived stress in an acute stressful situation.

In addition, egg powder intake is also associated with less increase of TSST-induced state anxiety at least in the high stress subsample. The treatment appears to facilitate stressed subjects' coping with the test situation.

The absence of changes in perceived stress and health-related quality of life across the four weeks of intake suggests that there is no effect of egg powder intake on these more general concepts.

The data of our exploratory analyses in chronically stressed subjects are encouraging, because they suggest that people may only profit both psychologically and physiologically from YTE™ once they are chronically stressed. This, however, needs to be confirmed in selected samples of chronically stressed subjects. In addition, such studies may control for effects of age and gender as well as eating habits.

Conflict of Interest Statement

DAaCRO, Germany performed this study for Med-Eq, Norway. Med-Eq provided all the funding for this study.

Acknowledgements

Statement of authorship: JH conceived of the study and co-wrote the manuscript. TH supervised the data analysis and co-wrote the manuscript. JS carried out the studies and data analysis and wrote the manuscript. All authors read and approved the final manuscript.

References

1. Mihaescu G, Olinescu R, Oancea F. Significant modification of lipid metabolism in aged persons following the treatment with a nutritive supplement containing embryonary peptides–preliminary results. *Rom J Intern Med* 2005;**43**:133–9.
2. Kovacs-Nolan J, Phillips M, Mine Y. Advances in the value of eggs and egg components for human health. *J Agric Food Chem* 2005;**53**:8421–31.

3. Eskeland B. *Effects of protein supplements on the natural production of hormones subsequent to hard training*. Oslo, Norway: Norwegian Academy of Sports; 1997.
4. Eskeland B, Thom E, Svendsen KO. Sexual desire in men: effects of oral ingestion of a product derived from fertilized eggs. *J Int Med Res* 1997;**25**:62–70.
5. Mason JW. A review of psychoendocrine research on the pituitary-adrenal cortical system. *Psychosom Med* 1968;**30**(Suppl.):576–607.
6. Dickerson SS, Kemeny ME. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull* 2004;**130**:355–91.
7. Kirschbaum C, Pirke KM, Hellhammer DH. The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 1993;**28**:76–81.
8. Kudielka BM, Hellhammer DH, Kirschbaum C. Ten years of research with the trier social stress test-revisited. In: Harmon-Jones E, Winkielman P, editors. *Social neuroscience: integrating biological and psychological explanations of social behavior*. New York: Guilford Press; 2007. p. 56–83.
9. Schulz P, Schlotz W, Becker P. *Trierer Inventar zum chronischen Stress (TICS)*. Göttingen, Germany: Hogrefe; 2004.
10. Laux L, Glanzmann P, Schaffner P, Spielberger CD. *State Trait Angstinventar (STAI)*. Weinheim, Germany: Beltz; 1981.
11. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav* 1983;**24**:385–96.
12. Steyer R, Schwenkmezger P, Notz P, Eid M. *Mehrdimensionaler Befindlichkeitsfragebogen (MDBF)*. Göttingen, Germany: Hogrefe; 1997.
13. Bullinger M, Kirchberger I. *SF-36. Fragebogen zum Gesundheitszustand. Handanweisung*. Göttingen, Germany: Hogrefe; 1998.
14. Bellingrath S, Kudielka BM. Effort-reward-imbalance and overcommitment are associated with hypothalamus-pituitary-adrenal (HPA) axis responses to acute psychosocial stress in healthy working schoolteachers. *Psychoneuroendocrinology* 2008;**33**:1335–43.
15. Hellhammer DH, Wust S, Kudielka BM. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* 2009;**34**:163–71.
16. Olff M, de Vries GJ, Guzelcan Y, Assies J, Gersons BP. Changes in cortisol and DHEA plasma levels after psychotherapy for PTSD. *Psychoneuroendocrinology* 2007;**32**:619–26.
17. Bonifazi M, Suman AL, Cambiaggi C, Felici A, Grasso G, Lodi L, et al. Changes in salivary cortisol and corticosteroid receptor-alpha mRNA expression following a 3-week multidisciplinary treatment program in patients with fibromyalgia. *Psychoneuroendocrinology* 2006;**31**:1076–86.
18. Mommersteeg PM, Keijsers GP, Heijnen CJ, Verbraak MJ, van Doornen LJ. Cortisol deviations in people with burnout before and after psychotherapy: a pilot study. *Health Psychol* 2006;**25**:243–8.
19. Fisher PA, Stoolmiller M, Gunnar MR, Burraston BO. Effects of a therapeutic intervention for foster preschoolers on diurnal cortisol activity. *Psychoneuroendocrinology* 2007;**32**:892–905.
20. Brotman LM, Gouley KK, Huang KY, Kamboukos D, Fratto C, Pine DS. Effects of a psychosocial family-based preventive intervention on cortisol response to a social challenge in preschoolers at high risk for antisocial behavior. *Arch Gen Psychiatry* 2007;**64**:1172–9.
21. Fehm HL, Kern W, Peters A. The selfish brain: competition for energy resources. *Prog Brain Res* 2006;**153**:129–40.

Please cite this article in press as: Schult J, et al., Effects of powdered fertilized eggs on the stress response, Clinical Nutrition (2009), doi:10.1016/j.clnu.2009.09.004

YTE AS ANTI DEPRESSANT

A placebo controlled, double-blind study on the efficacy of a nutritive supplement containing embryonic peptides in treatment of mild to moderate depressive mood.

Ester S. Kristoffer; Svein Gulbrandsen* and Erling Thom; Ph.D.**
Olav Duunsvei 15;3080 Holmestrand Norway
***Med- Eq Ltd; Tønsberg Norway, ** ETC Research and Development, Oslo, Norway**
Running title: Embryonic peptides and treatment of mood disorders
Corresponding author: Erling Thom;Stasjonsveien 5A; 0774 Oslo; Norway; Phones +47 22490664 or +4791710137. e-mail:erlingthom@etc.as

Background: Embryonic oligopeptides might have a therapeutic effect in the treatment of depressed mood.
Objectives: To investigate if a powder from fertilised eggs have a clinical effect on patients with mild to moderate depressed mood
Methods: In the present comparative, randomized, placebo controlled, double–blind, parallel group study we have investigated the effect of a nutritive supplement containing embryonic peptides (YTE™) in the treatment of mood disorders. Patients with mild to moderate mood disorders according to Hamilton rating scale (Ham-D) and Beck Depression Inventory (BDI) were included in the study according to the protocol. The patients were randomly assigned to receive placebo, Deprevent™ or Deprevent™ Forte for 12 weeks. The main outcome was change in the Ham-D total score from baseline to 12 weeks as well change in BDI from baseline to controls after 3, 6 and 12 weeks.
Results: 57 patients concluded the study. It was a significant effect in favour of the two active groups in the primary outcome measures as compared to placebo. Between the two active groups, however, it was no significant difference in the outcome measures, even if it was a weak tendency in favour of the Deprevent™ forte group. It was no reports of adverse effects in any of the groups during the study.
Conclusion: Based on the results from this study we conclude that these nutritive supplements might have a place in the treatment of mood disorders. With its excellent tolerability it might be an attractive supplement to the drug treatments used today.

Introduction

The avian egg is an important source of nutrients, containing all of the proteins, lipids, vitamins, minerals, and growth factors required by the developing embryo, as well as a number of defence factors to protect against bacterial and viral infections. Moreover, eggs are now understood to contain substances with biological functions beyond basic nutrition, and extensive research has been undertaken to identify and characterize these biologically active components (1).
It has also been demonstrated that the content of eggs can be manipulated, through various methods including diet and immunization, to target certain functionalities, leading to the hen as a bioreactor of the production of medically relevant substances (1). Continued research to identify new and existing biological functions of hen egg components will help to define new methods to further improve the value of eggs, as a source of numerous biologically active compounds with specific benefits for human and animal health, and secure their role in the therapy and prevention of chronic and infectious diseases.
We have also been interested in the medically use of manipulated eggs. The manipulation we are interested in is fertilisation. We have previously carried out one study with egg powder from fertilised eggs (2) in men with reduced sex drive with significant results as compared to placebo.
In addition a number of pilot studies have been carried out with the egg powder from fertilised eggs in people with depressed mood in Norway and US with quite promising results (Data on file at Med-Eq Ltd)
The results from these small pilot studies have been the basis for receiving an international patent on the utilization of an egg-based preparation as an antidepressant (EP094838).The egg powder has been shown to have an effect on cortisol (Data on file at Med-Eq Ltd)(3). In this way it is might be a pharmacological rational for the product to have effect on mood disorders.

In the blastodermel to protoembryonic stages of embryogenesis oligopeptides with molecular weights from 0.5 to 1.0 kD are formed and identified.
They are able to cross the digestive barrier intact. These embryonic peptides work via elevation of 17-ketosteroid levels in the adrenal glands which improve anabolism through increased synthesis of androgens and a decrease in the catabolic hormone cortisol, which offer multiple health benefits (3). In one of our earlier studies, the level of cortisol was reduced by about 50% after ingestion of similar doses of egg powder as used in this study (data on file Med-Eq Ltd). Certain of these peptides might also exhibit neurotransmitter effects.
Similar biological effects have been reported by others after intake of an egg powder from fertilised eggs (3) were it is a significant increase in the levels of DHEA-S and andostenedione while the increase in testosterone as slightly increased but not reaching statistical significance. Oxidative stress was also significantly reduced.
As the abovementioned pilot studies have been carried out as open studies it was decided to carry out a placebo controlled double – blind study in order to investigate the efficacy of the egg powder on mild to moderate depressed mood.

Material and Methods

Design of the Study and Sample Size
The study was designed and carried out as a randomized placebo controlled double-blind study with three parallel groups; a placebo group, an egg powder group and a group receiving egg powder and *Melissa officinalis*. The treatment period was 12 weeks, and the participants came to follow-up controls after 3, 6 and 12 weeks.
The patients were randomized to one of the three available treatments in the following way. The total number of patients was first randomized in 5 groups with 12 patients in each group. In the blocks of 12 the patients were randomized to receive one of three treatments. By using this type of block randomization we foresaw that the three treatment groups would be comparable at the start of the study with respect to the main parameters such as gender, age and disease score.
Recruitment of patients
The patients were recruited to the study through an advertisement in local newspapers in the Vestfold area in Norway where the investigator (ESK) has her practice.
A total of 60 patients was planned to be included in the study. As no relevant clinical data was available at this stage about the effect of the egg powder on the indications we were investigating, it was impossible to carry out valid sample size calculations. Our plan was therefore to include 10 patients in each of the three groups (a total of 30 patients). Based on this material we carried out a blinded interim statistical analysis on the efficacy parameters in the three treatment groups in order to confirm that the planned number of patients was valid or if we had to change it. It was also possible to stop the study at this point if we did not find any tendencies with respect to effect in the material between the three groups.

The investigational products

Fertilised eggs are collected daily from laying hens, subjected to a surface gassing to sterilize their outer surfaces and placed into storage at 11°C until a week's batch has been collected. Storage at 11°C halts the embryogenesis and during this period the eggs are rolled to prevent settling out of the contents. The week's batch is then placed in an incubator maintained at 39°C and a relative humidity of about 80 %. In the incubator an automatic turner turns the egg two or three times per day. After five days incubation the eggs are illuminated to determine which are not developing. These eggs are taken out. After 9 days incubation the eggs are removed ,dipped in 70% ethanol, cracked open in a sterile room, the liquid s removed and the solid phase is placed in a freeze drier. The content is freeze dried to about 2 % (weight %) over a period of two days at 56°C under vacuum. The crisp, flaky freeze dried product containing 2% humidity is then grounded and used as an ingredient in different food supplement product alone or mixed with other ingredients. The egg powder and the medical use of it are patented. The egg powder substance has the trade name YTE® (Young Tissue Extract) and depending on the indication different galenical formulations have been developed.
The eggs used are desirably the ones in the blastodermal and subsequent preembryonic to protoembryonic stages in which yolk transformation has begun, but the organs of the embryo are barely if at all discernible.

This corresponds essentially to the subembryonic stage of embryogenesis (generally 3 to 14 days incubation for a hen's egg), or the period up to acceleration of calcium uptake by the embryo. This occurs after about 15 days incubation for the hen's egg.
All investigational products used in this study were supplied by Med-Eq AS;Tønsberg. Three different types of capsules were used. Capsule type I is a placebo capsule. The placebo preparation contained lactose, while capsule type II contained YTE™ (Deprevent™) and capsule type III contained YTE™ and extract from the herb lemon balm (*Melissa officinalis*) (Deprevent™ forte) . Lemon balm extract has been shown in several studies to have a positive effect on mood disturbances (4-6). The idea behind combining the egg powder with the lemon balm extract was that the two components might have an additive effect.
In order to keep the blindness in the study the capsules had similar appearance and weight and were packed in similar boxes. The boxes were labelled in local language (Norwegian) with the following text: For clinical study: Take 3 capsules in the morning and 2 capsules in the evening. The capsules should be taken together with food and swallowed with water. The capsules should be stored at room temperature and out of reach of children.
Formal dose – response studies have not been carried out with the egg powder. In our earlier study (2) we have used daily doses of up to 6 g. However, during the later years a new production method has been introduced giving a more potent product with respect to the content of protein. The protein concentration is now at 80 % ±5% compared to 44-46% with the original separation method. This means that the efficacy of the product is improved as more of the inactive substances are removed by the new production process. The efficacy is tested in scientific studies and a daily amount of 1.6 g is equivalent to 3.0 g by the older method of extraction as far as the desired response is concerned.
We decided to use a daily intake of egg powder of 1680 mg (336 mg per capsule) and the daily intake of lemon balm was 600 mg per day. The total daily dose supplied in 5 capsules will mean that each capsule of contained 456 mg of active ingredients or placebo.
The patient received supply for 3 weeks of the actual preparation they are randomized to at inclusion visit (week 0). When they were coming back to the control visits at Week 3 and 6 they received a supply of the preparation for 3 and 6 weeks, respectively. On the control visits after 3, 6 and 12 weeks they brought with them and delivered the unused preparation. The capsules returned were counted and compared with the dose schedule in order to check the compliance to the recommended dose schedule. If the compliance requirement was not met this was considered as a protocol violation. The compliance to the recommended dose should be 80 % or more in order to include the actual patient in the statistical analysis.

Performance of the study

Candidates that gave a positive response to the advertisement were screened by the investigator (Week-1) in order to check if they met the inclusion criteria for the study. They also got written and verbal information about the study and the procedures involved. If they met the inclusion criteria and still were interested to participate after having received the information and were willing to sign the informed consent, they could be enrolled in the study.

Inclusion and exclusion criteria

The patients eligible for inclusion in the study should have mild to moderate depressive syndromes rated by Hamilton's scale and Beck's self rating scale (7,8). These tools are internationally accepted tools for diagnosing depression. Both scales have previously been used in several depression studies and have been validated as highly acceptable. The scales have been translated to Norwegian by qualified personnel and the Norwegian versions were used in this study. Participants of both genders could be included in this study.
The following inclusion criteria were valid:
Age in the range 18-65 years
Current diagnosis of mild to moderate depression rated by Hamilton /Beck rating scales
Cooperative and willing to meet at the control visits.

Willing to sign an informed consent form
Should not use antidepressants of any kind
No other chronic diseases that require continuous drug treatment
The following exclusion criteria were used:
Not meeting the score for mild to moderate depressions
Using antidepressants of any kind
If using benzodiazepam or hypnotics the dose should be stabilized
Allergy to egg products
Not willing to sign informed consent
Not able to participate in all follow-up visits

The patients received the medication and were asked to come back to control to the investigator after three weeks. At each of the control visits the Beck's rating scale was used to score the symptoms (8).
The Hamilton rating (HAM-D) scale (7) was used at inclusion in the study and at the last visit (after 12 weeks). In the statistical evaluation the scores during the study were compared with the initial scores.
Tolerability questions were asked at each of the follow-up controls. As tolerability is a main concern with all types of antidepressant therapy the enrolled patients were followed closely in that respect in this study. At each of the control the patients were asked the following question: " Have you had any nuisances that could be linked to the preparation you take ?". All reports from the patients were recorded in the clinical report forms.

Ethics

The study protocol, the patient information sheet, the informed consent , and the advertisement intended to be used in the patient recruitment as well as the evaluation tools used during the study were sent to the Regional Ethic Committee (REK) prior to starting the study. REK will be updated if any special events take place during the execution of the study. A final clinical report will be sent REK when the study is finalized.

Statistical methods

Mean will be used for estimation of continuous and near-continuous variables and Student procedure will be used for construction of confidence interval of mean. The one-sample t-test will be used for analyzing change over time within groups. Analysis of co-variance and two-sample tests will be used to compare between groups with regard to continuous variables.

Categorical variables will be reported using contingency tables. Fisher's extract test will be applied when testing 2 x 2 tables.
A Network Algorithm for Performing Fisher's Extract Test in r x c contingency tables will be used when testing tables greater than 2 x 2. A significance level of 5% will be used in tests, and two-tailed tests will be applied.

Results

As discussed earlier we carried out a blinded interim analysis when 10 patients had been included in the study. Based on this analysis it was decided to continue the study in order to enrol a total number of 60 patients. At this stage it was a clear tendency of effect differences between the three groups. Neither the investigator nor the patients were informed about the results of this analysis.
We were able to include a total of 60 patients in the study fulfilling the inclusion criteria as listed above. Of these 57, with 19 patients in each group, concluded the study according to the protocol. The drop-outs withdraw from the study for reasons not linked to the treatment they received.
The three groups are clinically comparable with respect to scores at the start of the study as well as with respect to age and gender distribution. These parameters are shown in table 1.

The clinical results after 12 weeks treatment show the following. The reduction in BDI score in the placebo group (N=19) is 5.0 , while the corresponding reductions in the Deprevent™ forte group (N=19) and the Deprevent™ group(N=19) are 11.6(SD2.2) and 11.2(SD2.0) respectively. The reduction in scores in the two groups receiving active treatments is statistically significant compared to the score reduction in the placebo group ($p< 0.01$). It is, however, not a statistically significant difference in scores between the groups receiving the two active treatments. The development in scores as a function time in the three treatment groups during the study period is shown in Table 2.

It is worthwhile to comment that already after a treatment period of three weeks we detected a substantial reduction in BDI scores in the two groups receiving the active treatments.
With respect to HAM-D scores that was obtained at the initially and at the end of the study. The score reduction was 2.0 in the placebo group and 10.1 in the Deprevent™ forte group and 8.3 in the Deprevent™ group, respectively. It is a statistically significant reduction in the two active groups as compared to the placebo group. However, between the two active groups the difference in reduction is not statistically significant ($p>0.05$)
Following the control of the returned capsules at each of the control visits we found that all of the participants had complied with compliance requirement of taking at least 80% of the recommended dose during the study period. All of them could therefore be included in the statistical evaluation.
None of the participants reported any side –effects of the treatments they received. The three patients that withdrew from the study did so for reasons not linked to the treatment.

Discussion

The results from this study show that egg powder produced as outlined and given in the doses used has a significant positive effect on depressed mood. By comparing the three treatments we can not detect any significant clinical differences between Deprevent™ and Deprevent™ Forte (the one with *Melissa officinalis*). The addition of this herb does not seem to give an additional effect on mood disturbances and will not be included in the product in the future.

With respect to the mechanism of action of the egg powder from fertilized egg on mood disturbances it might be that it is due to precursors for building sex hormones found in the egg powder. As we have reported from an earlier study we found positive response in sex drive after taking the egg powder and we also saw a normalisation of the teststosterone levels in participants having a low level at the start of the study

Chemical analyses carried out on egg powder from non-fertilized and fertilized eggs show remarkably little difference in composition with respect to aminoacid composition (9) .
Yolk steroid hormones have been documented to have growth and behaviour effects on hatchlings in several avian species (10). In this study it has ,however, been shown that the initial levels of androstenedione (A), dihydrotestosterone (DH), estradiol (E2) ,and testosterone (T) are decreasing significantly during the embryonic development.
After an initial decline , E(2) in the yolks of chicken eggs undergoes a significant increase at the end of the development, between embryonic stage 40 and 45 (days 14 and 20 of the development). As the increase is much larger than could be accounted for by hormones present in the yolk material , this may represent early embryonic production of steroid hormones by developing gonads. Similar results are reported in a later publication (11).

Clinical studies confirm that Deprevent™ contains biostimulators (oligopeptides) that are able to cross the digestive barrier (3). The most important target organ for these biostimulators is the adrenal glands. This is because the adrenal glands produce hormones like androgens, estrogens, glucocorticoids, mineralcorticoids, adrenaline, and noradrenaline. However. it is important not to overstimulate these glands since this can result in mental irritation, loss of muscle mass, and loss of strength. Egg powder is not an adrenal stimulator, and does not force the adrenals to produce more hormones but helps the adrenal output. Specifically, the egg powder will help to regulate the adrenal 17-ketosteroid sulphates and the 7-hydroxycorticosteroids (17-OHCS). The regulation of these hormones has been shown to enhance tissue function, including muscle, skin, GI, vascular, nerve , and immune system functions. Moreover it has been it has been shown to positively influence lipid metabolism (3), protein anabolism, and memory, as well as many other physical and mental functions. Regular use of the egg powder can also stimulate natural DHEA and testosterone production(3), reduce physical and mental stress (3), burn fat, promote more restful sleep, and increased libido (2).

The daily dose of egg powder used in this study is 2000mg (3) and is comparable to the dose used in our study of approximately 1700 mg

Treatment with egg powder from fertilized eggs thus give a number of interesting treatment possibilities and a these possibilities should be investigated in well designed clinical studies in the future.
As we have shown in this study the egg powder might be an effective and well tolerated food supplement for people with mood disturbances and could thus be an interesting supplement to conventional drug therapy: More documentation on the effect will be

needed before firm conclusions can be drawn with respect to mood stabilizing effect of the egg powder and the mechanisms leading to this improvement But as pointed out earlier it might be the growth hormones can be involved in the positive effect seen with the egg powder, as recent research has shown the growth hormones can have an effect to play in treatment of depressions (12). As we also know that oligopeptides have a relative strong antioxidative potential (3) this might also be a part of the mechanism of action of the product on mood disorders (13).

Table 1: Anthropometric parameters for the enrolled patients group wise. Standard deviations in brackets.

Group/Number	Initial score	Age	Gender distribution
Placebo(N=19)	20.7(3.5)	45.4(3.9)	3M/16F
Deprevent (N=19)	22.0(3.7)	45.2(4.1)	4M/15F
Deprevent Forte (N=19)	23.0(3.9)	44.2(3.9)	4M/15F

Table 2: The development of the average BID scores in the three treatment groups. Standard deviations in brackets.

		Change in score			
Group	**Initial score**	**3weeks**	**6 weeks**	**12 weeks**	**Diff**
Placebo	20.7(3.5)	-4.9	-0.6	+0.5	5.0(1.4)
Deprevent	22.0(3.7)	-6.9	-1.7	-2.6	11.2(2.2)
Deprevent forte	23.0(3.9)	-7.8	-1.4	-2.4	11.6(2.0)

Table 3: The development of the HAM-D scores in the three treatment groups. Standard deviations in brackets.

		Change in score	
Group/Number	**Initial score**	**12 weeks**	**Diff**
Placebo	18.3(2.7)	16.3(2.5)	-2.0(1.2)
Deprevent	19.5(2.8)	9.4(1.2)	-10.1(1.5)
Deprevent forte	18.9(2.9)	10.6(1.3)	- 8.3(1.4)

References:

1. Kovacs-Nolan J, Philips M & Mine Y. Advances in the value of eggs and egg components for human health. J Agric Food Chem. 2005; 53 : 8421-8431.
2. Eskeland B, Thom E & Svendsen KOB. Sexual desire in men: Effects of oral ingestion of a product derived from fertilized eggs. Int J Med Research 1997; 25 :62-70.
3. Mihaescu G, Olinescu R & Oancea F. Significant modification of lipid metabolism in aged persons following treatment with a nutritive supplement containing embryonary peptides-preliminary results. Rom J Inter Med 2005;43:133-139.
4. Kennedy DO, Shoe AB, Tidily NT, Perry EKE & Weans KA. Modulation of mood and cognitive performance following acute administration of Melissa officinalis (lemon balm). Pharmacology Biochem Behave 2002:72:953-964.
5. Kennedy DO,Wake G,Savelev S,Tilsesley NT, Perry EK,Wesnes KA,Scholey AB. Modulation of mood and cognitive performance following acute administration of single doses of Melissa Officinalis (Lemon balm) with human CNS nicotinic and muscarinic receptor binding properties. Neuropsychopharmacology 2003;28:1871-81
6. Wheatley D. Medicinal plants for insomnia: a review of their pharmacology, efficacy and tolerability. J Psychopharmacol 2005;19:414-21
7. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psyhiatry. 1960;23:56-62.
8. Beck AT, Steer RA & Brown GK. The Beck Depression Inventory. 2nd ed. San Antonio, Tex: Psychological Corp;1993
9. Paul AA & Southgate DAT: The Composition of Food, Fourth Revised Extended Edition of MRC Special report No297,pp.280, 1978
10. Elf PK& Fivizzani AJ. Changes in sex steroids in yolks of the leghorn chicken ,Gallus domesticus , during the embryonic development. J Exp Zool 2002; 293:594-600.
11. Eising CM, Muller W, Dijstra C & Groothuis TGG. Maternal androgens in egg yolks: relation with sex, incubation time and embryonic growth. Gen & Comp Endocrinol 2003;132:241-247.
12. Evans SJ, Choudray PV, Neal CR, Li JZ, Vawter MP, Tomita H, Lopez JF, Thompson RC, Meng F, Stead JD, Walsh DM, Myers RM, Bunney WE, Watson SJ, Jones EG & Akil H. Dysregulation of the fibroblast growth factor system in major depression. PNAS 2004;43:15506-15511
13. Yanik M, Erel O & Kati M. The relationship between potency of oxidative stress and severity of depression. Acta Neuropsychiatrica 2004;16:200-203.

YTE FOR STRENGTH & HORMONE PRODUCTION

EFFECTS OF NUTRITIONAL SUPPLEMENTS DESIGNED TO PROMOTE LEAN TISSUE ACCRETION ON BODY COMPOSITION AND STRENGTH PERFORMANCE.

This study (double blind and placebo controlled) was carried out at the University of Colorado in Arapaho Community during the spring and summer of 1996. The study's main purpose was to test the effect of two nutritional preparations in developing muscle strength and muscle mass. Their effect was also studied with regard to any influence they might have on volunteer's testosterone and LH hormone levels, body composition (muscle weight in comparison to fat weight), and a number of subjective conditions. The preparations being tested were "Multifactor" (a composition which includes among other things 5 g egg powder and 5g creatine per day) and creatine Monohydrate (5g/day).

The volunteers: thirty two (32) 19-35 year old men and women (mean age of 30) were recruited from Arapaho Community College Fitness Center in Colorado, USA, to take part in a 6 week test of how the above-named preparation would affect a number of different dependent variables. Nineteen (19) of these volunteers completed the study. Their mean training time was 17.5 months. 10 volunteers took Multifactor and 9 volunteers took creatine.
In addition, a group consisting of 7 advanced bodybuilders with a mean training time of 6 years was recruited. This group took Multifactor.
Altogether, therefore 26 volunteers were included in the test. Volunteers not completing the test totaled 33% (i.e. 13 out of 39 persons).

Measurement of the study's dependent variables:

1. *Muscle mass* was measured in two ways: (a) by measuring the following parts of the body with a centimeter tape-measure, in most cases both tensed and relaxed: shoulders, chest (men only), waist, hips (women only) and thighs, also (b) by measuring body composition, which was determined partly by underwater weighing and partly by caliper and ordinary weighing scales.
2. *Strength* was measured in two ways, with bench press and leg press (a) maximum weight for one repetition (1RM) of both bench press and leg press, and (b) maximum number of repetitions with 70% of 1 RM weight for bench press and 80% of the 1 RM weight for leg press.

3 *Testosterone and LH levels* were measured by blood tests taken by personnel at the university hospital's clinical research center.

4 *Dietary records* were kept for four days by the volunteers. They were analyzed later by the personnel at the university hospital's clinical research center. This analysis provided information on the number of calories, amounts of protein, carbohydrate, fat, vitamins, minerals, water etc. the volunteer in question normally consumed per day.

5 Information about a number of *subjective conditions* (for instance, energy levels, training motivation, mental alertness, well-being, health, libido, self-evaluation, muscle strength and muscle mass), *training experience, diet and certain living habits* were defined by questionnaire at three points: before the test, after three weeks, and after six weeks at the end of the program.

3: Subjective measurements
(a scale of 9 where 1 = least and 9 = most):

How effective was the preparation in comparison with the other, in the following:

	Increased muscle definition:	Fat reduction	Increased energy:
Multifactor group:	8,0	7,5	8,0
Creatine group:	6,2	5,6	6,2

There is an almost statistically significant difference between the Multifactor group and the Creatine group (p=0,09) regarding fat reduction.

There is an almost statistically significant difference between the Multifactor group and the Creatine group (p=0,09) regarding increased energy.

Søyleforklaring

Multifactor

Creatine

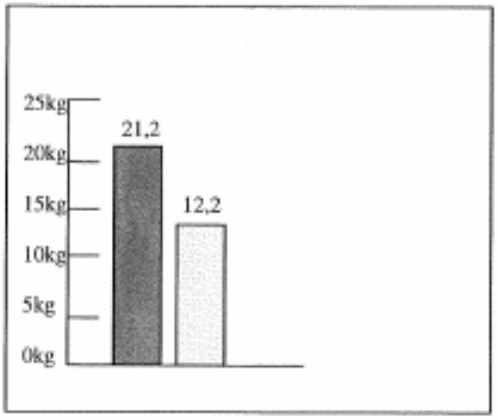


Fig.1 Strength increase in leg press after six weeks training and consumption of Multifactor and Creatine.

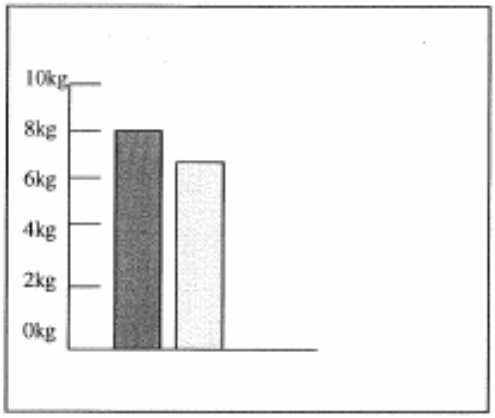


Fig.2 Strength increase in bench press after six weeks training and consumption of Multifactor and Creatine.

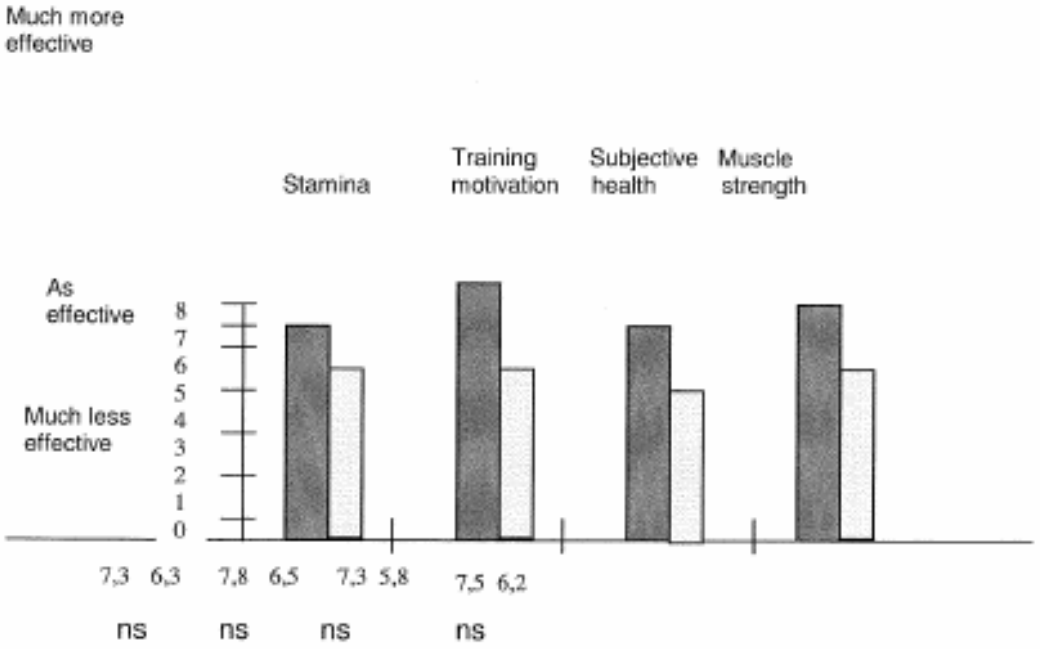


Fig 4 How effective were Multifactor and Creatine compared with other diet supplements?

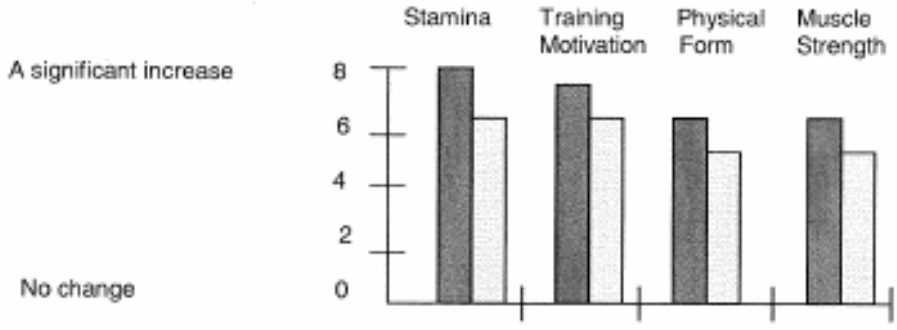


Fig 5 Increases after 6 weeks training with Multifactor and Creatine as diet supplement.

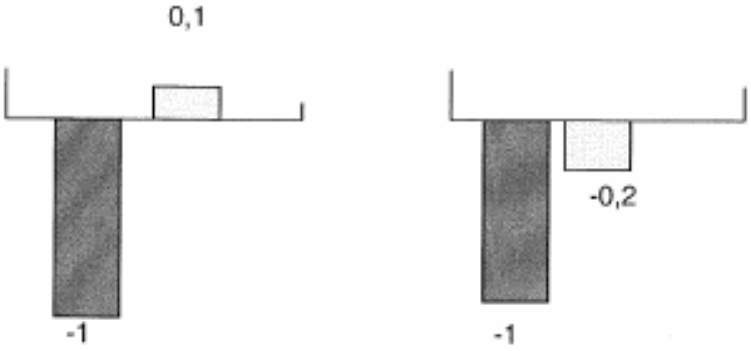


Fig. 3 Fat reduction after six weeks training with Multifactor and Creatine as diet supplement.

3. Subjective measurements

(a scale of 9 where 1= least and 9= greatest)

After 6 weeks on the program, changes in the following:

	Muscle mass	Strength	Physical shape	Training motivation
Multifactor group:	7,8	7,9	7,7	8,1
Creatine group:	6,9	6,9	6,3	6,6

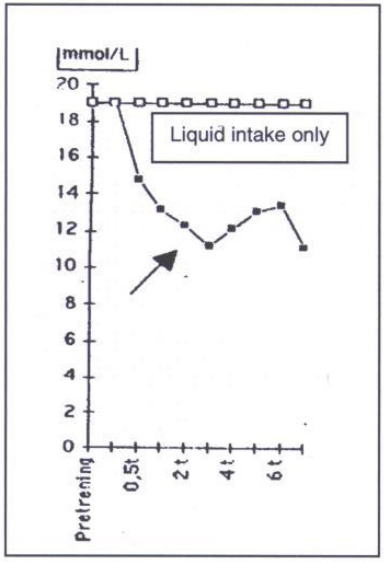
There is an almost statistically significant difference between the Multifactor group and the Creatine group (p=0,09) regarding strength.

There is an almost statistically significant difference between the Multifactor group and the Creatine group (p=0,059) regarding physical shape

There is an almost statistically significant difference between the Multifactor group and the Creatine group (p=0,02) regarding training motivation.

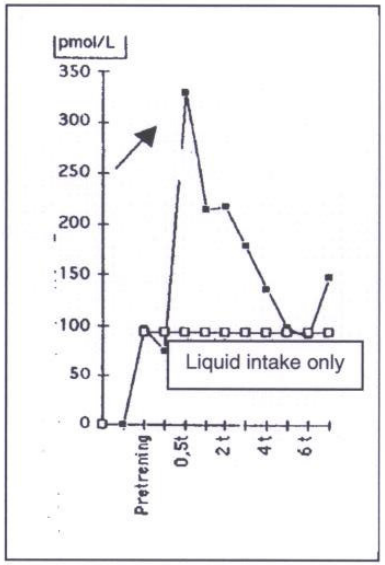
TESTOSTERONE

	Protein	Liquid
Pretraining	18,9	19
Posttraining	19,1	19
0,5hour	14,8	19
1hour	13,2	19
2hours	12,3	19
3hours	11,2	19
4hours	12,1	19
5hours	13,1	19
6hours	13,4	19
8hours	11,1	19



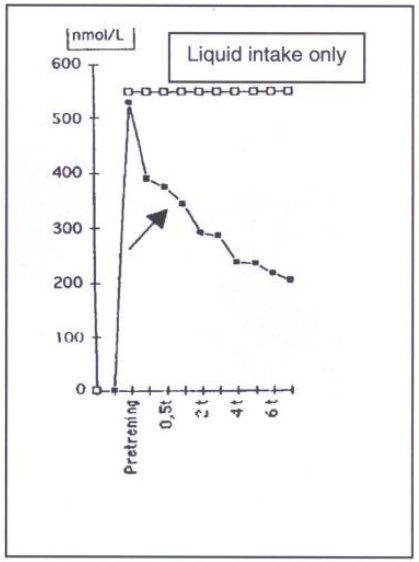
INSULIN

	Protein	Liquid
Pretraining	97,9	95
Posttraining	74,7	95
0,5hour	329,3	95
1hour	213,6	95
2hours	216,6	95
3hours	178,4	95
4hours	136,0	95
5hours	99,0	95
6hours	89,8	95
8hours	148,0	95



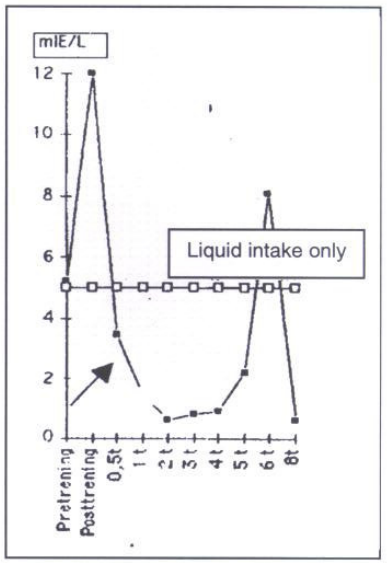
KORTISOL

	Protein	Liquid
Pretraining	530	550
Posttraining	390	550
0,5hour	375	550
1hour	345	550
2hours	290	550
3hours	285	550
4hours	238	550
5hours	236	550
6hours	218	550
8hours	205	550



GROWTH-HORMONE

	Protein	Liquid
Pretraining	5,2	5
Posttraining	12,0	5
0,5hour	3,5	5
1hour	1,4	5
2hours	0,6	5
3hours	0,8	5
4hours	0,9	5
5hours	2,2	5
6hours	8,1	5
8hours	0,6	5



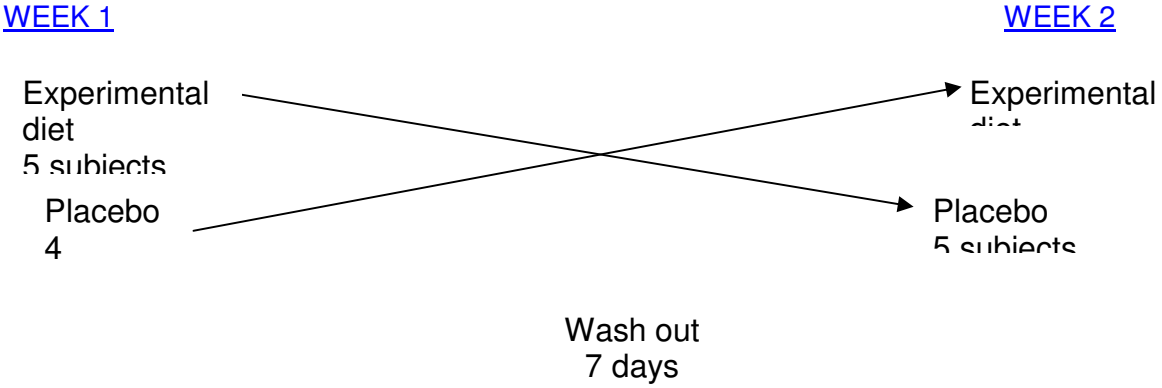
The product has been tested in a double blind study approved by an ethical research council. As the graph shows the following effects have been achieved with the test product.

1. The test product shows significant reduced kortisol values (stress value) which is important to achieve a positive nitrogen balance.
2. Reduced testosterone levels – shows that the hormone are used in the muscle synthesis process.
3. The test product increases the Insulin production, which leads to an increased amino acid absorption.
4. The growth hormone is positively effected when taking the test product.

OF HORMONES SUBSEQUENT TO HARD TRAINING.

(study conducted at the Norwegian Academy of Sport)

This study was carried out with 9 advanced bodybuilders between the age of 20 to 30. The criteria used in selecting the individual was at least 2 years strength training.
In the cross-over designed study the participants consumed a meal (500 Kcal in liquid form) of carbohydrates and protein in the ratio of 50:50.
Immediately after 2 hours hard training, and repeated 2 hours later.
Placebo group was given the same ratio on isoenergy basis, and the protein fraction to be studied was replaced by albumin from regular eggs.



Prior to the study, the participants did not train, and minimized their physical activity to the absolute minimum the last 48 hours.
They recorded their food intake 3 days prior to the study. As the study started as early as 7:00 AM, they had no food before the test.
Blood samples were collected from the jugular vein before exercise, immediately after, and 30 and 60 minutes after completed training. Thereafter every hour for 8 hours. The blood samples were analyzed for testosterone, insulin, kortisol, growth hormone and urea-N.
Increased cellular uptake of testosterone, higher insulin level and low kortisol values were recorded in individuals on the experimental diet, compared to control. The favorable cellular hormone state is likely to increase protein synthesis and decrease protein breakdown.
Interesting, the values of testosterone were declining after the hard work-out, and was even lower for the individuals on the protein supplement than the control, and may be caused by increased uptake of testosterone in the muscular tissue.
Since it is only free testosterone, and not the bounded that can be available for uptake in the muscle cells, it is important that the testosterone are not bound to SHBG. Higher uptake of testosterone results in more efficient protein synthesis via increased formation of mRNA.
Kortisol level were declining and more in the experimental group than the control. To keep this hormone as low as possible is of outmost interest in all physical training and competition, and important to obtain a positive N-balance and shortened time of restitution after a hard work-out.

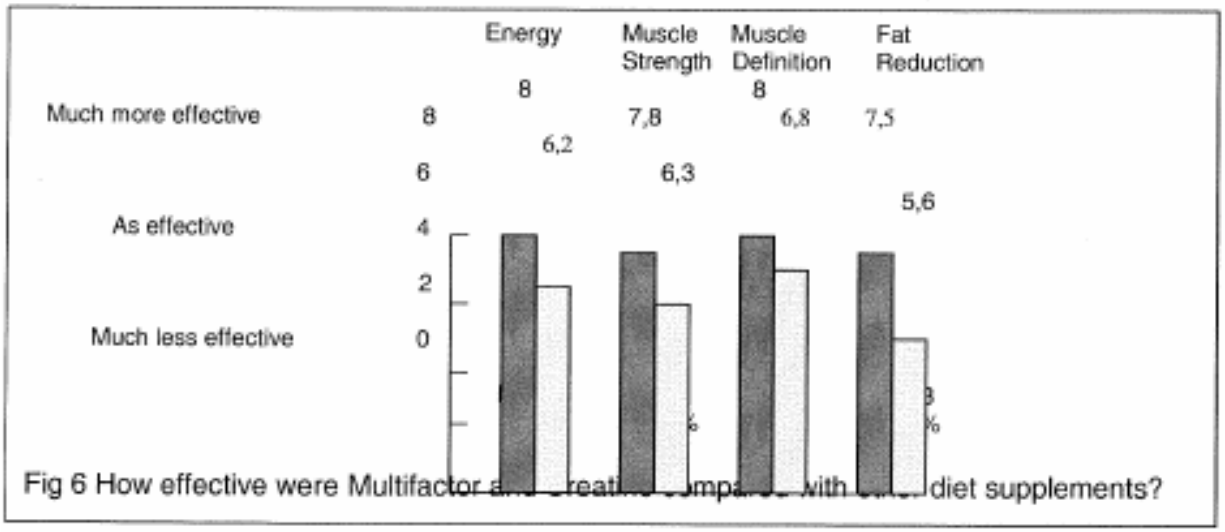


Fig 6 How effective were Multifactor and creatine compared with other diet supplements?

List of words in figures:

Fig 3
Calliper method
Hydrostatic weighing

Fig 4
Much more effective
As effective
Much less effective
Stamina
Training motivation
Physical form
Muscle strength

Fig 5
A significant increase
No change
Stamina
Training motivation
Physical form
Muscle strength

Fig 6
Much more effective
As effective
Much less effective
Energy
Muscle strength
Muscle definition
Fat reduction

YTE ON SEXUAL DESIRES

CLINICAL REPORT

PROJECT:

A randomized placebo controlled study of "Libido" on the sexual desire in middleaged men.

PROJECTHEAD:

Erling Thom; Ph.D;
Medstat Research A/S
Lillestrøm; Norway

Date: April 1992
647KL/TP185/AB.ET

MEDSTAT
RESEARCH

FOREWORD

The present study was carried out in the period october 1991 - february 1992, and should be jugded as a preliminary investigation. 16 persons participated in the study. The report presents and discusses the results. Additional investigations are needed in order to verify the results from this study.

Lillestrøm April 1992

Erling Thom; Ph.D.

I INTRODUCTION

Through the ages people have been investigating possibilities for finding substances having effect on the sexual drive and desire. Substances with such effects are named aphrodisiacs. Several remedies have been proposed and tried, and most of them have failed to show any effect in clinical studies.

Reduced sexual drive can be a complex problem, and by nature the sexual arousal decline by age. The libido can further be influenced by diseases and drug treatment. It is utopian to believe that is possible to find one remedy that will have good effect in all persons with reduced libido.

"Libido" is a natural product based on extract from eggs. It is a theoretical basis for its effect on libido. In open pilot-studies the participants have reported a clear-cut positive effect on the sexual drive. Open studies have, however, several weaknesses (bias, placebo-effects etc.) In order to verify, or invalidate, the indications about effect, it was decided to perform a controlled, double-blind study. The present report contains the results from the study.

II AIM OF THE STUDY

The aim of the study was to investigate and compare if Libido have an effect on sexual drive as compared to placebo in a group of middleaged men.

III DESIGN OF THE STUDY

The study was carried out as a randomized placebo-controlled double-blind study with a duration of 6 weeks (e.g. 3 weeks on each of the two preparations). The complete trial plan is enclosed.

IV EVALUATION OF EFFECT

The effect evaluation was performed by the participants themselves on a weekly basis using a visual analog scale of 10 cm with defined endpoints "No change" and "Very pronounced change".

RESULTS

THE MATERIAL

16 men aged 47 to 60 years (average 52,5 yrs) have participated in the study. The average bodyweight was 84.0 kg and the height 181 cms. The Body Mass Index (BMI) is on average: 25,6 kg/m^2, indicating normalweight.

The dosing of LIBIDO/placebo in the study is based on the experiences obtained in the previous pilot-studies. No dose/response studies have been performed so far.

None of the participants used drugs on a regular basis. Half of the patients received LIBIDO during the first periode (3 wks) and the rest received placebo. In the second part of the study the treatments were interchanged.

EFFECT

The study is performed, as previously mentioned, as a cross-over study without a wash-out period between the two treatments. This design is chosen because the number of subjects can be reduced when compared to a parallel group design.

Table 1 shows the average scores for all participants receiving LIBIDO in the first period and placebo in the second on a weekly basis.

Table 1: Average scores (n=8)

	LIBIDO → PLACEBO					
Week no.	1	2	3	4	5	6
Score (cm)	0,11	1,69	7,84	2,48	0,18	0,18

As can be seen from the table an increase in the VAS-score takes place during week 2 with high score in weeks 3 and 4.

In table 2 the average score for the participants having the opposite treatment scheme are listed.

Table 2: Average scores (n=8)

	LIBIDO → PLACEBO					
Week no.	1	2	3	4	5	6
Score (cms)	0	0.13	0.13	0.31	1.74	7.75

As can be seen from the table, low scores are recorded during the period with a significant increase in the second ad third weeks of the LIBIDO periode.

Table 3 lists the average scores for all participants on a weekly basis for both periods.

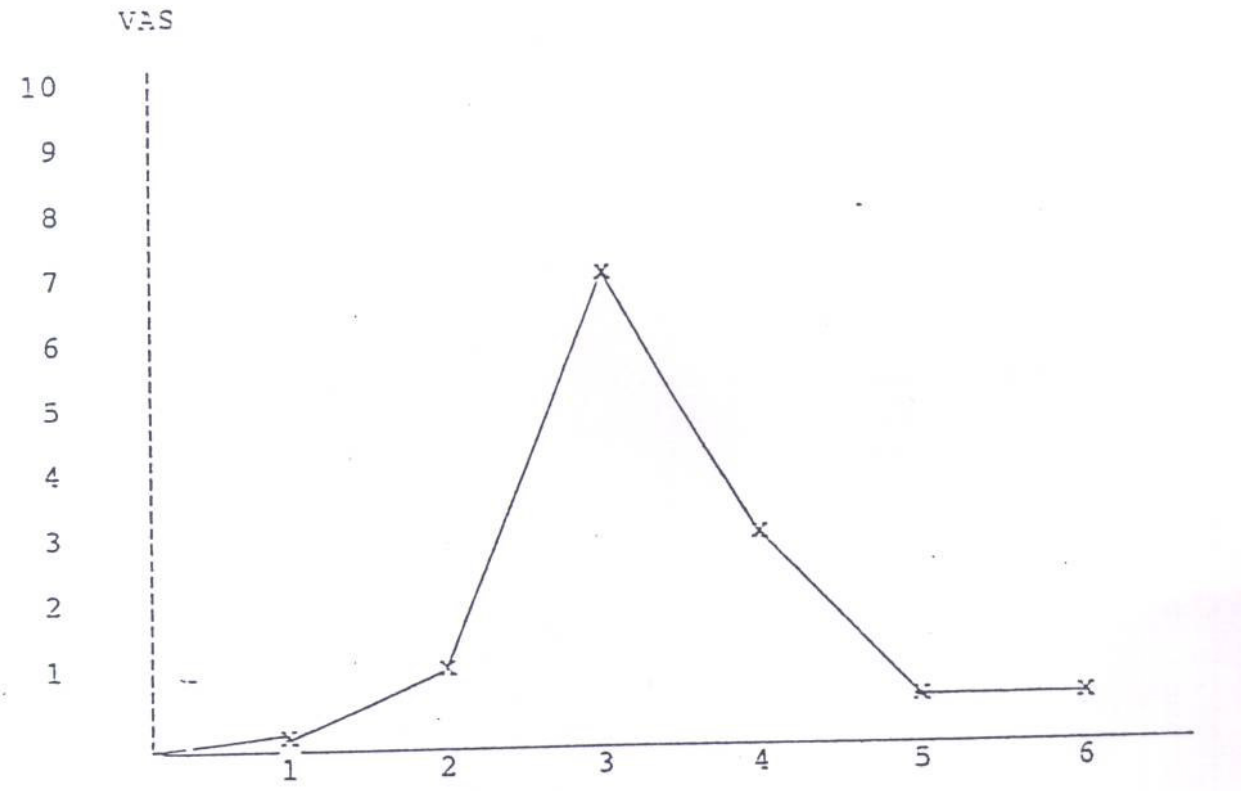
Table 3: Average scores for all participants (n=16)

MAIN SCORE			
	Week 1	Week 2	Week 3
Libido periode	0.21	1.72	7.80
Placebo periode	1.24	0.16	0.25

As can be seen from the table, Libido gives a significantly higher score than placebo in weeks 2 and 3. The relatively high score for placebo in week 1 is due the residual effect from patients randomized to the treatment scheme LIBIDO - placebo. This can also be seen from table 1. Some of the effect of LIBIDO is still present during the first week in the placebo periode because no wash-out periode is used between the two treatment periods.

In figure 1 the VAS-score for one of the participants receiving LIBIDO in the first period and placebo in the second period is illustrated.

Figure 1:



The effect of LIBIDO seems from the results of the study to come after 2 - 3 weeks treatment. Whether the effect will be even more pronounced later on can not be juged from the results of the study.

TOLERABILITY AND ACCEPTANCE

None of the persons participating in the study reported any side-effects or uncomfort related to the treatment. Six of the participants would favour that the tast of the product was changed. They expressed that the present tast was too strong (spicy). Two of the participants found that the solubility of the powder could be better, if possible.

CONCLUSION

The results from the placebo controlled double-blind study with LIBIDO indicate that the preparation has an effect on the sexual drive (libido) in middleaged men as compared to placebo. The study has been carried out in a relatively small sample (n=16) and the results should therefore be verified in a larger population.

Spezialised studies for investigating the mechanism of action for LIBIDO should be designed. The present study is a short-trem study (2x3 weeks) and can not give answer to the question whether the effect of LIBIDO will be long-term. Only carefully designes studies will give answer to this question.

The dose used in this study (3g x 2) seems to be satisfactory. The galenical formulation should however be somewhat changed especially with reference to taste.

APPENDIX 2

Preliminary Clinical report/MAY 1993.

COMPANY : DRYMED A/S; c/o HEROS A/S, BOX 30 Bygdøy 0211 Oslo Norway.

PROJECT : A randomised placebo controlled double-blind study of Libido and placebo on the sexual desire in heathy middel-aged men.

INVESTIGATORS: Kjell-Olav Svendsen;MD, Frogner Helsesenter,Oslo and Einar Christiansen;MD, Røde Kors Klinikken, Oslo.

PROJECTHEAD : Erling Thom;Ph.D., Medstat Research A/S, Lillestrøm.

FOREWORD

The study reported here between placebo and **LIBIDO** was performed in order to investigate the effect on sexual drive in middel-aged men. The study was carried out during autumn 1992 and spring 1993.
The study is a part of a research project aimed at investigating the effect of **LIBIDO** on sexual drive. At present only the effect on men has been investigated, but in the future investigations in females will also be performed. In future studies focus will be placed on trying to define which patients that could profit from taking **LIBIDO** and also trying to investigate somewhat more the mechanism of action for the product. Hopefully laboratory tests could be a valuable tool in defining these patients.

Oslo May 1993

Dr. Einar Christiansen
Røde Kors Klinikken,Oslo

Dr. Kjell-Olav Svendsen
Frogner Helsesenter,Oslo

Erling Thom,Ph.D.
Medstat Research A/S
Lillestrøm

SUMMARY

* The results from this study with **LIBIDO** and placebo confirm the results from a previous study. **LIBIDO** seems to have effect on the sexual drive in some middel-aged men.

* The results obtained in this study are not as pronounced as the ones in the first study. This might be due to the differences between the patient poulations in the two studies.

* At present we have no basis for defining which patients that could be expected to be responders to treatment with **LIBIDO**.

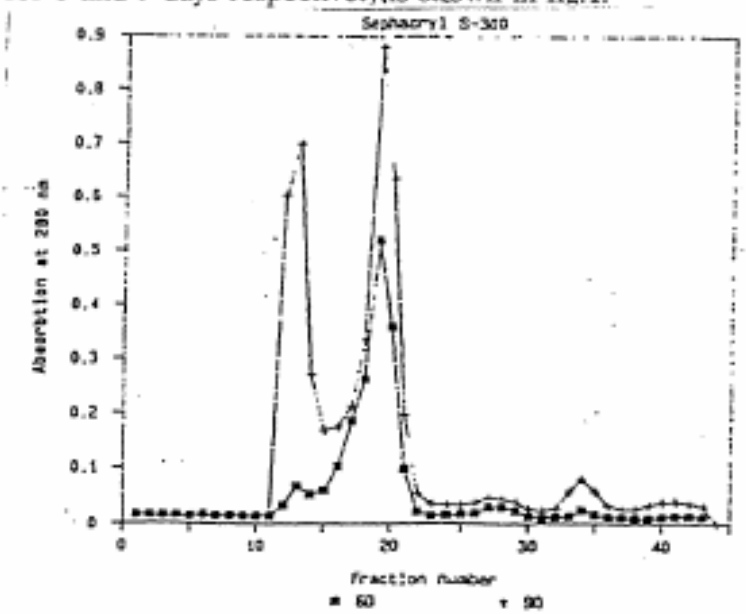
* Approximately 42% of the patients in the study did not respond to treatment with **LIBIDO**.

* The tolerability is excellent. Only one patient reported side-effects in the period treated with placebo.

INTRODUCTION

LIBIDO is a natural product based on fertilized eggs. The hatching is stopped after 9 days and from these eggs the raw material for **LIBIDO** is produced. Chemical analyses of the powder reveal that it has a special composition. On this background the hypothesis, that it may have an effect on the sexual drive in humans, was launched. The idea was put forward by a Norwegian research group with great expertise about eggs and the composition of fertilized eggs. An egg contains normally considerable amounts of cholesterol(in average 225 mg per. egg), while the amounts in fertilized eggs are greatly reduced as cholesterol is an important building block in the development of the fetus. The possible mecanishm of action behind the effect on sexual drive is not known in detail, but a possible explanation is that it can can have an effect on the hormone concentrations. Drymed A/S has developed the product to the present formulation and Bjødne Eskeland,Ph.D. and Peder Gjendemsjø have been responsible for development of the product. The composition of the product is shown in appendix 1.

Absorption studies of the powder have been carried out showing clear-cut differences between powder produced from eggs incubated for 6 and 9 days respectively. A marked increase in highmolecular substances is seen between these samples. These substances might be precursors to sexhormones. Spectra showing the differences in compositions of eggs hatched for 6 and 9 days respectively,is shown in fig.1.



Clinical studies aimed at investigating the effects of drugs and other substances on the sexual drive are challenging and are problematic to carry out. These kind of studies should always be performed as double-blind placebo controlled studies. A priori one can

expect a high placebo response in these patients.

A number of specialists working with sex problems are of the opinion that the number of patients having problems with sexual drive have increased during the last 10-15 years. Whether it is a real increase in the number of problems or is a result of a more openminded attitude towards these problems, can be discussed.
Relatively limited research has been carried out on sexology in Norway, and no studies on sexual drive and substances with potential to stimulate the sexual drive have been performed.

In a study carried out on sexual habits amongst Norwegians by the Norwegian Association for Sexology in 1987 (Svendsen,Almås,Benestad), 16% of the 666 participants said that they had had sexual problems ,which they needed professional help to solve. 13 % of the men and 38% of the females revealed that the main problem was lack of or low sexual drive. Low sexual drive is defined as " lack of desire ". In some persons the lack of desire is of a general nature, meaning that all sexual drive is lacking irrespective of partners. Others , however , lack the desire just toward the partner. The most common desire problems are those which develope in partnership over time.

The mechanisms behind sexual desire are complex, and physical as well as mental factors are playing a role. Somatic as well as psychiatric diseases, sexual problems , conflicts and aging are also important factors in this context, and will influence the desire.

Testosterone is the hormone with the most potent effect on sexual desire in both genders. A reduced level of testosterone will normally reduce the sexual desire. It is important to differentiate between reduced desire and reduced ability, for instance erection problems. In men castration, as an example, will reduce the desire while the erection not will be influenced in the same way. With reduced desire most men will have erection, but the desire problem may also secondary reduce the erection ability.

Testosterone circulates in the body bound to SHBG(Sexual Hormone Binding Globuline), and only the free fraction is biologically active. The testosterone concentration varies from day to day and even during the day, with the higher concentrations in the morning and with a reduction of 20-30% during the day. The testosterone levels in plasma are relatively stable from puberty to the age of 55-65 years and are thereafter slowly reduced. SHBG increases with age, and therefore the biologically active part of testosterone is rduced as function of age. The hormone system is also influenced by disease, physical and mental factors- as an example one can get a reduction of testosterone through stress and/or physical strain. This reduction will, however, be restored quickly. The correlation between desire and testosterone is complex. Supply of testosterone in men with normal concentrations has been shown to have no effect. In persons with low testosterone concentrations, treatment with testosterone can have an effect on sexual desire.

Libido has been tried by a number of men in open pilot-studies, and the response has been positive with reports on increased sexual desire. These experiences are ,however, worthless as documentation, and only indicate that it can be worthwhile to continue with investigations on the effect in a scientifically reliable way. Previously a double-blind placebo controlled study in 16 middle-aged men with cross-over technique has been carried out in Norway. The results from this study is very interesting. A clear-cut

difference was obtained in the effect on sexual desire between **Libido** og placebo, in favour of **Libido,** rated by the patients themselves on a visual analoqe scale. The persons participating in this study were healthy middle-aged men(N = 16) aged 47-60 years(average age 52.5 years). The study was carried out as pure efficacy study in order to see if it was possible to detect an effect on sexual desire. No clinical examinations or laboratory controls were carried out in this study.

Based on the result from this study, and with the arguments put forward above, it was judged as neccesary to carry out a separate double-blind study in order to verify or invalidate these results. In this study one would like to rely on the expertise of medical doctors with special knowlegde about sexology and andrology. Contact was therefore established with Samlivsklinikken Røde Kors Oslo, and Kjell-Olav Svendsen;MD and Einar Christiansen;MD were interested to participate in the project.

THE AIM OF THE STUDY

The aim of the study was to investigate and to compare the effect of **Libido** and placebo on the sexual desire in a group men with reduced sexual desire, but otherwise healthy. The selection of patients is defined in the section "Inclusion and exclusion criteria".

THE DESIGN OF THE STUDY

The study was planned and performed as a randomised placebo controlled double-blind study with multi-cross-over design(MCO). The total treatment period was 12 weeks. In each of the treament periods it was 6 cross-overs, meaning that the patient either could **receive the following treatment plan: A-P-A-P-A-P, where A is Libido** and P is placebo, or **P-A-P-A-P-A.**
Each of short treatment periods had a duration of 2 weeks. The treament schedule each of the patients received was randomised and thus unknown both for the patient and the doctor. The complete trial protocol is enclosed as appendix.

NUMBER OF PATIENTS

A minimum of 25 male patients with reduced sexual desire, but otherwise healthy was to be included in the study. Patients on drug treatments should not be included in the study. The patients were recruited through an advertisement in an Oslo newspaper(Aftenposten). Patients responding to the advertisement were asked to come to medical examination at Røde Kors Klinikken in order to see if they met the criteria for inclusion(see **below**).
All patients had a personal consultation with Dr. Svendsen, where the actual situation was discussed, clinical examination performed and relevant laboratory tests for the sexualfunction were carried out.

Inclusioncriteria

Healthy men with reduced sexual desire and where this reduction was not due to organic changes and/or drug intake, were included in the study. The patients had to be cooperative and able to undrstand and fill in the scores on analoqe scales.

Exclusioncriteria

Patients having one or more of the following exclusion criteria, were not included in the study:

- The reduced sexual desire must not be due to a number known mental and/or physical reasons.

- The patients should not be on continous treatment with drugs known to have a negative effect on sexual desire and erection. It can,however, be difficult to decide which drugs that can have such effects, and therefore all patients on continous drug treatment were excluded.

TRIAL PREPARATIONS AND DOSAGE

The trial preparations(active/placebo) were identical in apperance and taste. The prparations were packed in sachets of 3 g. The daily dose was 2 sachets, one in the morning and one in the evening. The content was solved in water, juice or milk and consumed immidiately.

RESULTS

Patientpopulation

Thirty-one(31) men aged 38 to 65 years(average 50.9 yrs.), meeting the inclusion criteria, and after having received information about the the project were included in the study. Twenty-nine(29) of the patients performed the study according to the protocol. One of the patients stopped the treatment due to side-effects on placebo,while two others stopped treatment for reasons not linked to the trial preparations. These patients are discussed in the section on tolerability and drop-outs. In all patients the following laboratory tests were carried out: Hb, TSH,B-glucose, FSH, prolactine and testosterone. If the patients had a low testosterone reading in the first test another test was performed at the conclusion of the study. This test also included SHBG.

EVALUATION OF EFFECT

Initially and at the end of each week, the patients rated their own status with regard to sexual desire using visual analoqe scales.
The following tables illustrates the change in scores for the two groups.

Table 1: Averages scores in the two groups with different treatment schedules.

Group A-P-A-P-A-P Average score		**Group P-A-P-A-P-A Average score**	
Initially	1.47	Initially	1.55
Week 1;A	2.47	Week 1;P	1.90
Week 2;A	3.65	Week 2;P	2.05
Week 3;P	2.54	Week 3;A	2.37
Week 4;P	1.97	Week 4;A	2.76
Week 5;A	2.21	Week 5;P	2.07
Week 6;A	2.04	Week 6;P	2.07
Week 7;P	2.36	Week 7;A	2.55
Week 8;P	2.73	Week 8;A	2.88
Week 9;A	2.73	Week 9;P	2.33
Week10:A	2.90	Week10;P	2.16
Week11;P	3.06	Week11;A	3.12
Week12;P	2.68	Week12;A	3.24

The table shows that higher scores are recorded in the periods where the patients are taking active preparations as compared to the placebo to the placebo periods. However,both groups show an increase in scores as compared to the initial values.

In table 2 the average values for the scores in the active and in the placebo periodes are shown respectively.

Table 2: Average values for scores in active and placebo periods respectively.

Group A-P-A-P-A-P Average score		**Group P-A-P-A-P-A Average Score**	
Period 1(week1&2)A	3.06	Period 1(week1&2)P	1.98
Period 2(week3&4)P	2.26	Period 2(week3&4)A	2.57

Period 3(week5&6)A 2.13	Period 3(week5&6)P 2.07
Period 4(week7&8)P 2.55	Period 4(week7&8)A 2.72
Period 5(week9&10)A 2.82	Period 5(week9&10)P 2.25
Period 6(week11&12)P 2.87	Period 6(week11&12)A 3.18

If the two groups are merged we get the following scores.

Table 3: Average scores in the placebo and in the active periodes respectively.

Average score

Initially 1.51

First Libido periode 2.82

First placebo period 2.12

Second Libido period 2.43

Second placebo period 2.31

Third Libido period 3.00

Third placebo period 2.56

Responders and none-responders

A critical review of the patientmaterial shows that some of the patients can be characterised as none-responders to treatment with **Libido**. These patients have the following numbers: 10,11,13,14,15,18,19,23,24,26 and 27. A total of 11 patients can be classified as none-responders, meaning that for these patients it is impossible to detect any difference in response between placebo and **Libido**. With 11 none-responders the total result of the study will of course be influenced in a negative direction. At present we do not know if the none-responders have one or more common denominators. As soon as we have the complete set of laboratory data analyses will be carried out to see if it is correlation between these data and the the response.

In the included figure(Fig.1) the responder profiles for two patients in the treatment

group P-A-P-A-P-A(Pat.no.17&20)are shown, while Fig. 2 shows the profiles for the two patients with the treatment schedules A-P-A-P-A-P(Pat.no.1&22). As can be seen from the figures and especially in Fig. 1 the patients show a marked and pronounced "saw tooth" pattern with regard to effect-profile, having high scores on **Libido** and low scores on placebo.

Testosterone concentration in serum

In all patients laboratory analyses of the testosterone concentration in serum were carried out before inclusion in the study and in some patients(N=11) these determinations were also performed at the end of the study. SHBH determinations were also repeated. The normal range for testosterone in serum is 12-40nmol/l. The mean value in our population was 16.1nmol/l and with a range from 8.1 to 31.6nmol/l.
In the patients were determinations were performed twice, the mean value initially was 11.8nmol/l and 14.7nmol/l at the end, meaning that the testosterone level on average had increased by approx. 25% during the trial periode. All blood samples were drawn at approximately the same time of the day, but sample times were not strictly standardized. Seven of the included patients had testosterone values below the lower value of the normal range(less than 12nmol/l). All these patients have to be classified as none-responders to treatment with **Libido.**
However, testosterone readings within the normal range do not automatically make the patient a responder to **Libido.** As mentioned previously the lack of desire can be complex and not dependent on only one factor as for instance the testosterone level.

Drop-outs

Two patients did not perform the study according to the protocol(Pat.no.8&27). For patient no. 8 we have not received any CRFs, while pat.no. 27 only participated for the first 6 weeks. On this basis pat.no. 8 is to be considered as a clear drop-out, while pat.no.27 has to be considered differently(see Side-effects).

Side-effects

One of the patients(Pat.no.27) reported side-effects of severe characther during the first 6 week of the study. The patient was hospitalised with suspicion on myocardial infarction. At that time no relation was suspected between treatment with placebo/**Libido** and the possible side-effect. However, provocation, twice, gave the same symptoms. The code was opened and showed that the patient had received placebo on all three occations. Several possibilities exist asregards the possible relation between the side-effect and the placebo powder. The placebo powder is composed of several flour-types and had the same ingredients with respect to taste as **Libido.**
No other reports on side-effects were received.

The galenical foermulation/Patient reports

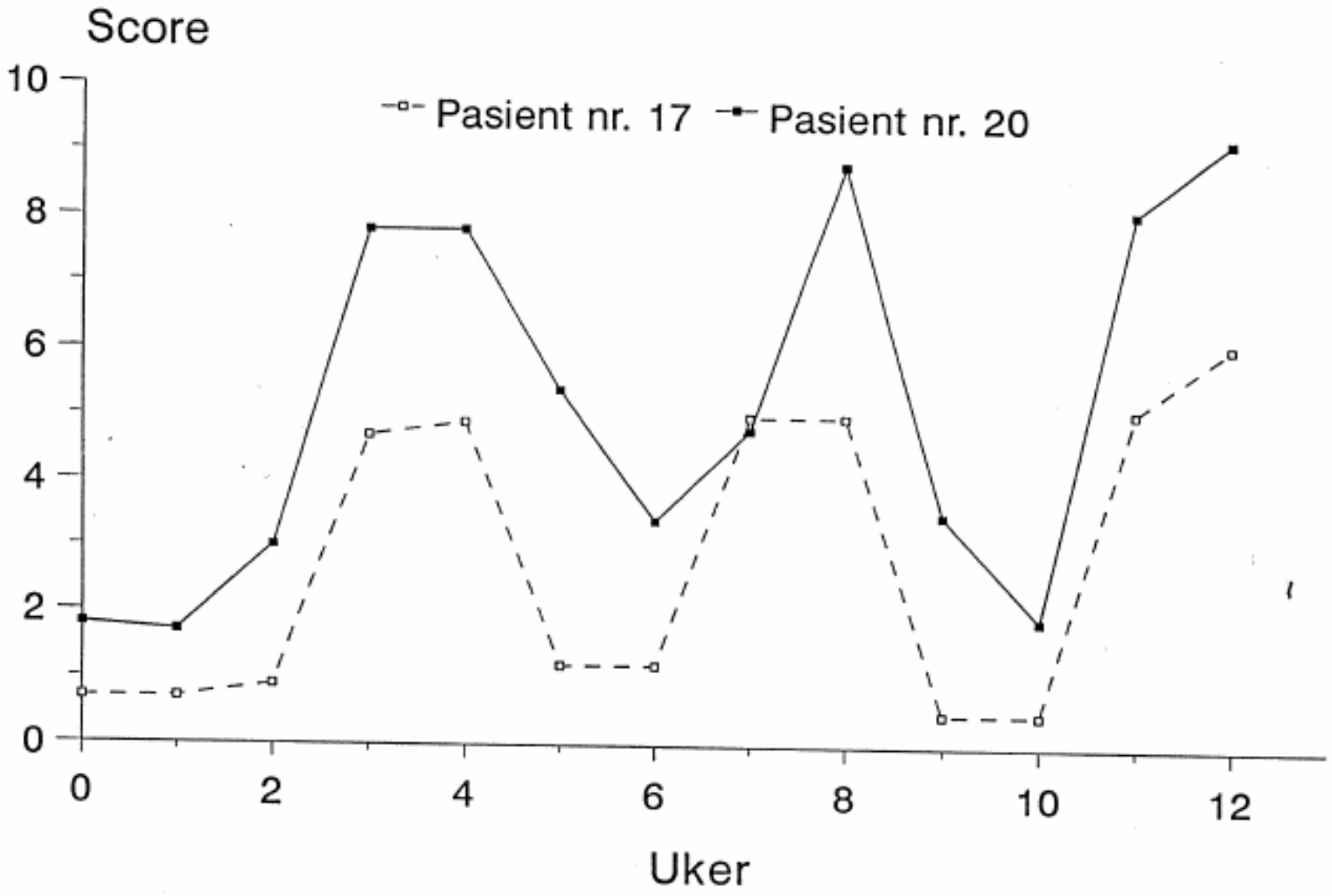
Three of the patients gave feed-back on the galenical formulations of the preparations. One patient reported a miscolouring of the teeth(Pat.no.7), one gave negative feed-back on the taste(Pat.no.14) and one was of the opinion that the solubility should be improved.

DISCUSSION

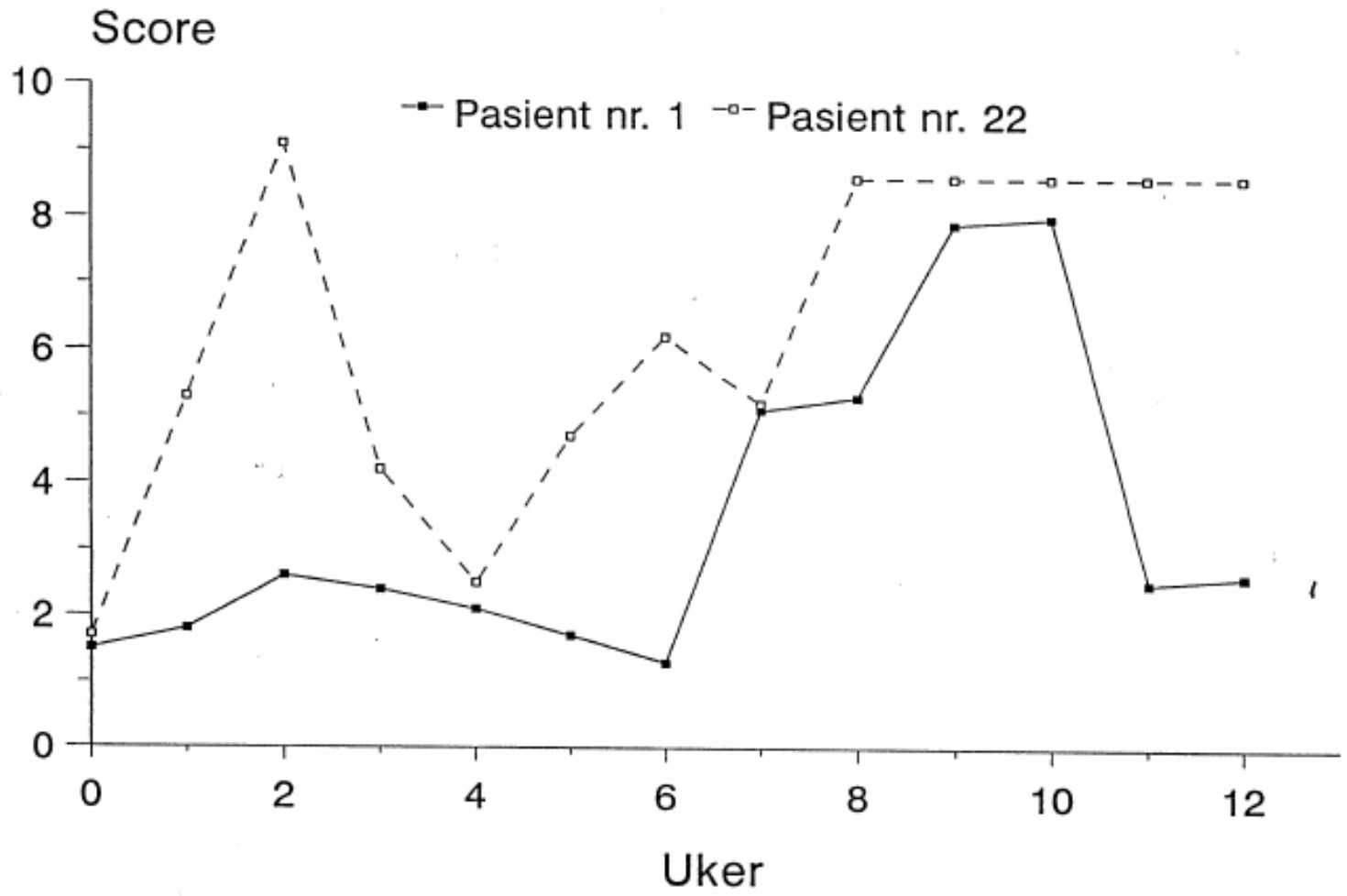
The result from the study confirm the results from the pilot-study. **Libido** seems to improve the sexual desire in middel-aged men. The effect is ,however, not as pronounced as in the pilot-study. One reason might be the difference between the two populations. In the pilot-study healthy middel-aged men without problems with the sexual desire was included. These persons reported,however, that **Libido** gave a booster effect on the sexual desire as compared to placebo.
In the present study the population is somewhat different and probably "sicker" with regard to sexual desire and sexual function. As can be seen from the enclosed figures , we also have persons with a similar effect profile as in the pilot-study. At this stage we have, however, no data indicating which patients that can expect to profit from treatment with **Libido**. This should be clarified before general recommendations on the use of **Libido** to male patients with lack of sexual desire is worked out.
It is possible that eligible patients have to meet certain criteria. Correlation between responsdata and other patientdata can possibily be of value. Another possibility might be that the dose has to be increased in the none-responders. At present we have no information on this item, since dose-response studies not have been performed. The dose used today is based on empirical data. The duration of the treatment period can of course be of importance especially in patients with low testosterone concentrations.
It is also interesting to investigate the effect on females in future studies.

The conclusion from this study is that **Libido** seems to have an effect on the sexual desire when compared to placebo. Consistent higher scores for sexual desire are recorded on **Libido** as compared to placebo throughout the observation period of 12 weeks.
It is , however , shown that 40-50% of the patients have no effect of the treatment as given in this trial. Today we have no possibilities to describe the none-responders a priori. This should, amongst other things discussed, be an item for future research on **Libido**.

Responder profiler



Responder profiler



APPENDIX 3

Report based on data from Sweden – Spring 1995

By
Dr. Bjødne Eskeland
Ph. D. Senior Researc Scientist

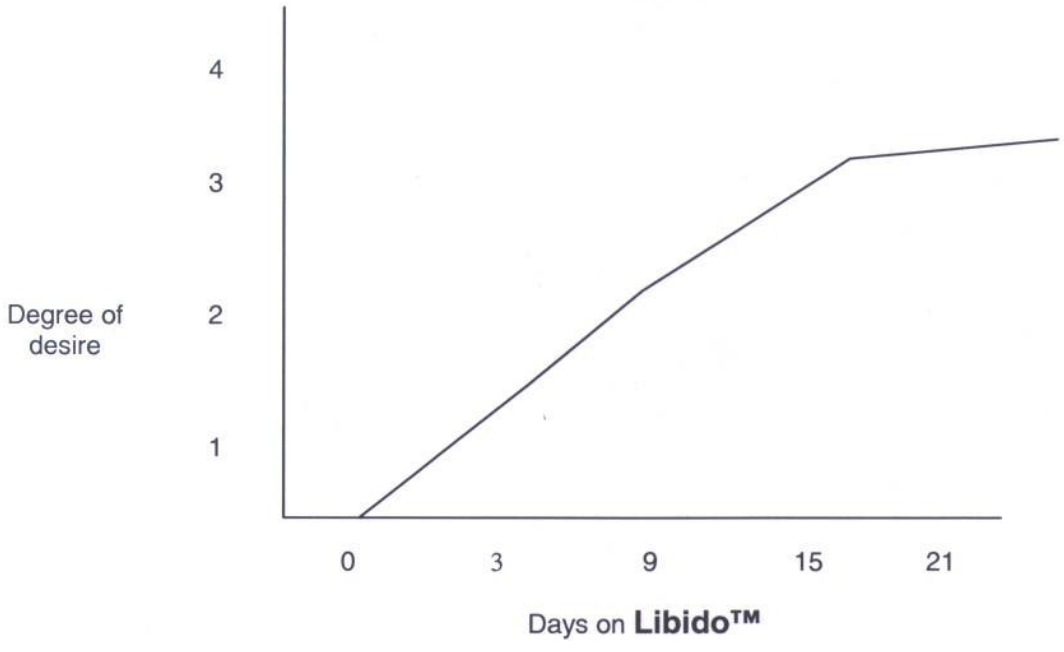
Table 1:

The effect of **Libido™** on sexual desire in 31 men during a three week period.

5. Very pronounced increase	25,8%
4. Major increase	19,4%
3. definitive increase	9,7%
2. Minor increase	29,0%
1. No increase	16,1%

The data indicates that 93,9% of the men during the three week period experienced a favorable effect with **Libido™** on their desire for sexual activity. 45% increased to the two highest activity levels.

Figure 1:
Increase in sexual desire during 21 days



APPENDIX 4

VOLUME 25, NUMBER 2
MARCH/APRIL 1997

THE JOURNAL OF

INTERNATIONAL MEDICAL RESEARCH

Cambridge Medical Publications
A division of PPS Europe Ltd
ISSN 0300-0605
JIMR BV 25 (2) 53 - 116 (1997)

The Journal of International Medical Research
1997; 25: 62 – 70

Sexual Desire in Men: Effects of Oral Ingestion of a Product Derived from Fertilized Eggs

B ESKELAND[1], E THOM[2] AND KOB SVENDSEN[3]

[1]Eskeland Electronics, Aas, Norway;
[2]Medstat Research, Lillestrøm, Norway;
[3]Frogner Health Centre, Oslo, Norway

A commercial product, Libido® (Libid, Libbido, Erosom and Ardorare, names used in different markets), which is based on components derived from fertilized, partly incubated chickens' eggs, has been used to treat diminished sexual desire in men. The results from two double-blind cross-over placebo-controlled experiments indicate that Libido has a significant enhancing effect on sexual desire in men with normal and reduced sexual drive. Over periods as short as 2 weeks, 58% of the participants with low sexual desire noticed improvement as assessed using a visual analogue scale. Data obtained from consumers suggest significant effects on the frequency of sexual intercourse, on increased self-esteem, on the level of happiness, and on stamina. In a separate Swedish study, 84% of 31 men reported increased sexual desire during 3 weeks on Libido. It takes 1 – 2 weeks of regular use of Libido (3g twice daily) before noticeable changes are observed.

KEY WORDS: LIBIDO®; ARDOR®; EROSOM®; APHRODISIAC; EGG PRODUCT; SEXUAL DESIRE

The Journal of International Medical Research
1997; 25: 62 – 70

INTRODUCTION

Through the ages people have searched for substances and dietary supplements that affect sexual drive or desire. A number of remedies have been developed and tried. For instance the rhinoceros population has been at the edge of extension because of the belief that rhinoceros horn is an effective aphrodisiac. Many of the early aphrodisiacs derived their reputation from physical qualities such as resemblance to genitalia (e.g. rhinoceros horn, ginseng root, cucumbers).

Over the years, hundreds of so-called aphrodisiacs have been tried and investigated; some have gained reputations for having real effects on sex drive, but the studies done lacked a placebo-treated group. The authors are of the opinion that the placebo effect may be considerable in this type of study, and may account for as much as 50% of the apparent effect.

Reduced sex drive is a complex problem; by nature, sexual desire and performance decline with age. Sex drive can also be effected by disease and drug treatment. It is unreasonable, therefore, to expect that an individual remedy will have a good effect on all those with reduced sex drive.

As people nowadays are more aware of and open about their sexual feelings, it is clear that reduced sex drive and performance is a larger problem than previously anticipated.

In a study of sexual problems carried out by the Norwegian Association for Sexology, 13% of men and 38% of women indicated that their main problem was lack of sex drive. Men may not be as honest as women when answering questionnaires about their sexuality, so the figures for men may, in reality, be similar to those for women. Rosselini estimated that approximately 38 of the 150 million American adults have no desire for sex. Since a relatively large part of the population is suffering diminished sex drive, a product that effectively increases sex drive would be valuable.

The rationale for using components derived from fertilized chickens` eggs incubated to a pre-embryonic stage, as enhances of sexual desire, is based on the assumption that these components are transformed into active substances in the body, e.g. stimulating steroidogenesis and/or acting as sex hormone precursors. Other possible mechanisms include a neutrophil effect, and stunting effects on the cholesterol derivatives, stimulating production of sex hormones instead of cortisol, as the precursors are identical. The main ingredient in the product , Libido® used in the present studies is derived from chickens` eggs.

The purpose of this study was to investigate in placebo-controlled studies whether the product Libido® is effective in enhancing the sex drive of men.

SUBJECTS AND METHODS

The product, Libido®, is derived from partly incubated fertile chickens` eggs and protein fractions extracted, lyophilized and made into a powder. This is main constituent of Libido® (> 80%). To make the product more acceptable and complete from a sensory and nutritional point of view, some plant ingredients, vitamins and minerals were added. Dose-response studies were not available when our studies began. The daily dose was decided on the basis of preliminary empirical data collected from samples distributed among friends and volunteers, which showed that 5 – 6 g daily produced an appreciable effect. In our studies the dose was therefore set at 3g twice daily (morning and evening). The investigational products (Libido®/placebo) for the two double-blind studies were packed in identical sachets containing 3g, and labeled with the instruction that two sachets should be taken every day, one in the morning, and one in the evening. The product was to be dissolved in 200 ml of liquid (juice or water), stirred and swallowed immediately. The taste, appearance and solubility of the two products were identical.

STUDY 1

This study was initiated to investigate the effect of Libido® on sex drive, compared with placebo, in a group of middle-aged men. This was a randomized placebo-controlled double-blind study with a duration of 6 weeks. (i.e. 3 weeks on each of the two preparations).

Only healthy middle-aged men (47 – 60 years) were included in the study. Patients receiving drug therapy were excluded.

The subjects required to this study came from a local rotary club.

Volunteers were given written and verbal information about the aims of the study, and gave written consent. As the study involved only healthy volunteers, using an approved nutritional product, it was not considered necessary to obtain Ethics Committee approval.

The participants came once a week to the research institute conducting the study, to report their scores and any adverse events that could possibly be linked to the treatment. Compliance with the treatment schedule was also checked at each visit; volunteers were required to have taken at least 80% of the recommended dose of the powder to be included in the evaluation of the effect.

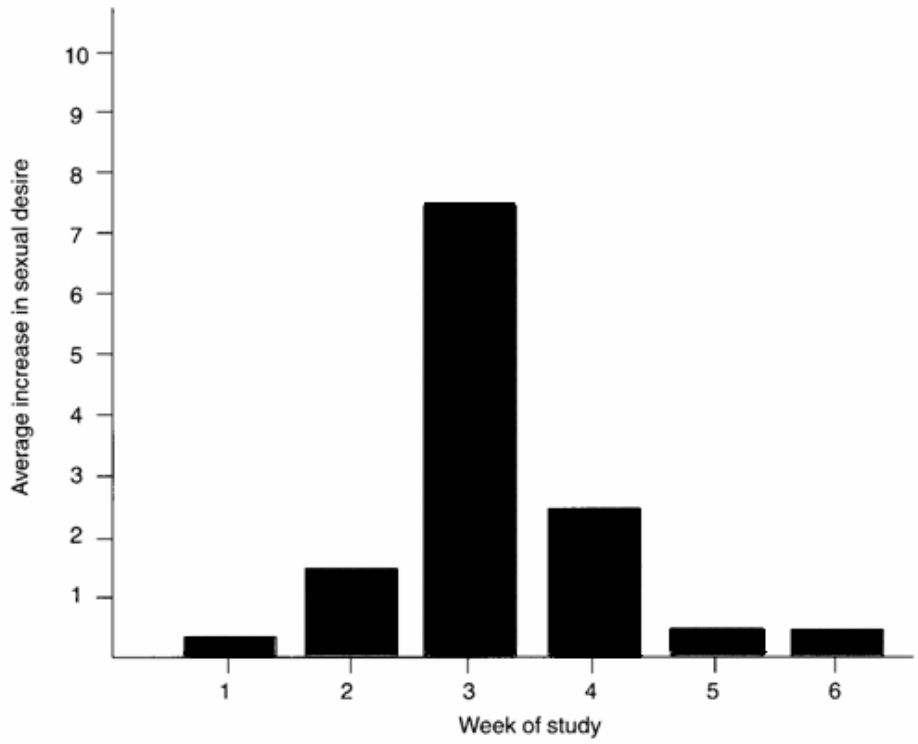
Sixteen men aged 47 – 60 years (average 52.5 years) participated in the study. Half of the participants received Libido® during the first period of three weeks, and the rest received placebo. In the second part of the study, the treatments were reversed. There was no wash-out period between the two treatments. The patients scored their feeling of sexual desire on a weekly basis, using a visual analogue scale ranging from 0 cm, no change, to 10 cm, very pronounced change. This was the only efficacy parameter used. The statistical comparisons were made using Student`s t-test with a significance level of 5%.

Based on the positive results from this placebo-controlled cross-over pilot study, the decision was taken to carry out a further placebo-controlled study in a larger male population.

B Eskeland, E Thom, KOB Svendsen

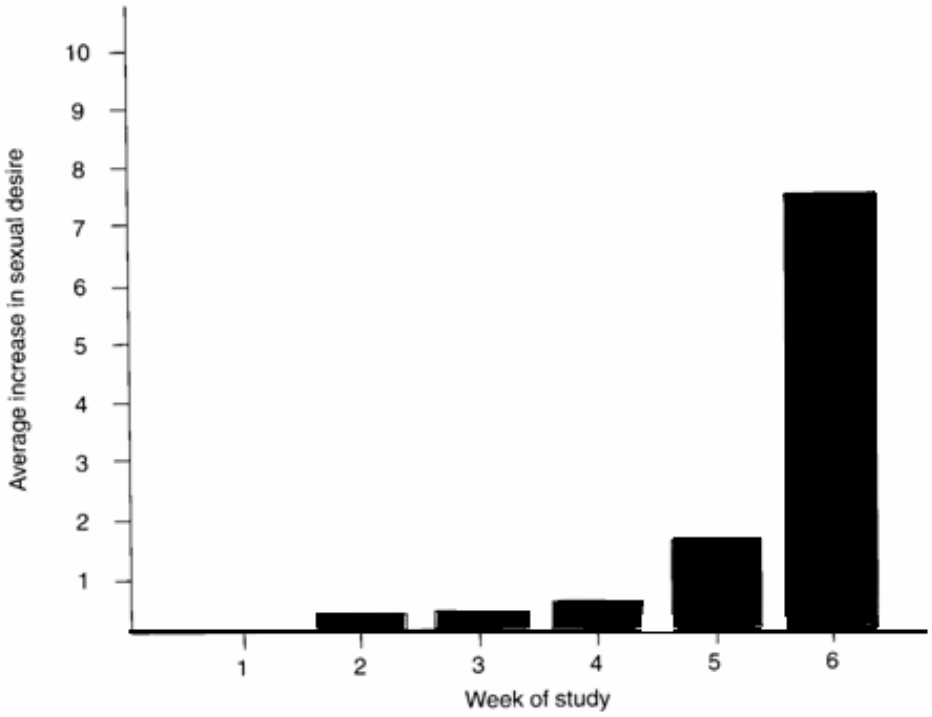
Sexual desire in men: effects of an egg product

FIGURE 1



Average scores for increase in sexual desire, based on a visual analogue scale from 0 (no change) to 10 (very pronounced change), of 16 healthy men (aged 47 – 60 years) who were treated with Libido for the first 3 weeks and with placebo for the following 3 weeks.

FIGURE 2



Average scores for increase in sexual desire, based on a visual analogue scale from 0 (no change) to 10 (very pronounced change), of 16 healthy men (aged 47 – 60 years) who were treated with placebo for the first 3 weeks and with Libido for the following 3 weeks.

STUDY 2

The volunteers for this study were required through an advertisement in a local newspaper, in which the aim of the study was presented. The responders were asked to the clinic for an interview and a clinical examination. The study was approved by the clinic`s Ethics Committee.

Only healthy men with reduced sexual desire not caused by organic changes, mental impairment or drug intake were included. Patients were excluded if the reduced sexual desire was of chronic and/or mental origin, or if they were receiving drug therapy.

B Eskeland, E Thom, KOB Svendsen

Sexual desire in men: effects of an egg product

TABLE 1

Average scores for sexual desire in 31 otherwise healthy male volunteers with reduced sexual desire. Volunteers were given alternating 2-week phases on Libido or placebo treatments for 12 weeks. Half of the group started with the placebo and half with the Libido treatment

	Average score for sexual desire[a]
Initial	1.51 ± 0.30
First Libido period	2.82 ± 0.65
First placebo period	2.12 ± 0.48
Second Libido period	2.43 ± 0.56
Second placebo period	2.31 ± 0.50
Third Libido period	3.00 ± 0.60
Third placebo period	2.56 ± 0.63

[a] Scores were recorded weekly, based on a visual analogue scale from 0 (no change) to 10 (very pronounced change).

B Eskeland, E Thom, KOB Svendsen

Sexual desire in men: effects of an egg product

The clinic is a specialized clinic for people with sexual problems, and all interviews and examinations were carried out by the same investigator on all occasions. If the volunteers met the inclusion criteria for the study, they were given written and verbal information about the aims and procedures of the study, and gave written informed consent.
Thirty-one otherwise healthy men with reduced sexual desire aged 38 – 65 years (average 50.9 years), were enrolled.
This study was planned and performed as a randomized placebo-controlled double-blind study with a multi cross-over design. The total treatment period was 12 weeks. Half of the group started on 2 weeks treatment with the "active" product, switching every 2 weeks to "placebo", or back to "active" product. The other half started on "placebo".

In each of the treatment periods there were six cross-overs, meaning that the patient could either receive the following treatment plan: A-P-A-P-A-P-A-P-A-P-A. the effect was recorded weekly using an analogue scale, as described above, using sexual desire as the efficacy parameter. Possible side-effects were also recorded by the subjects.

THE SWEDISH STUDY

The product Libido was introduced to the Swedish market by an invitation to participate in a trial for a 3 – week period. Men with reduced sexual desire were enrolled (n=31) and asked to record their degree of sexual desire according to the following categories:

(1) no increase in desire for sexual activity (2) minor increase (3) definite increase (4) major increase, and (5) very pronounced increase in desire for sexual activity.

MARKET RESPONSE

Information was obtained on the effect on sexual performance and the feeling of well being of the individuals consuming the first commercial samples produced, and 32 men reported their responses on a weekly basis, on an evaluation form included with the product. After completing the treatment, they returned the evaluation form to the production company. These men initially had low sex drive. The age of the participants raged from 37 – 74 years. Fourteen of the men were between 57 and 74 years and initially recorded almost complete lack of sex drive to satisfy their partners and themselves. Four of those had not been able to complete a sexual satisfying act during the last 2 – 4 years. The rest of the group were younger and middle-aged men, ranging from 37 – 55 years of age, who wanted to increase the frequency of sexual intercourse.

B Eskeland, E Thom, KOB Svendsen

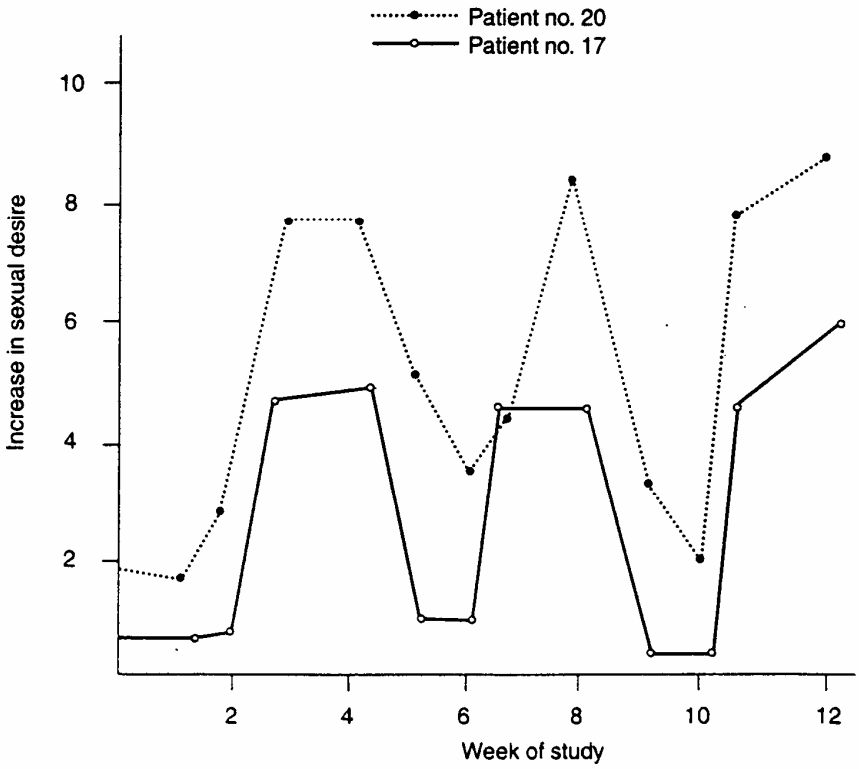
Sexual desire in men: effects of an egg product

TABLE 2

Percentage of men showing increased sexual desire when 31 male volunteers, with reduced sexual desire, were treated with Libido for 3 weeks

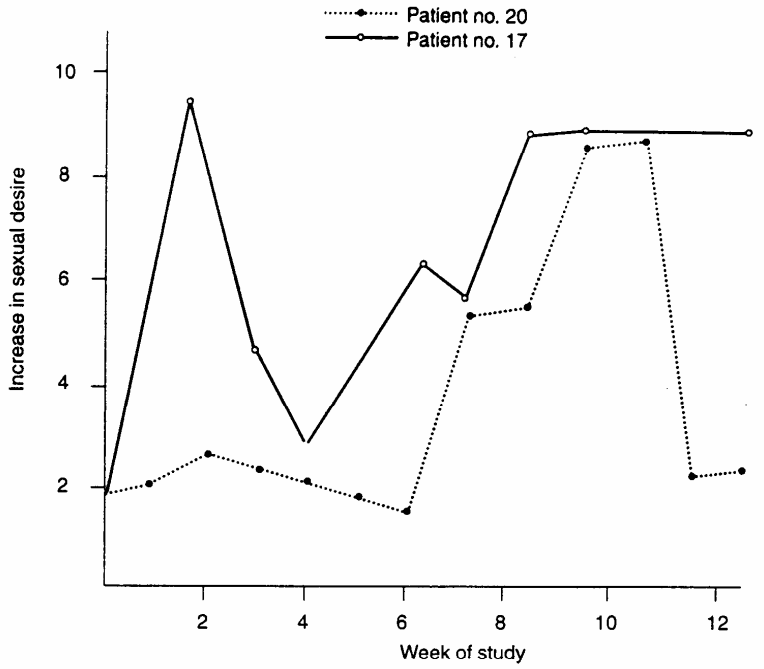
Change in sexual desire	Percentage of men showing change
No increase	16.1
Minor increase	29.0
Definite increase	9.7
Major increase	19.4
Very pronounced increase	25.8

FIGURE 3



Changes in the scores for increase in sexual desire, based on a visual analogue scale from 0 (no change) to 10 (very pronounced change), in two men from a group of otherwise healthy men with reduced sexual desire who were given alternating 2-week phases on active treatment with Libido or on placebo for 12 weeks, starting with placebo (P-A-P-A-P-A).

FIGURE 4



Changes in the scores for increase in sexual desire, based on a visual analogue scale from 0 (no change) to 10 (very pronounced change), in two men from a group of otherwise healthy men with reduced sexual desire who were given alternating 2-week phases on active treatment with Libido or on placebo for 12 weeks, starting with Libido (A-P-A-P-A-P).

RESULTS

STUDY 1

The results for the group that started with Libido and continued with placebo are illustrated in Fig. 1, and those for the group that started on placebo and transferred to Libido are shown in Fig. 2. Fig 1 shows an increase in the reported sexual desire during the first 3 weeks of the trial when the patients were given Libido. The relatively high score for the first week of placebo treatment is due to the residual effect of the preceding Libido treatment since there was no wash-out period.

The average score for sexual desire during the third week of treatment was with Libido on a visual analogue scale was 7.80 cm (SD +/- 1,6; n=16), while the score for the third week of placebo treatment was 0,25 cm (SD +/- 0.05; n=16), a statistically significant difference in favor of Libido (P> 0.001). These results suggest that Libido leads to significantly better stimulation of sexual desire than does placebo treatment. The study also indicated that the major increase in sexual arousal occurs 2 – 3 weeks after the start of treatment and that this treatment has a declining residual effect during the weeks after the treatment is stopped. None of the participants reported any side-effects either on Libido or placebo treatment.

STUDY 2

Table 1 summarizes the result of this study. A statistical comparison of the average scores in the three periods using Students t-test (first Libido period versus first placebo period, etc.) shows a significant difference in favor of Libido in all three periods ($p < 0.05$). Switching from Libido to placebo a carry-over effect was always observed during the first week, and even after 2 weeks on placebo. None of the individual volunteers returned to their baseline score during the placebo period. A 2-week interval, without a wash-out period is rather a short period as, in most cases, it is 6 – 10 days before any changes are observed. As there was no wash-out period between treatments, the carry- over effects from the Libido treatment to the placebo are considerable.

Over periods of treatment as short as 2 weeks, 58% of the individuals who basically had a very low sex drive, experienced enhanced sex drive.

The response profile of two of the patients in the treatment group P-A-P-A-P-A are shown in Fig. 3. Figure 4 shows the profile of two patients treated with the schedule A-P-A-P-A-P. the response profiles of these patients, especially those in Fig. 3, show a marked pattern of response, generally having higher scores on Libido and lower scores on placebo.

The illustrated patient profiles were selected randomly. Similar response profiles were seen for the majority of the patients.

THE SWEDISH STUDY

The data from this study are summarized in Table 2 .

These data indicate that during the 3 -week period of Libido treatment, 83.9% of the men experienced an increase in their desire for sexual activity; 45.2% experienced major or very pronounced increases. None of the participants reported any side-effects on the Libido treatment.

MARKET RESPONSE

Data collected from consumers of the first produced samples of Libido were very encouraging, especially among the older and middle-aged men. The four oldest men were able to have successful intercourse after 2 weeks` consumption, and during the third week they reported 2 – 3 satisfactory sex acts.

For men who had been unable to satisfy their partners sexually for extended periods, this was a great change which affected their self -esteem and level of happiness.

Of the 14 men between 57 and 73 years of age, 12 experienced great improvements and wanted to continue taking the product, and two recorded no effect. One of these two was, however, receiving treatment for coronary problems. Eight of the younger men experienced an increase to 3 – 5 times their initial sex drive(on the scale from 1 – 10). The rest (10 men) reported smaller, but noticeable and favorable changes in their sex drive (1-2 times), and wanted to continue taking the product.

APPENDIX 5

THE EFFECT OF "LIBIDO" ON DECREASED SEXUAL DESIRE ASSOCIATED WITH ANTI-DEPRESSANT MEDICATION.

BY
Kjell Y. Törnblom
University of Colorado at Denver

INTRODUCTION

Anti depressant medication is estimated to cause a partial or total loss of sexual desire in about 25-30% of patients. Thus, users have to face the irony of taking a medication designed to alleviate certain severe emotional problems just to find out that a new and often psychologically and interpersonally devastating problem of hypoactive sexual desire is created as a side effect. The patient ends up in a negative dilemma forced to make a choice between two evils: learning to live with the original emotional problems or with the loss of sexual desire.

To be sure, people on anti-depressants are only one of many categories of (potential) sexually hypoactive persons that would welcome a product with aphrodisiac-like properties. Unfortunately, it seems that the perennial search for such substance have delivered nothing but a wide variety of snake oils – up till now.
Users have, as a rule, lost their money rather than embarrassing dysfunction.

When it comes to impotence (the inability to achieve or maintain an erection, believed to afflict about 20% of men in their early forties), there are at least four major approaches to its treatment: Vacuum pumping which draws blood to the erectile tissue of the penis, injection of a muscle relaxant directly into the penis, implants to provide rigidity of the penis, and a new drug, sildenafil, that apparently blocks an enzyme that may prevent erection.

However, treatments for impotence may not necessarily increase sexual arousal or desire. Thus, perhaps the most effective treatment might consist of a two-pronged strategy within which the attempt at curing impotence is coupled with treatment of hypoactive sexual desire. Indeed, in some cases it may be reasonable to assume that successful restoration of sexual desire would also contribute to the cure of the impotence (or reduce its severity).

Recent studies (see Eskeland, Thom and Svendsen,1996, for a partial overview) suggest that a product named Libido (basically made from fertilized and incubated eggs) is able to increase sexual desire in most otherwise healthy men with reduced as well as normal sexual desire. Therefore, exploring the effect of Libido on decreased sexual desire associated with anti-depressant medication is a logical next step in the testing of this promising product. This study reports the results from such a test involving both males and females.

The invaluable assistance of Eva Ahlin in conducting this study is gratefully acknowledged.

METHODS

Subjects:

SUbjects were recruited via an advertisement that was placed two days in two local Denver papers (one daily and the other weekly). The ad invited men and women "….to participate in a 6-week study on the effects of a new Scandinavian egg-derived natural supplement that has increased sexual desire in non-depressed people." The ad also stipulated that participants had to meet three basic qualifications: they must (1) have had normal sex drive before taking medication, (2) have been on anti-depressant medication for at least 6 weeks, and (3) be experiencing a loss of sexual desire after starting medication. A phone number to the senior researcher was to be used for enrollment.

Twenty-two subjects had originally volunteered to participate in the study in response to the newspaper ads.

However, only sixteen persons (7 males and 9 females) showed up for introductory meetings during which information about the study was provided. Five subjects (1 male and 4 females) dropped out of the study almost immediately; they only completed the pre-study questionnaires.

Eleven persons , 6 men and 5 women (mean ages 47.0 and 32.8, respectively) completed two weeks of the study. All of them ingested Libido during that time period.

Another three persons (1 male and 2 females) dropped out into the third week of the study, leaving a total of 5 males and 3 females (mean ages 47.0 and 32.7, respectively) who completed the study in its entirety.

Attrition.

The drop-outs offered the following reasons for terminating their participation in the study: one man cited marital problems, the second man and one woman experienced stomach problems (gas and indigestion), another woman had too many crises going on in her life right now, a third female had nightmares and lost sleep and was told to discontinue the supplementation by her doctor. The remaining three women gave provided no information in this context.

If attrition is calculated on the basis of the eleven people who actually started on the study(of whom 3 dropped out), rate was 27.3%. (if calculated on the 16 who turned up for introductory meetings, the rate increases to 50%).

Procedure.

Before being accepted as a participant, responders to the ad were further screened to minimize the possibility (a) that other medical conditions and/or (b) that anyone allergic to eggs would be admitted by mistake.

Approved participants (singly or in small groups) were called to a meeting with the investigators during which they were informed about the purpose and nature of the study. Subsequent to personal introductions participants were asked to read through and sign a standard consent form. The study required them to ingest Libido during 3 weeks and a placebo during 3 weeks. The original design called for half of the participants to ingest Libido during the first 3 weeks and the other half to take this supplement during the final 3 weeks. However, this design had to be altered in such a way that all participants started out with Libido[2]. All supplements were provided free of charge. Participants were asked to make themselves available in person or by phone for interviews at four occasions during the study. They would also agree not to make any changes in their diets during the six-week test period.

Next, subjects were provided a written description of the Libido supplement and given the opportunity to ask questions about any aspect of the study and their involvement in it. Both oral and written instructions were provided as to how many and how often pills should be ingested. The dosage was six pills in the morning and six in the evening every day for six weeks. Unknown to the participants, they were taking Libido for 3 weeks and Placebo pills during the subsequent three-week period. Instructions included a schedule according to which participants were to call the investigators and make themselves available for telephone interviews, namely after the second, third, fifth and sixth week.

Finally, subjects were given the supplement and were asked to complete two questionnaires before the meeting was ended.

After the second, third, fifth and sixth week of taking the supplement participants were telephone interviewed about their progress.

Instruments.

Questionnaire 1, the pre-study questionnaire contained 93 items asking about the participants` medical history, life style, subjective physical and emotional / mental status, and sexual functioning.
Some of the questions required respondents to rate (along 9-point Likert scales) their present situation in terms of 17 qualities:

General well being	Energy level
Endurance	Mental sharpness
Feelings of happiness	Physical health
Psychological health	Amount of sleep
Intensity of sexual desire	Frequency of sexual desire
Mental alertness	Level of anxiety
Level of confidence	Self – esteem
Physical condition.	

(Ratings from these qualities were also done after weeks 3 and 6 of the study).

Presentation of results scheduled for a press conference after only three weeks into the study necessitated this change in design

The statuses on 5 of the above 17 qualities (items 41, 42, 45, 51, and 52) were monitored at five different occasions throughout the study (before the start as well as after 2, 3, 5, and 6 weeks.

General well being	Energy level
Feelings of happiness	intensity of sexual desire
Frequency of sexual desire.	

Furthermore, respondents rated (along 9-point Likert scales) their satisfaction with 7 sexual aspects (items 87 – 93) – before as well as after weeks 3 and 6 of the study:

Sexual fantasy life	Frequency of sexual desire
Intensity of sexual desire	Solitary sex practices
Ability to have an orgasm	Intensity of orgasm
General sexual function.	

Questionnaires 2 and 4 (administered after the second and fifth week of the study) were identical and contained only the group of 5 items mentioned above (i.e., General wellbeing, Energy level, Feelings of happiness, Intensity of sexual desire and Frequency of sexual desire, i. e., items 41, 42, 45, 51, and 52) in addition to an open-ended question about what changes the participant might have experienced since the beginning of the study.

Questionnaires 3 and 5 (administered after the third and sixth / final week of the study) were identical and encompassed 36 questions. Like in the pre-study questionnaire, respondents were asked to rate their present situation with regard to the 17 qualities listed above (i.e., items 41-57). Further, satisfaction ratings of the 7 sexual aspects (that appeared in the pre-study questionnaire, i.e., items 87-93 – see above) were obtained. In addition, respondents were asked how strongly they did or did not believe that their sexual desire would return to normal, and if / how much their relationships with their partners had improved.

Questionnaire 1,3 and 5 contained additional items asking respondents to rate (along 9-point Likert scales) their immediate past (during last month for questionnaire 1 and during last week for questionnaires 3 and 5) with regard to :
how often they had fantasized about sex,
how often they had felt a desire to engage in sexual activities,
how intense these desires were,
their ability to have an orgasm,
the intensity of their orgasms,
their overall sexual functioning, and
the quality of their lives.

Finally, the “Derogatis Affects Balance Scale” (DABS) was administered to all respondents at three occasions: before the start of the study, after three weeks into the study, and after the sixth/final week. DABS (Derogatis 1975) is a brief multidimensional self-report mood and affects inventory designed to measure the affects profiles of community, medical and psychiatric respondents. The inventory was developed on the premise that positive and negative affectivity represent the two fundamentals axes of human emotional experience.

The balance between positive and negative affective states is assumed to represent a highly valid characterization of personal well-being. Positive affect (PA) appear to be associated with feelings of high energy, enthusiasm, activity, a capacity for high concentration and pleasurable engagement with the environment. States of indifference, lethargy and melancholy are associated with a relative absence or decrease of PA. Negative affect (NA) is conceptualized in terms of general physiological distress, e.g., anxiety, anger and depression (Derogatis, 1996) .

O = never, 1 = rarely, 2 = sometimes, 3 = frequently, and 4 = always.

Each DABS item (i.e., tapping the frequency by which the respondent have experienced a particular emotion) is measured on a 5-point scale ranging from 0 to 4[3]. The DABS measures affectivity and affects balance via 4 positive and 4 negative primary affect dimensions that reflect basic emotional states; the positive affects dimensions are termed joy, contentment, vigor and affection, while the negative affects dimensions are labeled anxiety, depression, guilt and hostility[4].
DABS also measures affective status in terms of 5 global scores which serve as overall summary measures: (1) the Positive Total Score (PTOT), i.e., the sum of the four positive affects dimensions; (2) the Negative Total Score (NTOT), i.e., the sum of the four negative dimensions; (3) the Affects Balance Index (ABI), i.e., the difference between the PTOT and the NTOT scores; (4) the Affects Expressiveness Index (AEI), i.e., total affective expression expressed as the sum of PTOT and NTOT;
and (5) Positive Affects Ratio (PAR), i.e., the proportion of AEI that is positive.

RESULTS

Based on the information offered with regard to medical history, life style, physical and mental status the participants in this study did not seem to be an out of-the-ordinary group of people. With respect to sexually relevant information, the following background information may be of interest as a partial description of this li

sphere (n = 11):

- mean frequency of intercourse during the month preceding the study: 1.4;
- the extent to which orgasmic ability has decreased since starting antidepressant medication: 7.7 on a scale of 9;
- the extent to which orgasmic intensity has decreased since starting anti depressing medication: 7.9 on a scale of 9;
- the extent to which relationship with partner has been negatively affected by their decreased sexual desire: 6.1 on a scale of 9;
- the extent to which their decreased sexual desire has negatively affected their overall quality of life; 6 on a scale of 9;
- the degree of sexual activity before desire decreased: 7.3 on a scale of 9;
- the degree of importance of good sexual functioning: 7.5 on a scale of 9;
- the degree of satisfaction with sexual functioning before starting on anti depressant medication: 7.6 on a scale of 9.

[4] Each affect dimension is conceptualized in terms of five affects items: Joy (= happy, glad, cheerful, delighted and joyous), Contentment = pleased, calm, satisfied, relaxed and contented).
Vigor (= exited, energetic, active, vigorous and lively). Affection (= passionate, loving, friendly, affectionate and warm). Anxiety (= nervous, timid, tense, anxious and afraid). Depression (= sad,hopeless,worthless,miserable and unhappy). Guild (= regretful, blameworthy,ashamed,guilty and remorseful). Hostility (= irritable,resentful,angry,enraged and bitter).

Ratings of present situation.

As explained above, subjects rated their present situation at five different occasions throughout the study (before the start of the study as well as after 2,3,5 and 6 weeks) with regard to 17 qualities (General well being, Energy level, Endurance, Mental sharpness, Feelings of happiness, Physical health, Psychological health, Amount of sleep, Quality of sleep, Feelings of relaxation, Intensity of sexual desire, Frequency of sexual desire, Mental alertness, Level of anxiety, Level of confidence, Self-esteem and Physical condition).
Improvements occurred on all qualities, without exception. Some of these improvements reached their highest values during the Libido supplementation period and the remained at approximately those levels throughout the three week period of placebo ingestion. This held true for the following qualities; Energy, Happiness, Psychological health, Intensity of sexual desire and Physical condition. However, improvements continued and culminated during the placebo period for some qualities; Wellbeing, Endurance, Mental Sharpness, Physical health, Quantity of sleep, Quality of sleep, Relaxation, Frequency of sexual desire, Mental alertness, Anxiety and Confidence).

Statistically significant differences (using paired two-tailed t-tests[5]) were obtained for Intensity of sexual desire, Frequency of sexual desire, Energy (see table 1 and fig. 11-3), Confidence and self esteem (see table 2 and fig. 4&5).
Table 1 and fig. 1&2 show that after only two weeks on Libido, respondents` Intensity as well Frequency of sexual desire have increased from 3.1 to 4.7 (p= < . 009) and from 2.8 to 5.0 (p 0 < .008), respectively.

Treatment	Week	Mean intensity of sexual desire	Mean diff.	Mean frequency of sexual desire	Mean diff.	Mean energy level	Mean diff.	n
	0	3.1 [a *]		2.8 [a]		4.6 [a]		11
			1.6		2.2		1.7	
LIBIDO	2	4.7 [b]		5.0 [b]		6.3 [b]		11
			.9		-.2		-.8	
	3	5.6 [b]		4.8 [b]		5.5 [a]		8
			.2		.4		.8	
PLACEBO	5	5.8 [b]		5.2 [b]		6.3 [ab]		9
			-.2		.1		-.2	
	6	5.6 [b]		5.3 [b]		6.1 [ab]		7

- Means that do not share a common letter are significantly different at the p < .05 level.

TABLE 1: the effects of Libido and Placebo pills on “intensity of sexual desire”, “frequency of sexual desire” and “energy level”.
(No measurements were taken after weeks 1 and 4)

Fig 1

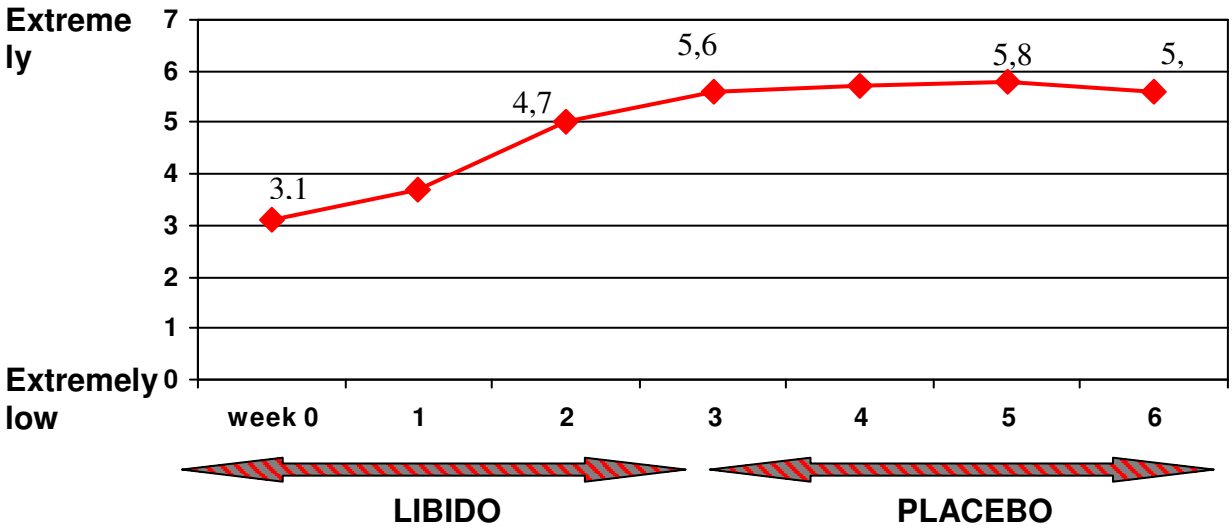


FIGURE 1

INCREASES IN
INTENSITY OF SEXUAL DESIRE
AFTER 2 AND 3 WEEKS OF INGESTING LIBIDO PILLS
AND AFTER 2 AND 3 WEEKS OF INGESTING PLACEBO PILLS

FIGURE 2

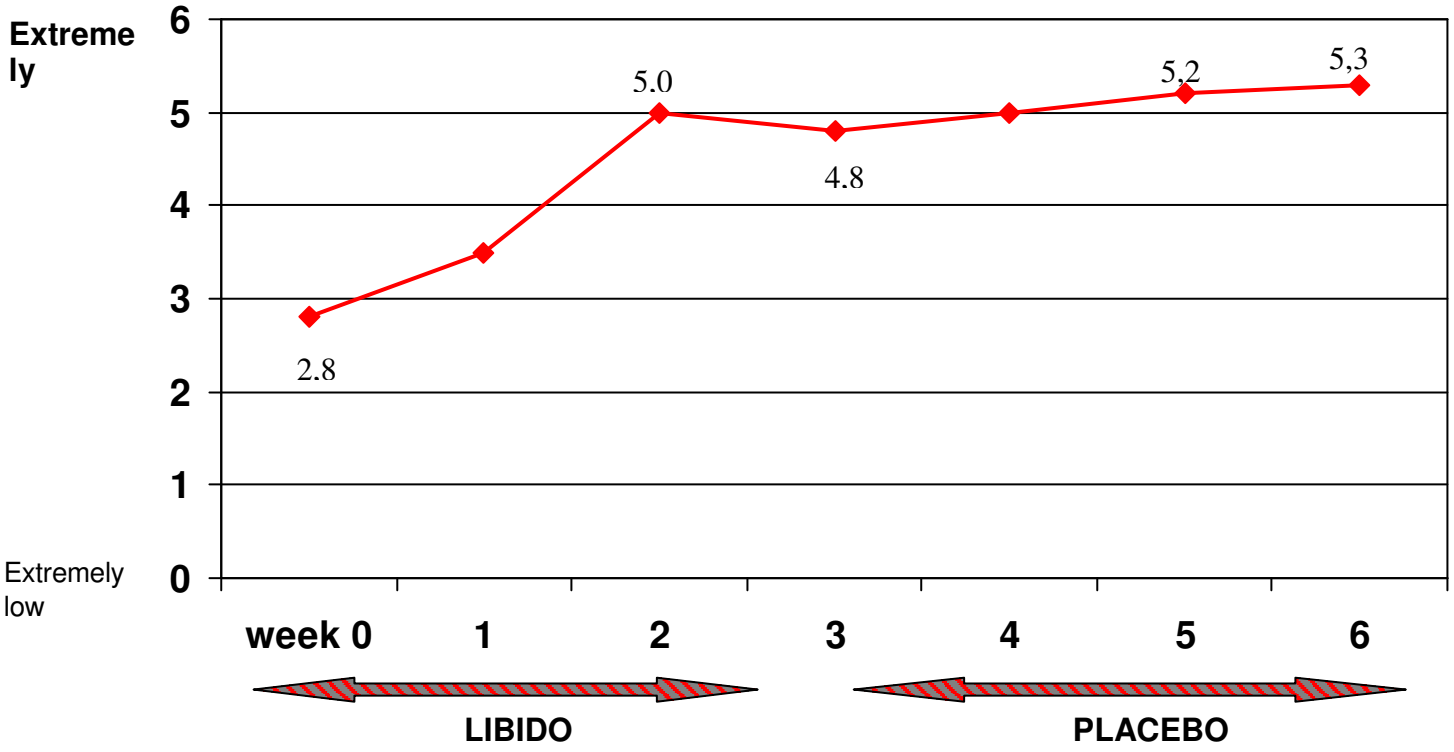


FIGURE 2

INCREASES IN
FREQUENCY OF SEXUAL DESIRE
AFTER 2 AND 3 WEEKS OF INGESTING LIBIDO PILLS
AND AFTER 2 AND 3 WEEKS OF INGESTING PLACEBO PILLS

FIGURE 3

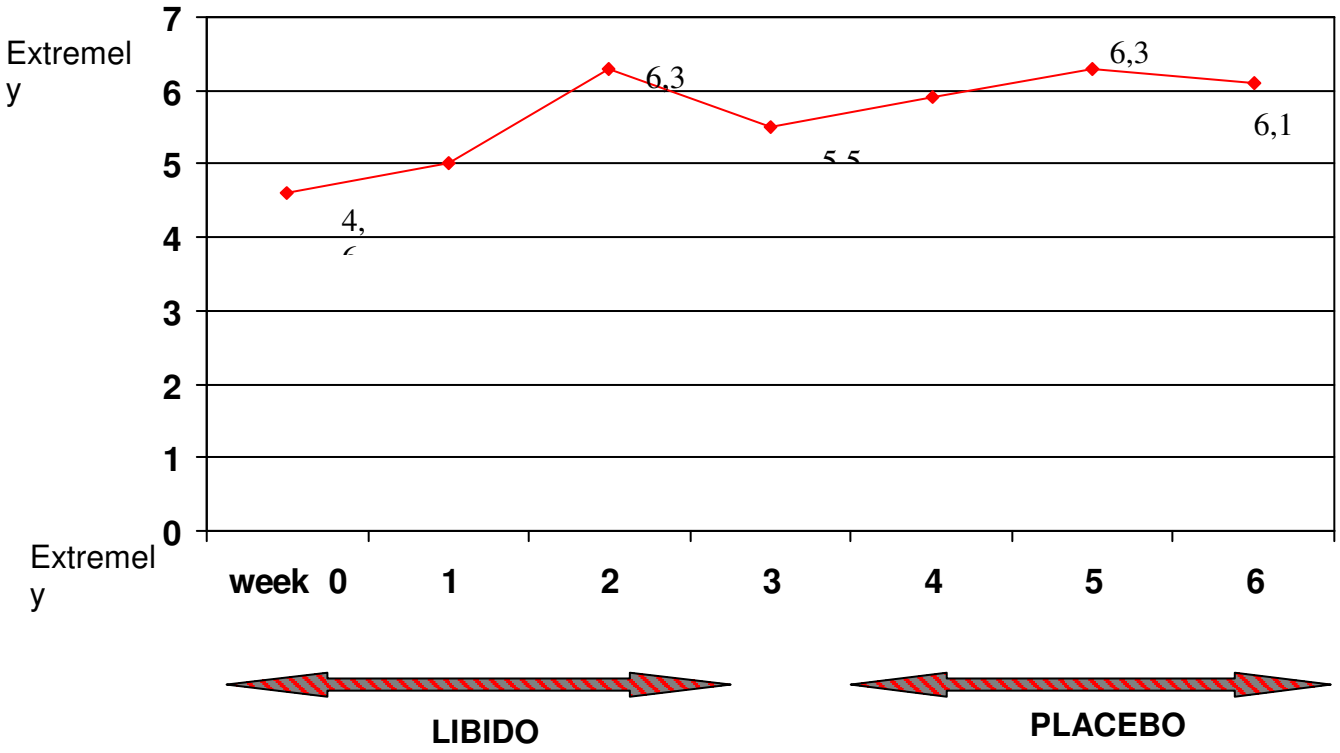


FIGURE 3

INCREASES IN
ENERGY LEVEL
AFTER 2 AND 3 WEEKS OF INGESTING LIBIDO PILLS
AND AFTER 2 AND 3 WEEKS OF INGESTING
PLACEBO PILLS

Both of these gains represent an advance from a “low” presrudy level to near the midpoint between the “extremely low” and the “extremely high” endpoints on the Likert scale. Further improvements (allthough not statistcally significant) were experienced throughout the remaining 4 weeks of the study, even though respondents were taking a placebo powder during the final three weeks.

A similar pattern is seen for respondent`s **Energy** levels (see Table 1 and Figure 3, p< .002 for the change sfter two weeks), although there is a slight dip in energy level after the third week on Libido (this decrease is recovered during the subsequent 3-week placebo period).

Finally, Table 2 and figures 4 & 5 show that a statistically significant increase (p< .05) has taken place in the level of **Confidence** by the end of the study, whereas **Self-esteem** has increased significantly (p< .02) already after 3 weeks and then remains at that level throughout the placebo period.

	Treatment week	Mean amount of confidence	Mean diff	Mean amount of Self-esteem	Mean diff	n
LIBIDO	0	5,0 ^{a} *		4,7 ^{a}		11
	3	6,1 ^{ab}		7,1 ^{b}		8
PLACEBO	6	6,7 ^{b}		6,9 ^{b}		4

- Means that do not share a common letter are significantly different at the p < .05 level.

TABLE 2: The effects of Libido and Placebo pills on “ amount of confidence” and “self-esteem”.

(No measurements were taken after weeks 1,2,4 and 5.

Fig. 4

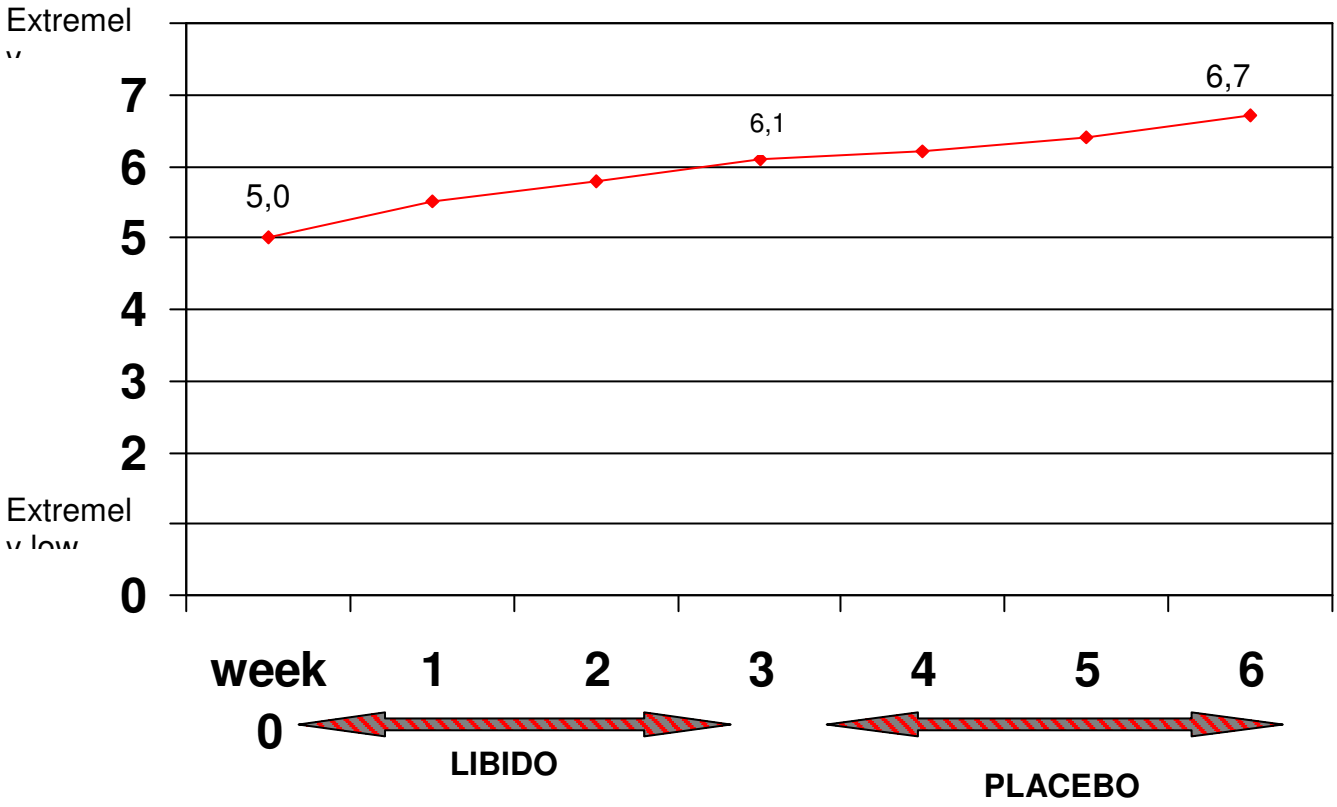


FIGURE 4

INCREASES IN
CONFIDENCE
AFTER 3 WEEKS OF INGESTING LIBIDO PILLS
AND AFTER 3 WEEKS OF INGESTING
PLACEBO PILLS

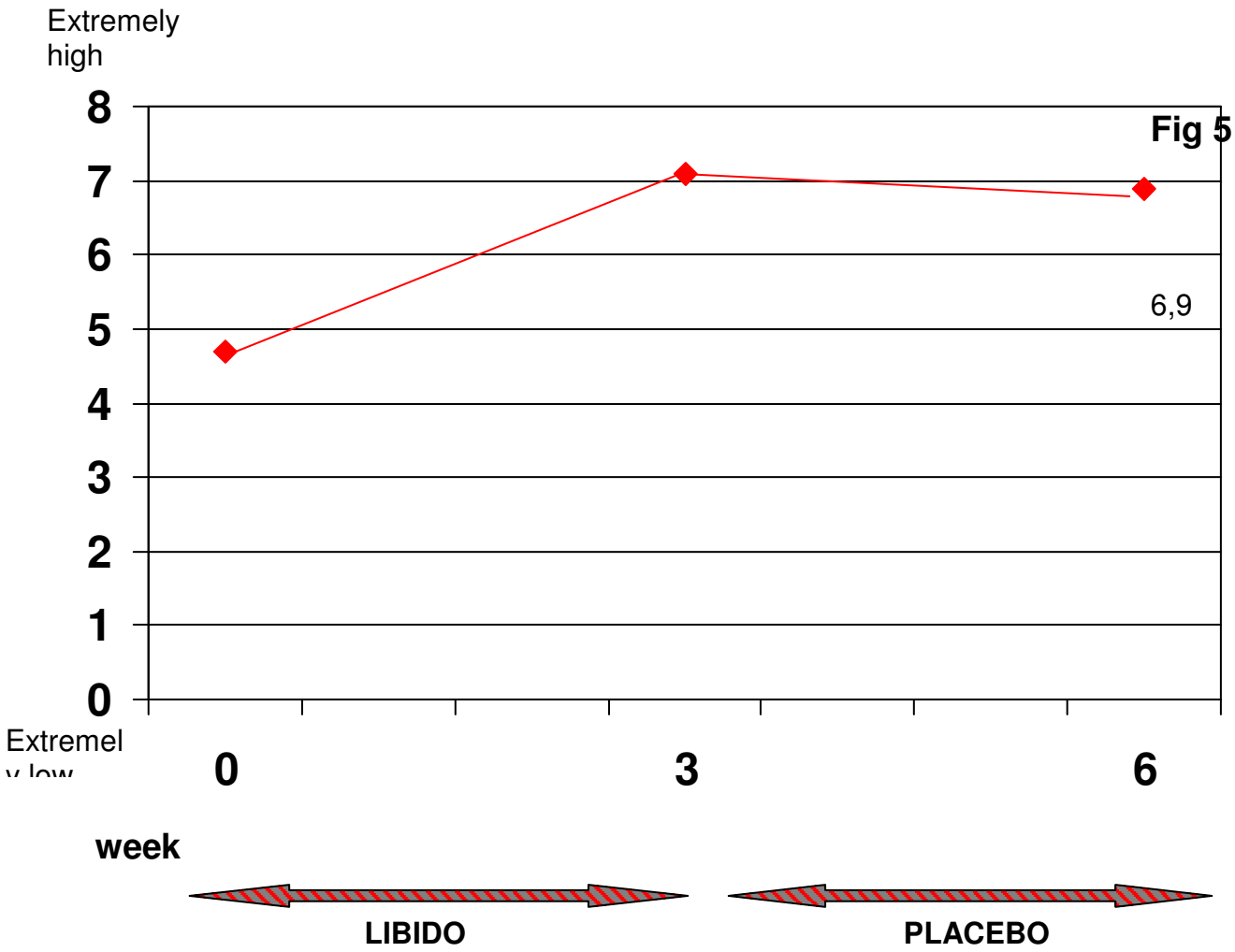


FIGURE 5

INCREASES IN
SELF-ESTEEM
AFTER 3 WEEKS OF INGESTING LIBIDO PILLS
AND AFTER 3 WEEKS OF INGESTING
PLACEBO PILLS

Satisfaction with present situation.
Subjects rated their present situation with regard to how satisfied they were with 7 aspects of their sex life: Sexual fantasy life, Frequency of sexual desire, Intensity of sexual desire, Solitary sex practices, Ability to have an orgasm, and General sexual functioning.
Statistically significant differences (using paired two-tailed t-tests) were obtained for all aspects except Solitary sex practices (see Tables 3 & 4 and Figures 6 – 11).
Table 3 and Figures 6 – 8 show that respondents` satisfaction with their Sexual fantasy life and the Intensity of their sexual desire underwent statistically significant improvements after only three weeks of ingesting Libido ($p < .05$ and $p < .03$, respectively).

TABLE 3

Treatment week	Satisfaction with Sexual fantasy life	Mean diff	Satisfaction With frequency Of sexual desire	Mean diff	Satisfaction with Intensity of Sexual desire	Mean diff	n
LIBIDO 0	3,7[a]		2,9[a]		2,6[a]		11
	2,3		2,1		1,8		
PLACEBO 3	6,0[b]	0	5,0[b]		4,4[b]		8
	1,1				2,3		
6	6,0[b]		6,1[b]		6,7[b]		7

- Means that do not share a common letter are significantly different at the $p < .05$ level.

TABLE 3: Satisfaction with "sexual fantasy life", "frequency of sexual desire", and "intensity of sexual desire" before the study (week 0), after three weeks of ingesting Libido pills, and after three weeks of ingesting Placebo pills.

(No measurements were taken after weeks 1,2,4 and 5.

Fig 6

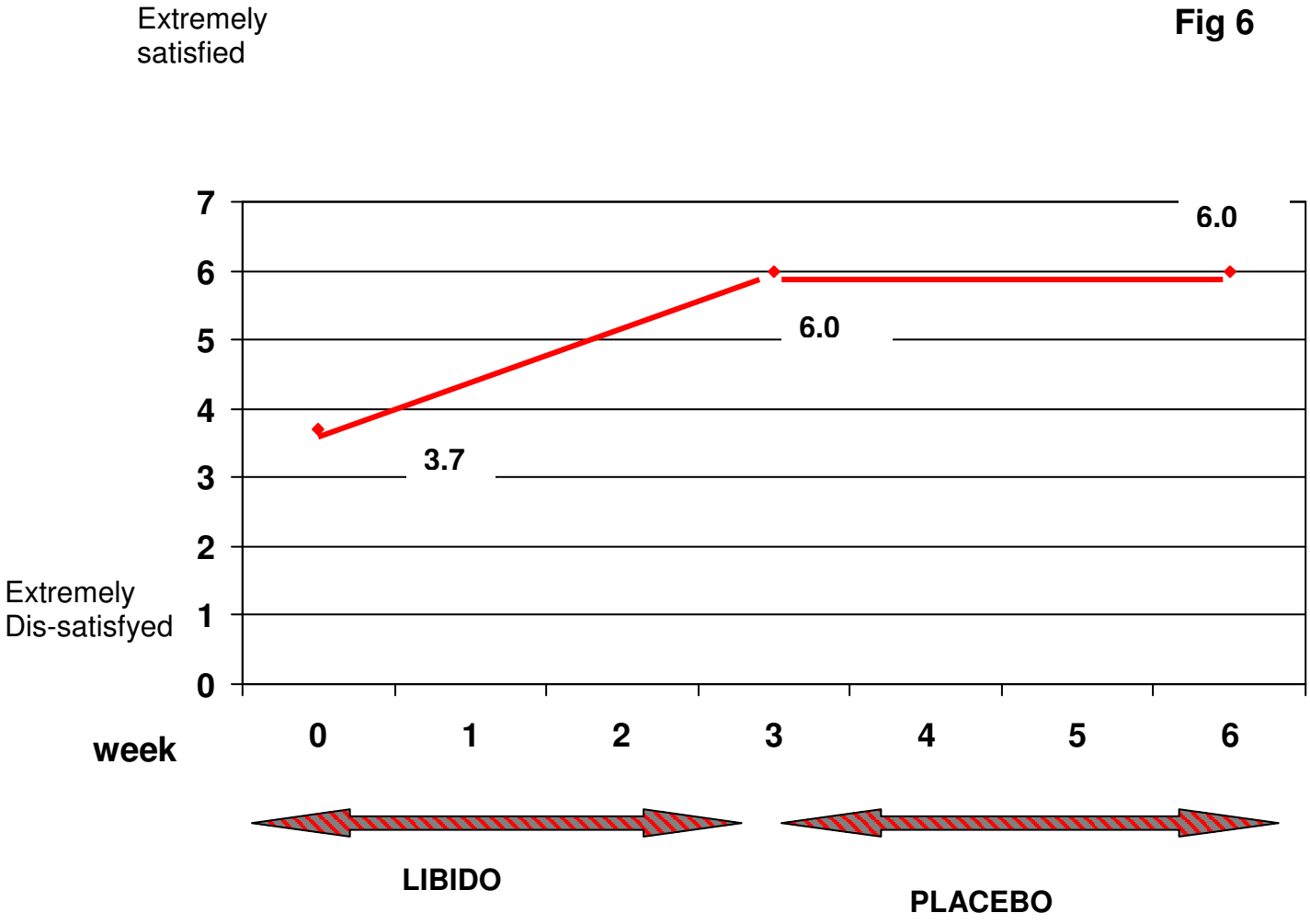


FIGURE 6

DEGREE OF SATISFACTION WITH **PRESENT SEXUAL FANTASY LIFE** BEFORE THE STUDY, AFTER 3 WEEKS OF INGESTING LIBIDO PILLS AND AFTER 3 WEEKS OF INGESTING PLACEBO PILLS.

Fig 7

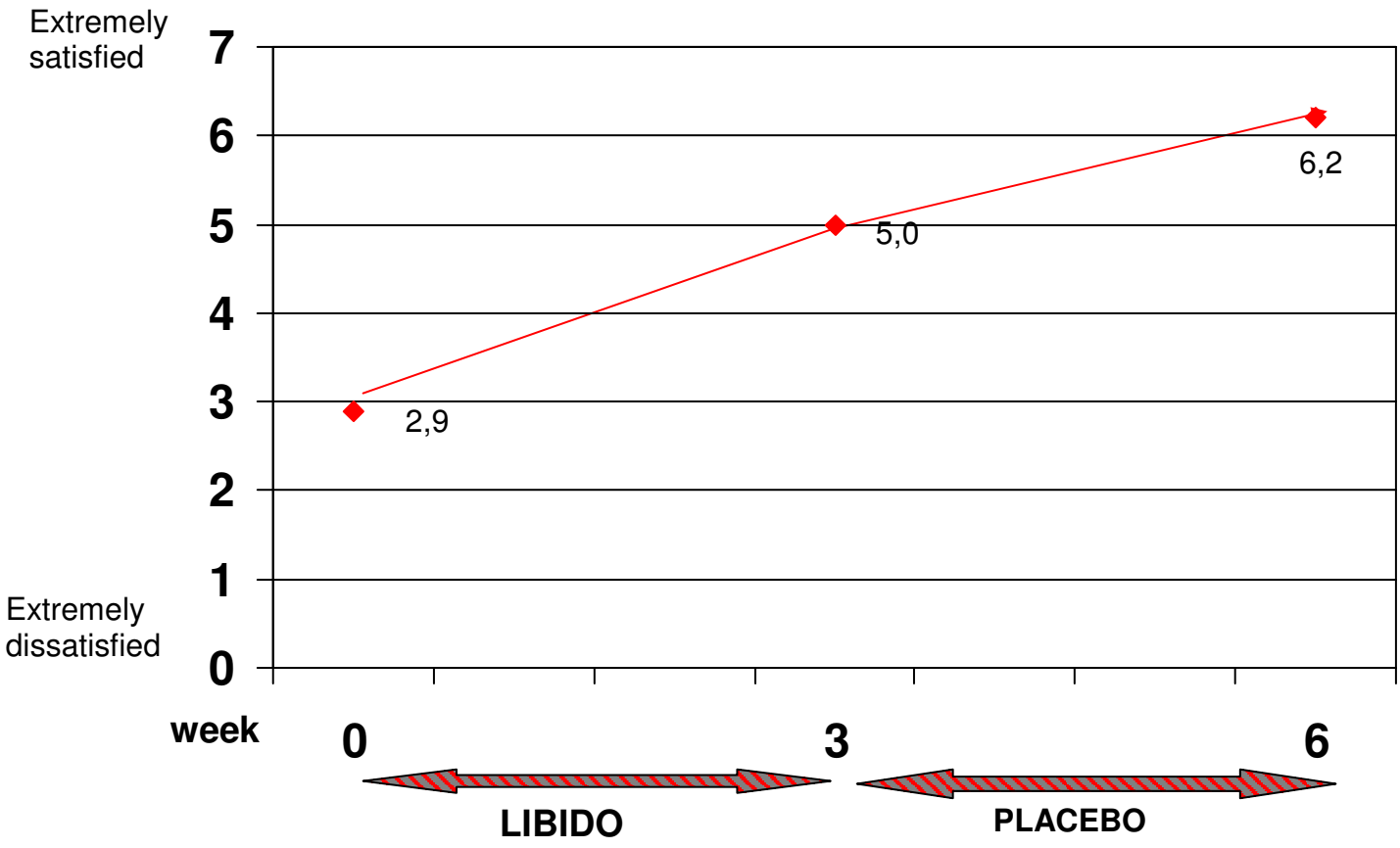


FIGURE 7

DEGREE OF SATISFACTION WITH
FREQUENCY OF SEXUAL DESIRE
BEFORE THE STUDY, AFTER 3 WEEKS OF INGESTING LIBIDO PILLS AND AFTER 3 WEEKS OF INGESTING PLACEBO PILLS.

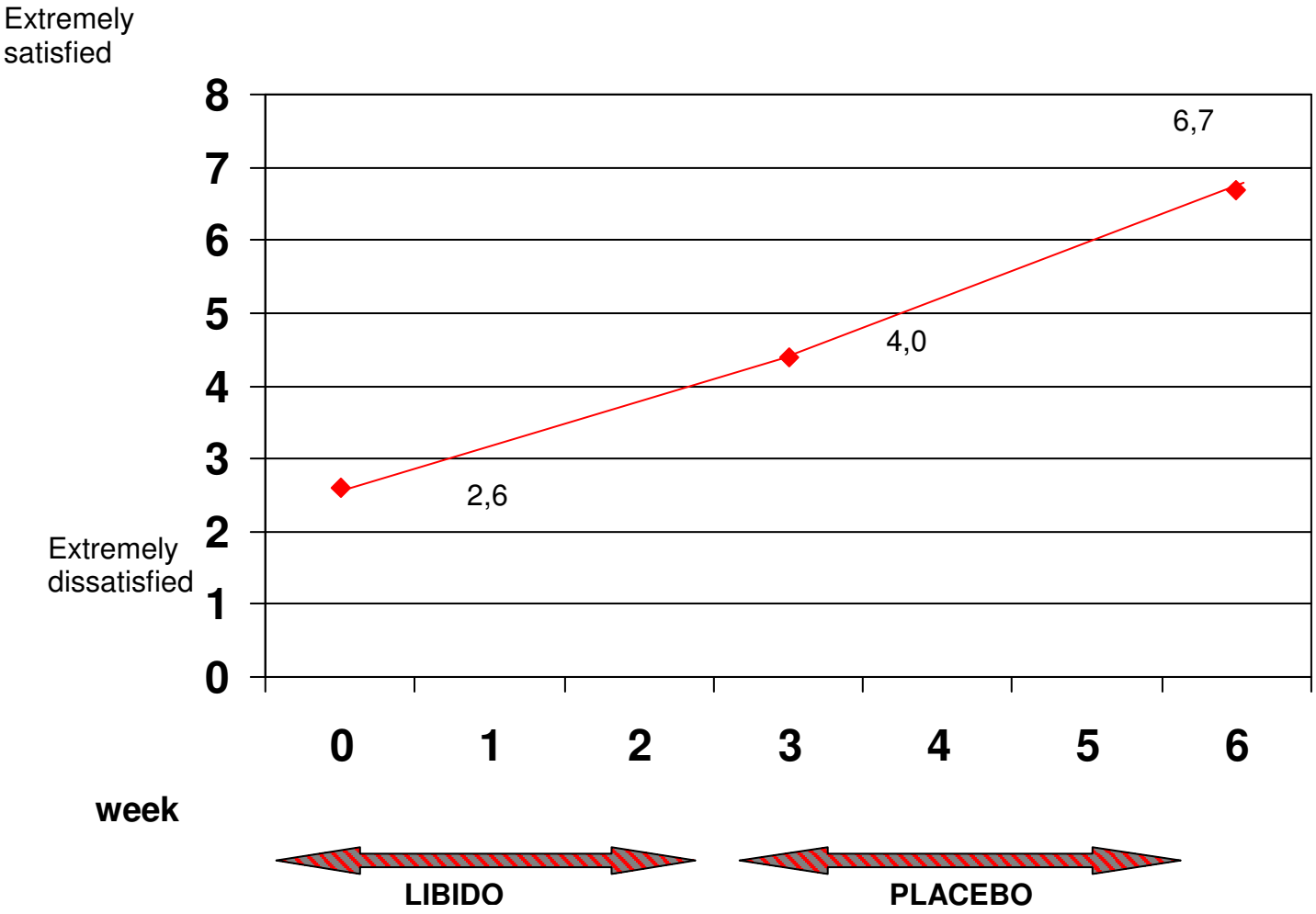


FIGURE 8

DEGREE OF SATISFACTION WITH
INTENSITY OF SEXUAL DESIRE
BEFORE THE STUDY, AFTER 3 WEEKS OF INGESTING LIBIDO PILLS AND AFTER 3 WEEKS OF INGESTING PLACEBO PILLS.

Frequency of sexual desire showed a significant increase at $p < .05$ after the sixth week. Satisfaction with the Frequency and Intensity of sexual desire continued to increase even further throughout the placebo period.
Similarly as shown by Table 4 and Figures 9 – 11, satisfaction with Orgasmic intensity and General sexual functioning increased significantly already after the first three weeks on Libido ($p < .01$ and $p < .05$), respectively), while satisfaction with Orgasmic ability had increased sufficiently to reach statistical significance
(at $p < .004$) at the sixth week of the study. Satisfaction with all three aspects continued to increase throughout the three weeks on Placebo.

TABLE 4

Treatment week	Satisfaction with ability to have orgasm Mean diff	Satisfaction With intensity Of orgasm Mean diff	Satisfaction with General sexual Sexual functioning Mean diff	n
LIBIDO 0	2,7[a] 1,4	2,6[a] 2,3	2,4[a] 2,0	11
LIBIDO 3	4,1[ab] 2,4	4,9[b] 2,4	4,4[b] 1,9	7
PLACEBO	6,5[b]	7,3[c]	6,3[b]	6

- Means that do not share a common letter are significantly different at the $p < .05$ level.

TABLE 4: Satisfaction with "ability to have orgasm", "intensity of orgasm", and "general sexual functioning" before the study (week 0), after three weeks of ingesting Libido pills, and after three weeks of ingesting Placebo pills.

(No measurements were taken after weeks 1,2,4 and 5.

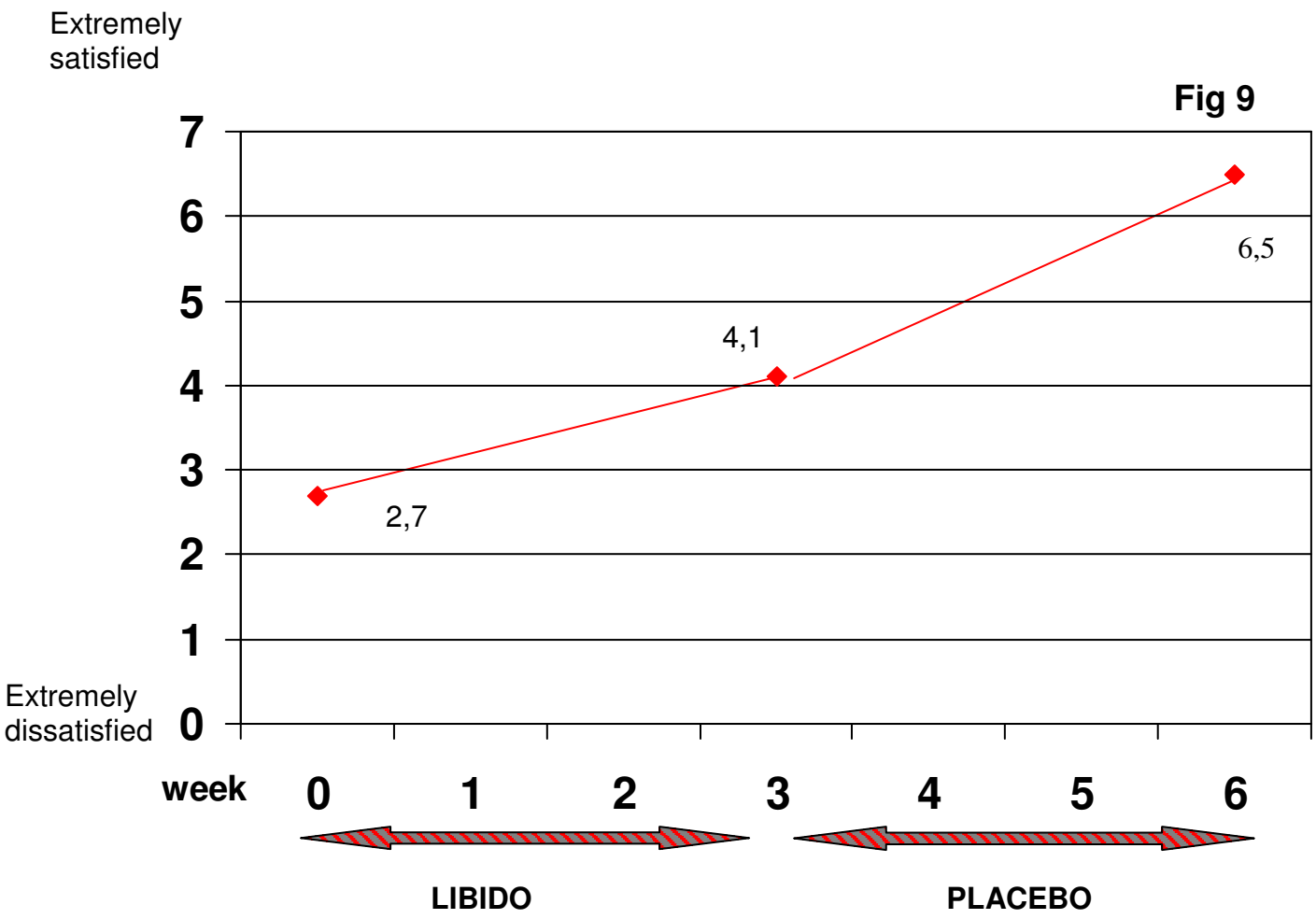


FIGURE 9

DEGREE OF SATISFACTION WITH
ABILITY TO HAVE ORGASM
BEFORE THE STUDY, AFTER 3 WEEKS OF INGESTING LIBIDO PILLS AND AFTER 3 WEEKS OF INGESTING PLACEBO PILLS.

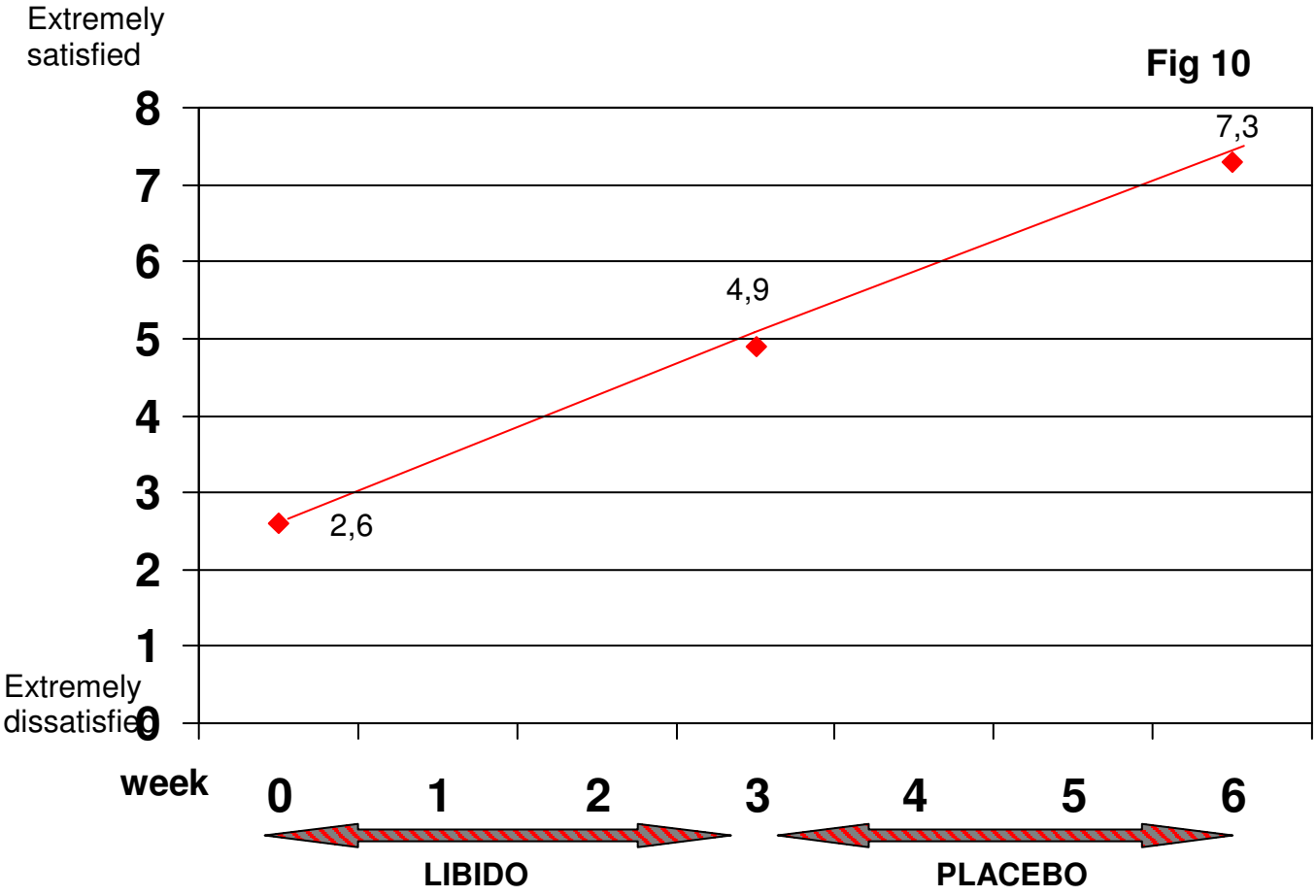


FIGURE 10

DEGREE OF SATISFACTION WITH
THE INTENSITY OF ORGASM
BEFORE THE STUDY, AFTER 3 WEEKS OF INGESTING LIBIDO PILLS AND AFTER 3 WEEKS OF INGESTING PLACEBO PILLS.

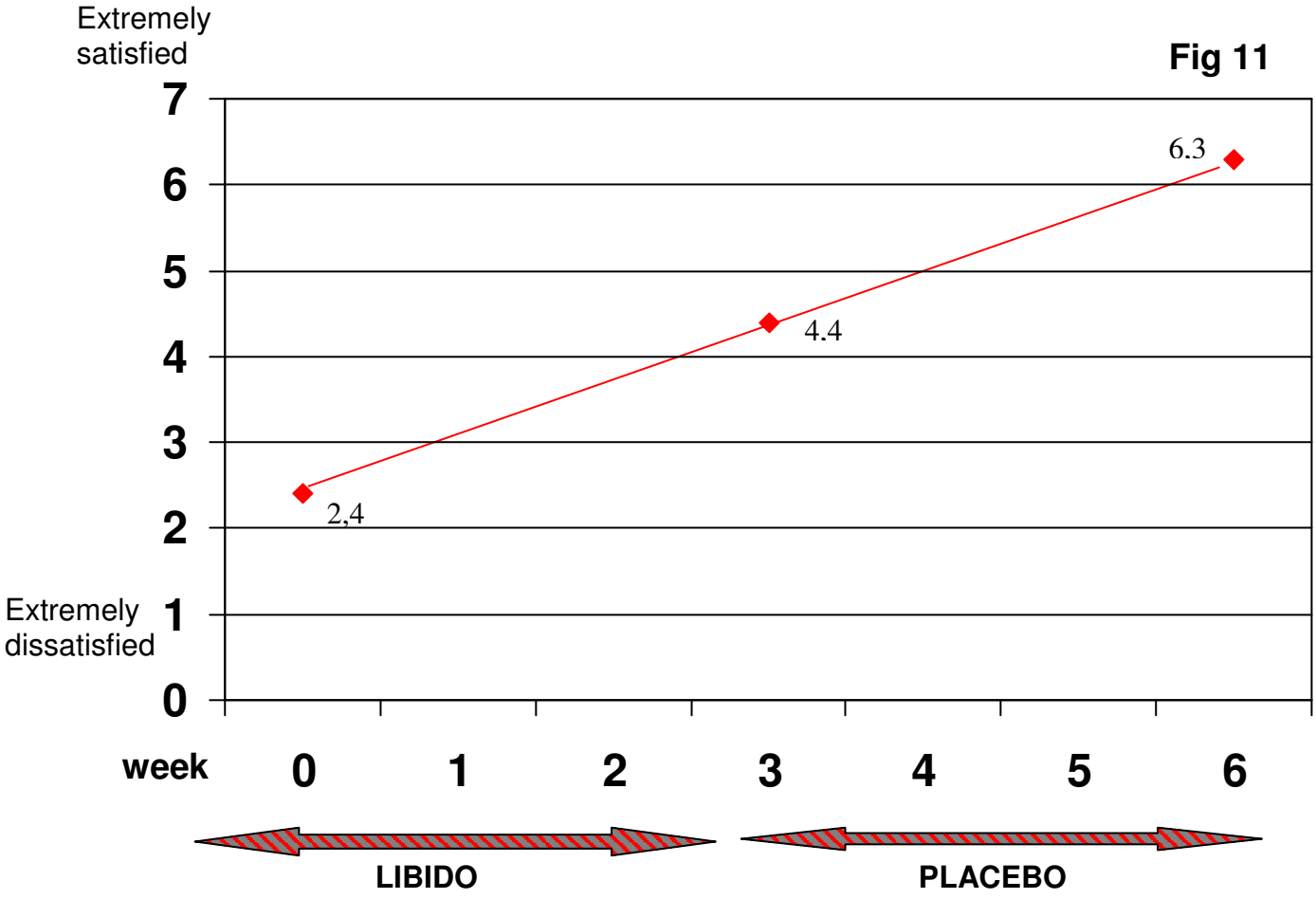


FIGURE 11

DEGREE OF SATISFACTION WITH **GENERAL SEXUAL FUNCTIONING** BEFORE THE STUDY, AFTER 3 WEEKS OF INGESTING LIBIDO PILLS AND AFTER 3 WEEKS OF INGESTING PLACEBO PILLS.

Assessments of sexual experiences in the immediate past.
Subjects rated various aspects of their sex lives as experienced during the month preceding the start of the study, as well as during the week preceding the 3rd week of ingesting Libido and during the week preceding to the 3rd week on the Placebo supplement (i.e. after 3 and 6 weeks of the study).

Statistically significant increases were obtained with regard to intensity of desire to engage in sexual activities, Ability to have an orgasm, Intensity of experienced orgasms, and Overall sexual functioning. (No significant increases were, however, obtained for three aspects; Quality of life, Frequency of sex fantasies, and Frequency of desire to engage in sexual activities). These results are addressed in Table 5 & 6 and Figures 12 – 14.
Table 5 and Figures 12 & 13 report on Intensity of sexual desire ($p < .01$) as well as Orgasmic ability ($p < .03$) and Orgasmic intensity ($p < .08$). all three aspect of sexuality steadily increased over the course of the study , and the first two aspects show statistically significant difference between the before and after ratings.

TABLE 5

Treatment week	Intensity of Sexual desire Last week	Mean diff	Ability to Have orgasm Last week	Mean diff	Intensity of Orgasm Last week	Mean diff	n
LIBIDO 0	3,9[a*]	1,0	3,1[a]	1,3	3,5[a]	1,0	11
3	4,9[ab]	8	4,4[b]	6	4,5[ab]	8	8
PLACEBO 6	5,7[b]		5,0[bc]		5,3[b]		7

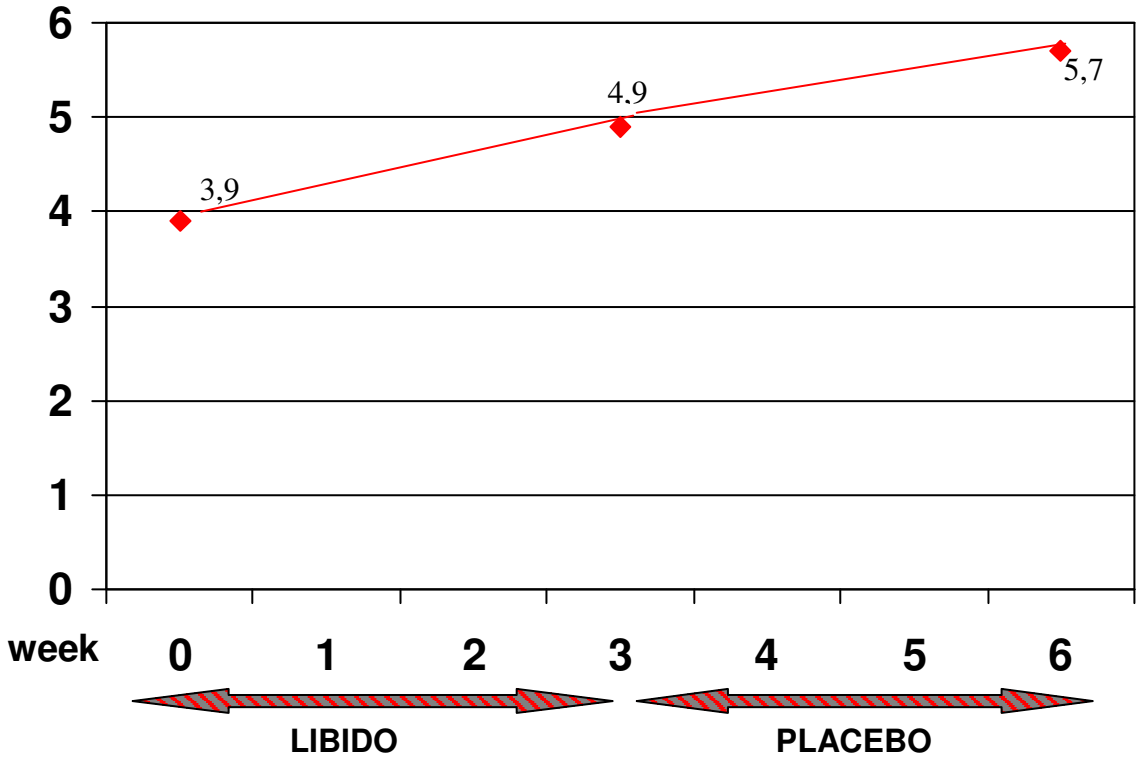
- Means that do not share a common letter are significantly different at the $p < .05$ level.

TABLE 5: "Intensity of sexual desire", "ability to have orgasm", and "intensity of orgasm " before the study (week 0), after three weeks of ingesting Libido pills, and after three weeks of
ingesting Placebo pills.

(No measurements were taken after weeks 1,2,4 and 5.

Extremely intense

Fig 12



Hardly noticeable

FIGURE 12

MEAN RATINGS OF LAST WEEK`S
INTENSITY OF SEXUAL DESIRE
BEFORE THE STUDY, AFTER 3 WEEKS OF INGESTING LIBIDO PILLS AND AFTER 3 WEEKS OF INGESTING PLACEBO PILLS.

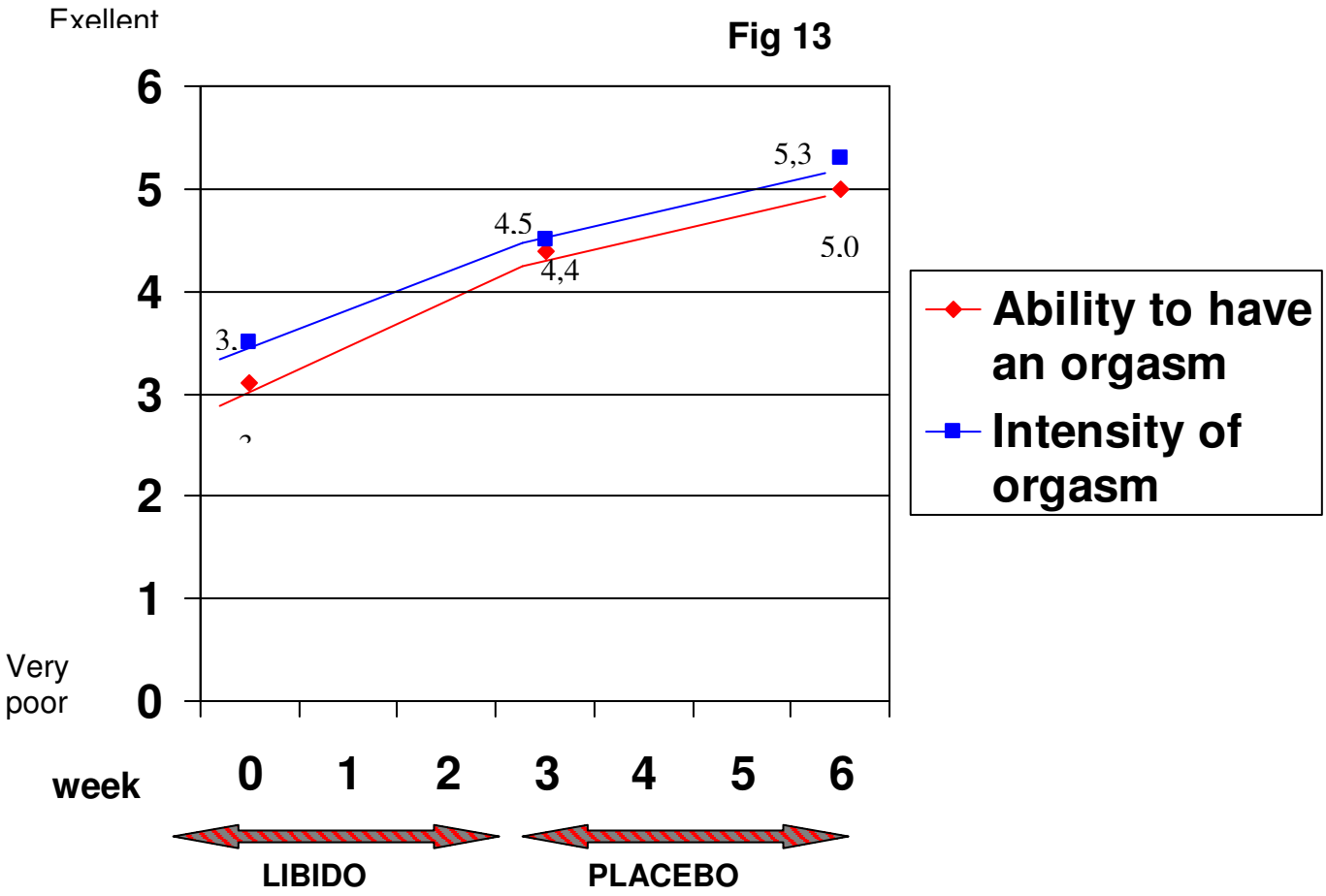


FIGURE 13

MEAN RATINGS OF LAST WEEK`S
ABILITY TO HAVE AN ORGASM AND
INTENSITY OF ORGASM
BEFORE THE STUDY, AFTER 3 WEEKS OF INGESTING LIBIDO PILLS AND AFTER 3 WEEKS OF INGESTING PLACEBO PILLS.

The most rapid change occurred for Ability to have an orgasm, showing statistically significant improvement already after three weeks of ingesting Libido (see Figure 13). Table 6 show the results pertaining to respondents` overall sexual functioning (see Figure 14) and their beliefs about the Likelihood that sexual desire will return (see Figure 15).

TABLE 6

Treatment week	overall Sexual functioning Mean diff	n	Belief sexual Desire will Return to normal Mean diff	n
LIBIDO 0	2,4[a*] 1,9	11	5,5[ab] 1.3	11
LIBIDO 3	4,3[ab] 1,9	7	6,8[b] 8	8
PLACEBO 6	6,2[b]	6	7,6[a]	7

- Means that do not share a common letter are significantly different at the $p < .05$ level.

TABLE 6: "Overall sexual functioning" and "belief sexual desire will return to normal again", before the study (week 0), after three weeks of ingesting Libido pills, and after three weeks of ingesting Placebo pills.

(No measurements were taken after weeks 1,2,4 and 5.

Fig 14

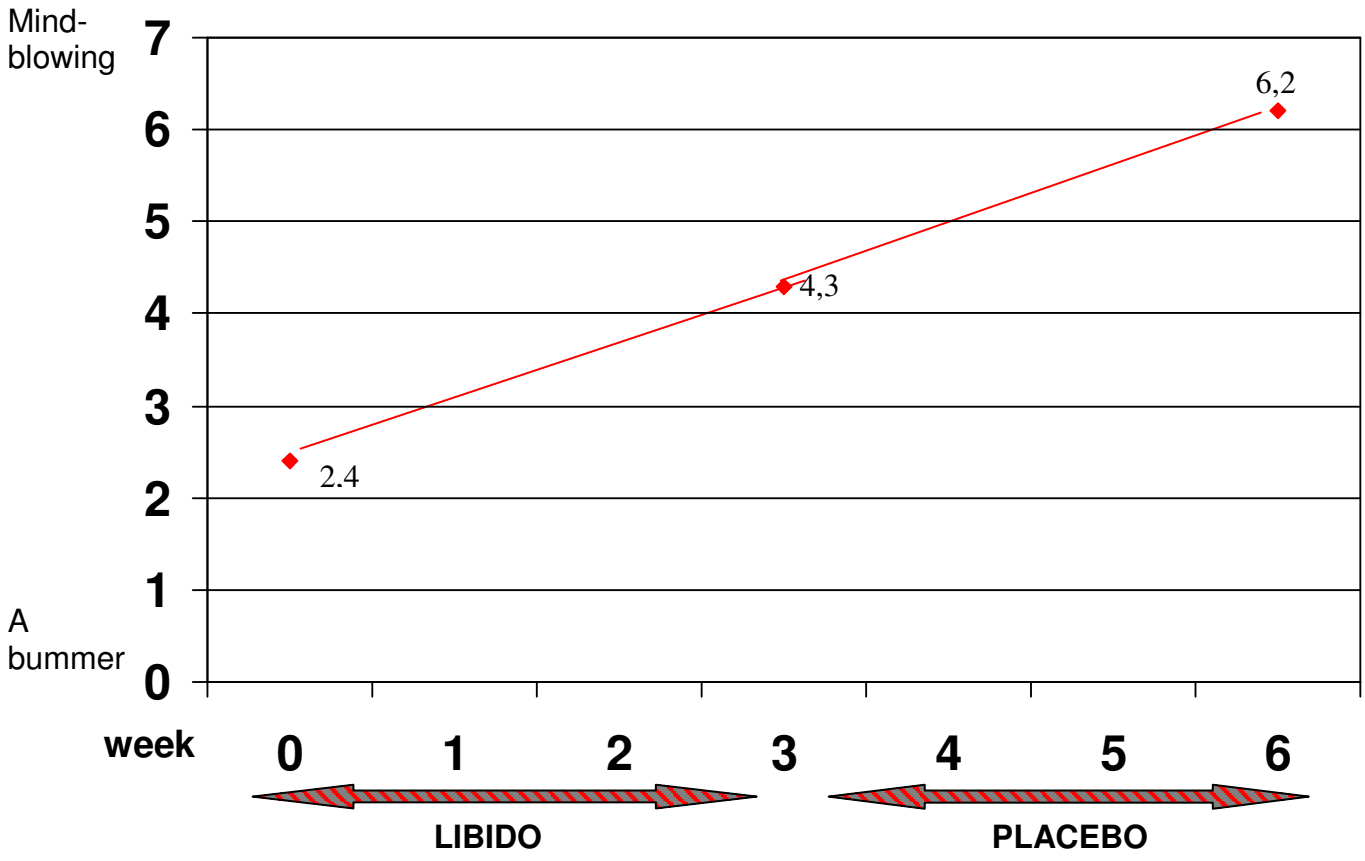


FIGURE 14

MEAN RATINGS OF
OVERALL SEXUAL FUNCTIONING
BEFORE THE STUDY, AFTER 3 WEEKS OF INGESTING LIBIDO PILLS AND AFTER 3 WEEKS OF INGESTING PLACEBO PILLS.

Fig 15

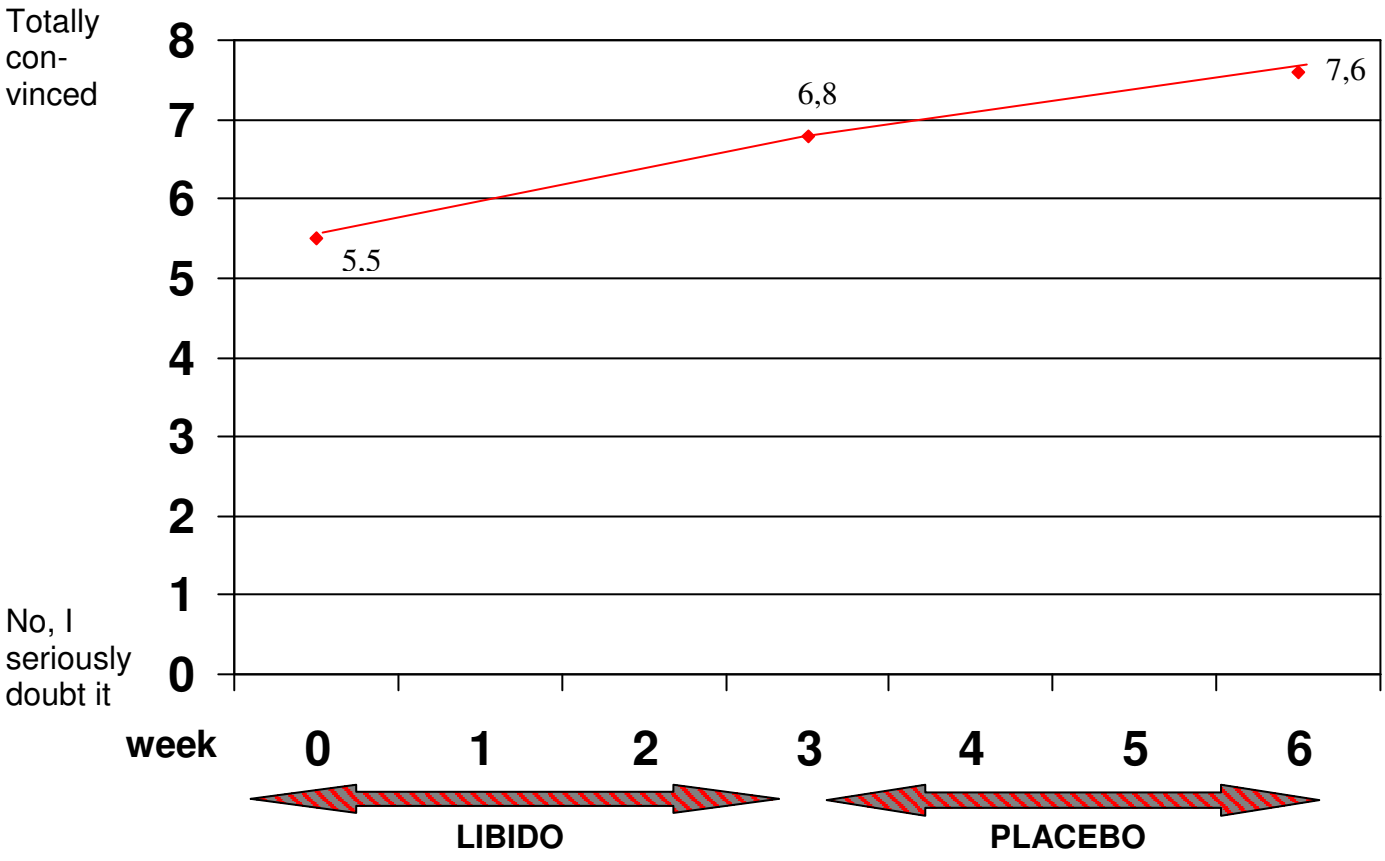
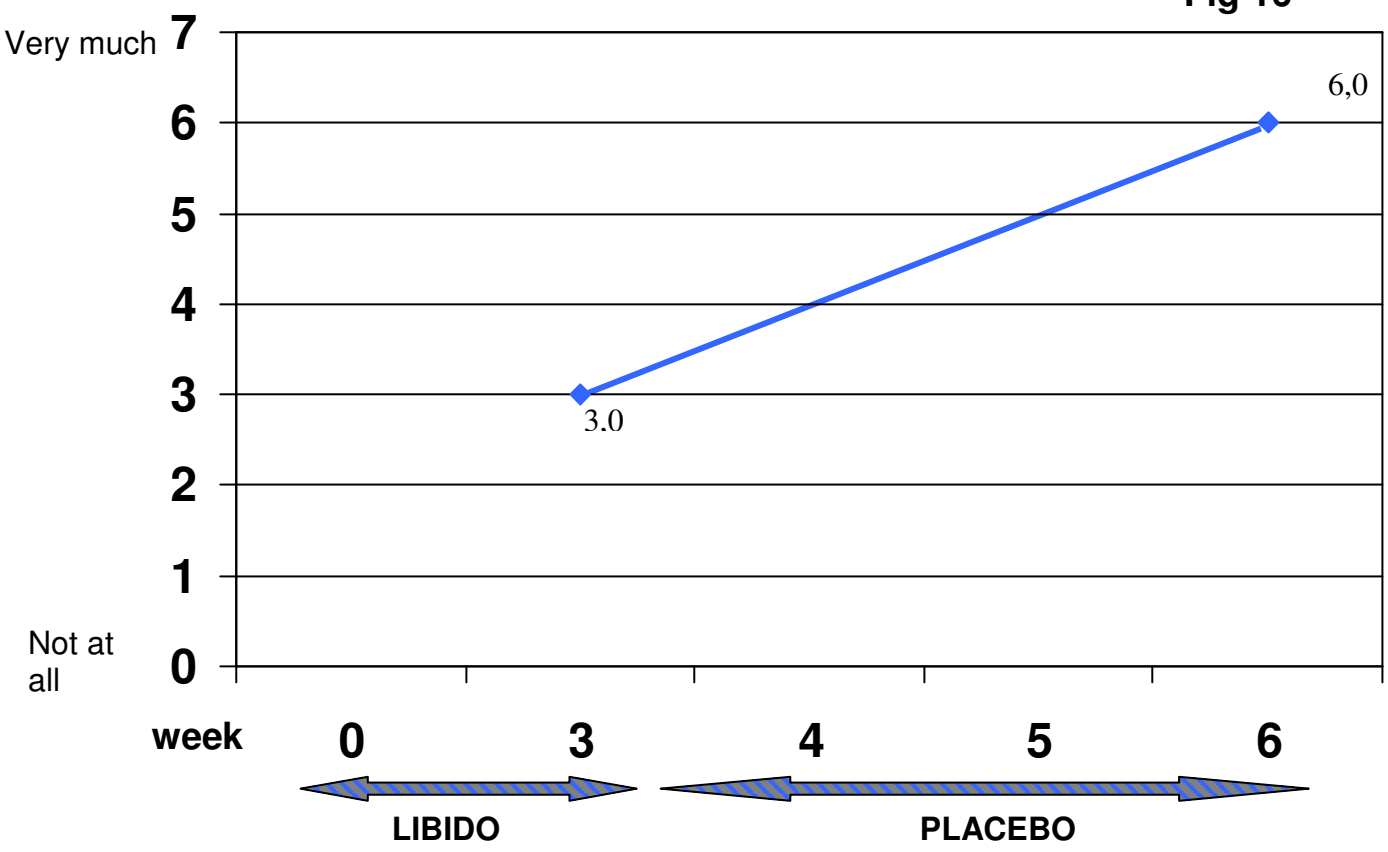


FIGURE 15

MEAN RATINGS OF BELIEF THAT **SEXUAL DESIRE WILL RETURN TO NORMAL** LEVEL AGAIN AS MEASURED BEFORE THE STUDY, AFTER 3 WEEKS OF INGESTING LIBIDO PILLS AND AFTER 3 WEEKS OF INGESTING PLACEBO PILLS.

Overall sexual functioning climbed 3.8 scale units from being close to "a bummer" (before the start of the study) to 2.8 units away from "mind-blowing" ($p < .002$). Respondents also gained an optimistic view of their situation with regards to their **"Beliefs that they would regain their sexual desire"** ($p < .03$).
Finally, respondents were asked after the third week and at the end of the study if and how much **Relationship with their partners** had improved. **Figure 16** addresses this aspect . Improvements continued to climb throughout the study and reached a peak at the end of the study, showing statistical significance at the $p < .03$ level for the difference between the ratings after the third and sixth weeks of the study.

Fig 16



(The difference between the third and sixth week is statistically significant at the $p < .03$ level).

FIGURE 16

MEAN RATINGS OF
IMPROVEMENT OF RELATIONSHIP WITH PARTNER
BEFORE THE STUDY, AFTER 3 WEEKS OF INGESTING LIBIDO PILLS AND AFTER 3 WEEKS OF INGESTING PLACEBO PILLS.

Mood and affect inventory scores.
Positive and negative affect states or emotions were assessed via the "Derogates Affects Balance Scale" (DABS). Recall that eight basic emotional states are reflected by four positive and four negative "primary affect dimensions" (each of which is measured via four DABS items – (see Appendices 8 & 9). While five "global outcome measures" assess affectivity at a more general conceptual level (see Appendix 10). The results are given in Tables 7 & 8.

TABLE 7

	Treatment week	NEGATIVE AFFECT DIMENSIONS			GLOBAL SCORES				n
		Anxiety	Guild	Hostility	NTOT[1]	ABI[2]	AEI[3]	PAR[4]	
LIBIDO	0	9,31[a*]	8,0[b]	8,23[a]	33,92[a]	38[a]	73,39[a]	,56[a]	13
LIBIDO	3	7,11[b]	6,22 [ac]	7,89[ab]	27,0[ab]	,90[a]	72,22[a]	,64[ab]	9
PLACEBO	6	4,71[b]	3,71[bc]	4,86[b]	18,71[b]	1,41[b]	65,71[b]	,72[b]	7

- Means that do not share a common letter are significantly different at the $p < .05$ level.

1. Primary measure of negative affectivity (i.e., sum of all 20 negative item scores).
2. Principal measure of affects balance (PTOT – NTOT)/20.
3. Affective expressiveness or intensity (PTOT + NTOT).
4. Proportion of total affective expression due to positive emotions (PTOT/ AEI).

TABLE 7: Derogates Affects Balance scores before the study (week 0), after three weeks of ingesting Libido pills, and after three weeks of ingesting Placebo pills (expressed as raw scores).

TABLE 8

Treatment week	POSITIVE AND NEGATIVE AFFECT DIMENSIONS					GLOBAL SCORES	n
	Joy	Content-ment	Vigor	Affection	Depression	PTOT 1	
LIBIDO 0	9,77[a]	10,0[a]	9,92[a]	11,54[a]	8,39[a]	41,46[a]	13
3	10,78[a]	10,56 [a]	10,89[a]	13,0[a]	5,78[a]	45,22[a]	9
PLACEBO 6	10,43[a]	11,86[a]	10,71[a]	14,0[a]	5,43[a]	47,0[a]	7

- Means that do not share a common letter are significantly different at the $p < .05$ level.

1. Primary measure of positive affectivity (i.e., sum of all 20 positive item scores).

TABLE 8: Derogates Affects Balance scores before the study (week 0), after three weeks of ingesting Libido pills, and after three weeks of ingesting Placebo pills (expressed as raw scores).

As shown in Table 7, two of the four primary negative effects dimension scores (guild and hostility) show statistically significant decreases by the end of the study ($p< .003$ and $p< .009$, respectively), while one (anxiety) had decreased already after 3 weeks on Libido ($p< .04$). Thus, the frequencies by which these negative emotional states were experienced by participants before the start of the study were significantly lower after two or six weeks. However, as Table 8 shows, no significant differences were obtained for the fourth negative affect dimension (depression) or for the four positive affects dimensions (joy, contentment, vigor and affection). However, although statistical significance is not reached, there is a definite trend of improvement for each one of the positive emotional states.

Table 7 also displays the results pertaining to the global measures. As expected on the basis of the observed decreases in the three primary negative affects scores the global negative measure, Negative Affects Total (NTOT), showed a statistically significant decrease between the start and the end of the study ($p< .006$).
The global positive affects measure (PTOT) did not increase significantly. All three remaining global measures (the Affects Balance – ABI, the Affective Expressiveness – AEI, and the PAR) exhibited statistically significant changes between weeks
1 and 6 ($p< . 02$, $p< . 006$, and $p< . 006$, respectively).
In summary, the major emotional effects (measured via DABS) occurred as decreases in the severity of negative emotional states rather than as increases of positive ones.

Additional information obtained via open – ended comments.
Five men and five women volunteered additional information about their experiences during the test. The comments offered by men were typically fewer and less lengthy than those offered by the female participants. Participants could provide open – ended comments at five different occasions (i.e., at every scheduled point of telephone contact with the researchers): before the start of the study, after 2, 3, 5 and 6 weeks, at which point they sum up their overall experiences throughout the study.
I will, in the following, relate each individual's experiences throughout the six weeks, using much their own words. Thereby the reader will gain a better sense of the process of change that they underwent.

Comments offered by male participants:

Male 1, age 67:	Week 5:	"Raised sexual desire".
Male 2, age 42:	Week 2:	"Felt calmer".
	Week 3:	"Desire doubled in strength", "I fantasize about sex", "orgasms much stronger" and felt "closer to people".
	Week 5:	"Sleeping more soundly".
	Week 6:	"I feel strong physically and mentally", "Desire definitely stronger", "Less fear of past situations", "Dreams increased", "Able to sleep through the night", "Less smoking desire", and "No question this has had a positive effect on my life.
Male 3, age 29:	Week 2:	"No change".
	Week 3:	"Wetter dreams".
	Week 5:	"More sexual desire".
	Week 6:	"Ability to have sex increased", "feelings of closeness", and "more focused on various projects".
Male 4, age 29:	Before:	"difficulty to climax (45 min – 1 hour)".
	Week 2:	"More control over climax", "greater intensity of orgasm", "I masturbate", and "I feel more confident".
	Week 3:	"I take less time to climax".
	Week 5:	"More intense climax", "I feel healthier", and "increased sex desire".
Male 5, age 47:	Before:	"Dull sex life".
	Week 2:	"stabilized sexual desire", and "pills had effect after a few days".

Comments offered by female participants:

Female 1, age 26:	Before:	"Non – existent sexual functioning".
	Week 2:	"I have dreams about sex".
	Week 3:	"Very affectionate", "more energy", and "now hopeful that desire will return".
	Week 5:	"Need not concentrate as hard to reach Orgasm", and "energy level higher".
	Week 6:	"Noticeably better sex functioning (but not great)", "more energy" "I take more initiative", and "I don't get as frustrated".
Female 2, age 33:	Before:	"No sexual feelings at all with Prozac".
	Week 2:	"Increased sense of well – being", "I'm more connected with sexual desire", "Desire comes more easily", "I feel good", "Orgasm comes easier", and "More intense orgasm".
	Week 3:	"I feel more comfortable with body and psychological responses", "I feel more comfortable with who I am overall", "Increase in happiness and well-being", "Increase in intensity of sexual desire", and "Increase in frequency of sexual desire".
	Week 5:	"Nothing happened on second batch (= placebo)", and "back to square one".
	week 6:	"No change during the second 3-week period (i.e., placebo period)", and "I want to order Libido".
Female 3, age 39:	Before:	"No interest in sex other than once a Month when ovulating".
	Week 2:	"More desire", more lubrication", and "I feel positive".
	Week 3:	More affectionate", and "more desire".
	Week 5:	"Increased desire".
	Week 6.	"Increased desire", and "increased lubrication (I had problems before)".
Female 4, age 34:	Before:	"Non-existent sex functioning except prior to menstruation and during ovulation"
	Week 2:	"Increased intensity of orgasm", "Increased ability to reach orgasm", and "dreams of sex".
	Week 5:	"No change since week 2"
Female 5, age 32:	Before:	"No desire".
	Week 2:	"I think more about having sex", "I have dreams about sex", "I have nightmares".

DISCUSSION

There is a striking consistency throughout the study with regard to the effects of Libido as compared to the placebo: In practically all cases where there are statistically significant increase in our parameters during the 3 weeks on Libido, the subsequent 3 weeks on placebo do show increases, but they are not statistically significant (except in two cases out of 20: “satisfaction with the intensity of orgasm”, and “ belief that sexual desire will return to normal”). This finding should be particularly noted when we recall, that the original crossover design had to be sacrificed due to need for announcement of early results at a press conference. Taken together, the two facts (a) that statistically significant findings were consistently obtained during the Libido but not during the Placebo period, and (b) that participants of the study were “blind” to (i.e., ignorant of) the order between the Libido and Placebo periods lend confidence to the proposition that Libido is a product with significant positive effects on anti-depression medication induced decrease in sexual functioning. More specifically, the ingestion of Libido appears to (within the very short period of 2-3 weeks) significantly increase lowered intensity and frequency of sexual desire, increase ability to have orgasm as well as the intensity of orgasm, and increase overall sexual functioning.

Due to the above mentioned positive effects of Libido in this study, participants experienced significant increase in their satisfaction with their sexual desire, with their orgasmic ability and intensity, and with their general sexual functioning.
In addition, it appears that participants were not the only beneficiaries of Libido`s positive effects: the relationships with their partners improved 100%, i.e., it jumped from a mean value of 3 to a mean of 6 on the 9-point scale!

Of great importance for the prognosis of these patients is the finding that they acquired new hope for the future: their beliefs that their lost sexual desire would return to normal again rose dramatically during the study. Such a conviction will, of course, greatly empower the individuals in question and increase the probability that they will be mentally open to available remedies and actively take charge of their situation. Perhaps it was because of this positive outlook that significant increments also occurred in their self-confidence and self-esteem.
Of course, participants` satisfaction with fact that their sexual lives had started on its way back towards normality might be the major explanation for improved self evaluations.

It is interesting to note that the effect of Libido frequently occurred after only 2 weeks of ingestion. This is consistent with findings from studies in different context (see reviews in Eskeland, Thom and Svendsen 1996). On the other hand, some effects were not powerful enough to be statistically significant until the 6th week of the study. However, this may be due to the nature of those parameters, in that they may not be inherently amenable to quick sizable changes. After all, it seems reasonable to assume that characteristics like confidence, for example, needs some time before the accomplishments of a significant change is possible. Similarly, it is likely that one might be reluctant to dare to be too satisfied too soon (after 3 rather than 6 weeks) with one`s overall sexual functioning. And before one will dare to believe that one`s decreased/lost desire will return to normal, most people would probably avoid forming strong expectations in this direction until after the passage of some time out of fear for painful and frustrating disconfirmation.

Last but not the least is a rather though provoking finding obtained via the Derogates Affects Balance scale (DABS). Recall that improvements in the four positive affect dimensions of joy, contentment, vigor and affection did not reach statistical significance – contrary to what one might have expected, considering the findings we have discussed so far. However, it turned out that three of the four negative affect dimensions (i.e., anxiety, guild and hostility) showed a decrease in intensity. Thus, one might speculate that the process of recovery from an emotionally depressed state involves at least two major stages – in the fashion of a hydraulic mechanism.
First, the intensity of negative effects is brought down to a level of sufficiently low intensity, the level at which the individual will be able to start focusing on positive rather than negative emotions. At first, freed from preoccupation with negative affect, perhaps mental energy may be redirected towards gaining awareness of existing positive affect deficiency (rather than the pain associated with high levels of negative affect). At that point, the stage might very well be set for continuos increments in positive affect.

REFERENCES

Derogates, L.R. The Affects Balance Scale. Baltimore: Clinical Psychometric Research 1975.

Derogates , L.R. Derogatis Affects Balance Scale: Administration, scoring and procedure manual. Towson, MD: Clinical Psychometric Research Inc., 1996.

Eskeland, B., Thom, E and Svendsen, K.O.B. sexual arousal in men: Effect of oral ingestion of a product derived from fertilized eggs.
The Journal of International Medical Research, 1996, in press.

PRE CLINICAL STUDY

Preliminary Laboratory Report / May 1996
Ref.: Project LIBIDO
Company: Zenyaku Kogyo Co. Ltd.

PROJECT:

Effects of Fertilised Incubated Shell Eggs on Sexual Behavior of male Rats

INVESTIGATORS: S. Kawashima (Director of Res. Lab.),
M. Takano, N. Otha, M. Higashi, M. Takahashi
T. Karakida and Y. Koide.

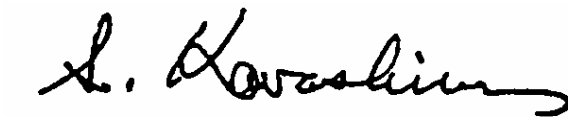
This study was carried out at: Researc Laboratory, Zenyaku Kogyo Co. Ltd. 2-33-7 Ohizumi, Nerima-ku, Tokyo 178, Japan.

Tel: 81-3-3922-5131
Fax: 81-3-3922-5065

FOREWORD

The present study was carried out during the period of March – May, 1996, and should be regarded as preliminary in nature. This report describes and discusses the results.
Repeated and additional experiments are needed to verify the present concussions.
However, such experiments are currently not planned.

May 1996



Seiichiro Kawashima, DSc.

INTRODUCTION

"LIBIDO" was developed by Matforsk Norwegian Food Research Institute with the aim to stimulate sexual drive and desire in human. The principal ingredient of LIBIDO is dried egg powder incubated to the blastodermal to proto-embryonic stage, which for hen's egg is about 10 days. Clinical tests in a randomized placebo controlled study of LIBIDO in middle-aged men by Medstat Research A/S showed that it acted to enhance sexual potency (or libido) and elevate blood testosterone levels.
In order to evaluate the effects of "LIBIDO" on sexual activity before finalizing contract between us, Zenyaku will observe whether it is effective on sexual behavior and serum testosterone levels in an animal model (male rats).

MATERIALS AND METHODS

Animals

Sexually naive immature male and female rats of the Sprague-Dawley strain were purchased from Charles River Ltd., Japan and maintained under controlled photo-period (12 hr light, 12 hr dark cycle) and temperature (22 º C). Thirty male rats were used for behavioral tests beginning at the age of 70 days. Fiftheen female rats ovariectomized (OVX) at 60 days of age. Two weeks after OVX, all OVX animals received subcutaneous implantation of a Silastic medical grade tube (2mm x 4mm inner and outer respectively, 4mm long) containing estradiol-17 beta (E_2).
About 3 weeks later, OVX female rats bearing E_2 implants were used for behavioral tests. They were made highly receptive to males by giving a single subcutaneous injection of progesterone (0,5 mg/rat/0,1 ml sesame oil) in the morning of test day.

Experimental design

The experimental design is shown in Figure 1. Thirty male rats were divided into three groups. Ten male rats each were used for the test either of egg powder (bulk egg powder at 10-day incubation, designate Libido in this paper for convenience), placebo (egg powder of fertilized but no-incubated eggs), or solvent vehicle (deionized water).

Behavioral tests were carried out once per week. Blood was collected from the caudal vein once weekly , and serum levels of testosterone were determined by radioimmunoassay (RIA).

Administration of Libido and placebo

Libido and placebo were dissolved in deonized water (146 mg/ml) and were administered to male rats at the dose 583 mg/kg twice daily by forced oral intubation for 3 weeks. The dose was about ten-fold that for human use (3,5 g sachet x 2 daily, body weight regarded as 60 kg). intubation was terminated at the end of 3 weeks, and rats were given neither test powder nor vehicle afterwards.

Behavior tests

Behavioral tests were carried out at the beginning (immediately before the first intubation of Libido, placebo or vehicle, day 0), and on days 7,14,21 and 35 of experiment. In the afternoon (1:30-4:30pm), each male rat was adapted in an observation cage (transparent acrylic resin plate, 60(w) x50(d)x40(h) for 5 min. then, a receptive female, which had been ovariectomizedand pretreated with E_2 and progesterone prior to the test was placed with the experimental male.
The observation for each male was continued for 30 min. the receptive female was

replaced by another individual at every 10 min in order to lessen the influence of variance of affinity between male and female.

The following parameters were recorded:

a. Mounting frequency (MF, number of mounts with thrust and with or without intromission during 30 min).
b. Intromission frequency (IF, number of mounts with intromission during 30 min).
c. Ejaculation frequency (EF, number of ejaculation during 30 min).
d. Mount, intromission, and ejaculation latencies (ML, IL, EL, time from the introduction of receptive female to the first occurrence of each behavioral pattern).

MF and IF were expressed as the number per 5 min.

Reflexive erection test

Spontaneous reflexive erection was measured after 3 weeks of treatment of Libido, placebo or vehicle. Rats were placed on a board in the supine position (=on their back) without anesthesia. Head and chest of the rat were covered by opaque plastic semicircular tube. The penis was exposed from the prepuce, and the frequency of reflexive erection during 10 min after the first erection was counted on the following ratings:

E1: Congestion, but no swelling of penis.
E2: Congestion with swelling of penis, and little or moderate swelling of glands.
E3: Maximum swelling of penis and glands, the latter showing cup-like extension.
F: Recurvature of the penis called “flip”.

Autopsy

Male rats were killed by decapitation at about day 42, and weight of some organs was recorded.

RIA

Blood samples (about 0,3 ml each) were collected from the caudal vein of conscious rats. The serum was separated by centrifugation at 3,000rpm for 20 min at 4º C, and stored at -80º C until RIA. Serum level of testosterone was measured by RIA using a commercial kit (Sorin Biomedica, Italy). Standard curve was constructed in the range of 0, 25-10,0 ng/ml).

RESULTS

Male sexual behavior

Mounting frequency (MF) per 5 min was almost constant during observation period in vehicle and placebo-treated groups. In contrast MF in Libido-treated group significantly increased at 2 and 3 weeks, the peak frequency being observed at 3 weeks (Fig 2). The peak value returned to the initial control level 2 weeks after the cessation of Libido treatment. The pattern of changes in MF expressed as percentage of the initial value (Fig 2b) was similar to that expressed in absolute values (Fig 2a). overall significant difference was detected between Libido and placebo groups in the data expressed as percentage ($p=0.049$, two-way ANOVA).

Intromission frequency (IF) per 5 min is illustrated in Fig 3. Although Libido-treated rats showed highest value at 3 weeks, differences from other two groups were statistically not significant.

Figure 4 shows the effect of Libido on ejaculation frequency (EF). EF gradually increased during the observation period in all the three groups, reaching a peak at three weeks (Fig 4a). Percentage increase in EF was the largest in Libido-treated group, and the differences between Libido and other two groups were statistically significant
(Fig 4b)

Mounting, intromission and ejaculation latencies were so variable among individuals. Therefore, all the data of the three groups were combined in order to see profiles of the changes of these parameters. As a result, overall data revealed that mounting latency did not show any appreciable change, while, intromission and ejaculation latencies decreased as a function of time (Fig 5). Post ejaculatory refractory period, when male rats are not responsive to soliciting behaviors of female counterparts) e.g. ear wiggling, hopping), were almost constant regardless of treatments (Fig 6).

Reflexive erection

There were no significant differences among the three groups in any rating of the reflexive erection frequency (Fig 7). The numbers of flips appeared to be greater in Libido and placebo groups then vehicle control group, but the differences were statistically not significant.

Testosterone concentration

Serum testosterone concentrations during the observation period are shown in Fig 8. No significant differences were detected among the three groups at any point. At the beginning of experiment (0 week), testosterone concentrations were variable among the three groups (Libido, 2,28ng/ml; placebo, 3,38ng/ml; vehicle, 4,26ng/ml), but the differences were not statistically significant.

Body weight and weight of some organs

The body weight of rats steadily increased as a function of time (Fig 9). No differences were detected among the three groups. Similarly, there were no significant differences in the weights of the liver, kidney, testis, ventral prostates, seminal vesicles, pituitary and adrenal (Fig 10).

DISCUSSION

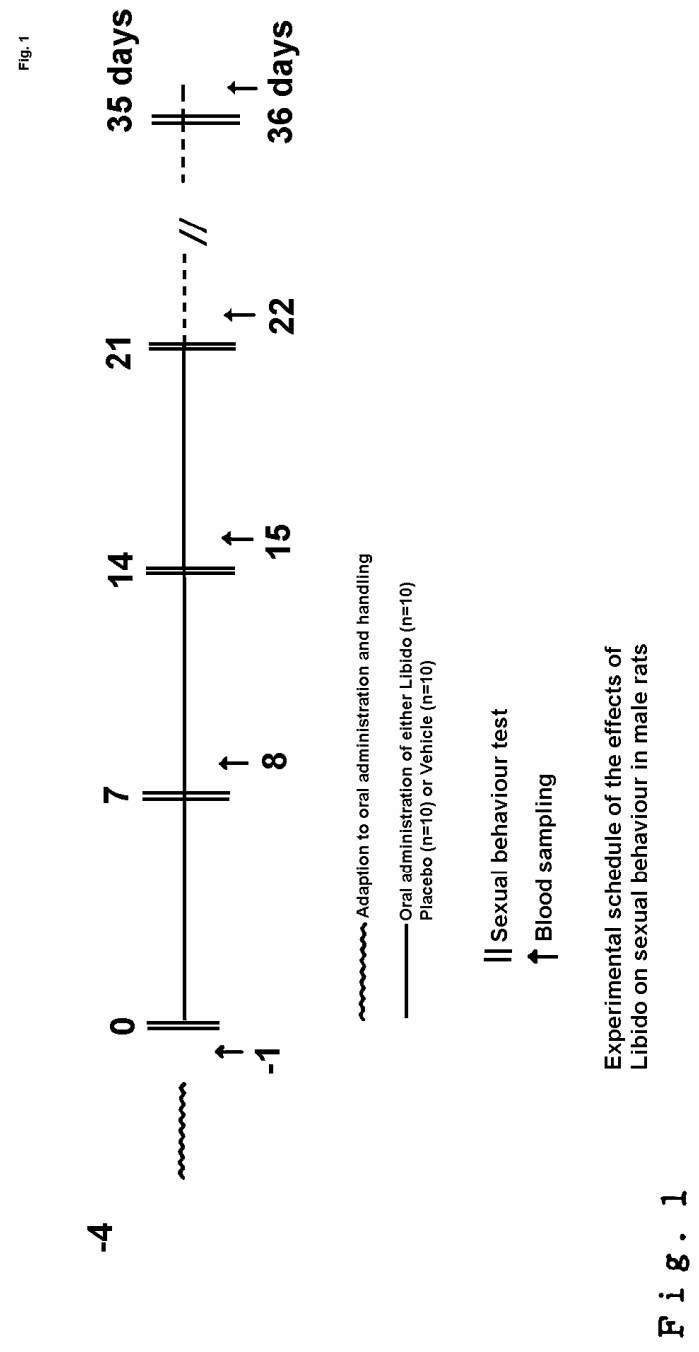
The present study showed that the rate increase in mounting frequency was the greatest in Libido-treated group at 2 and 3 weeks of treatment. This result indicates that incubated fertilized shell eggs (designated here “Libido”) contain some active principles, which can enhance sexual activity in male rats. Intromission and ejaculation frequencies increased more rapidly during 2-3 weeks in Libido-treated group than other groups, indicating also that Libido stimulates sexual activity of male rats toward female counterparts.

Reflexive erection frequency on a few parameters of erection was measured by the method of Sachs et al. (1994) and Dr. K. Yamanouchi of Waseda University (personal communication and his kind guidance). Because the reflexive erection has been regarded as a reliable index of sexual desire or libido, we had expected that we might observe stimulatory effects of “Libido” in the present study. However, this effect was not statistically verified.

Testosterone levels were not increased by treatment with Libido nor by placebo. When the data of testosterone concentrations were expressed by percentage of the initial levels, both Libido and placebo appeared to stimulate testosterone secretion, but the effect was only a tendency.

The effect of “LIBIDO” on rats may be different from that on human (enhancement of testosterone secretion).

To conclude, the stimulatory effects of “Libido” on male rat sexual behavior observed in the present study may not be through the stimulatory effects on pituitary-gonadal system. Some other sites of action should be considered.



Experimental schedule of the effects of Libido on sexual behaviour in male rats.

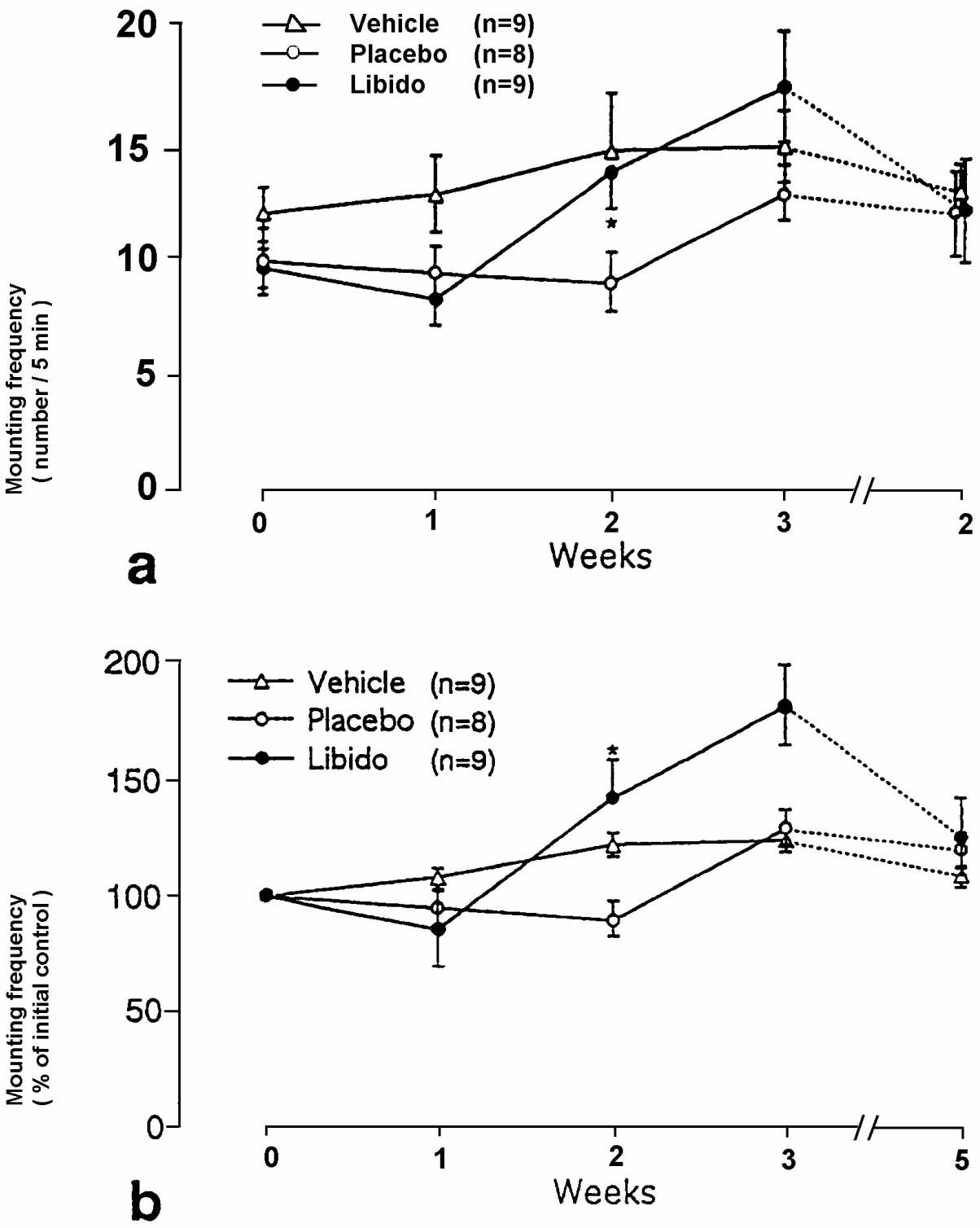


Fig. 2. Effect of Libido on mounting frequency.
A number per 5 minutes, B % of initial control.
……. no treatment during 3-5 weeks.
* p<0,05 (compared with placebo, t-test).

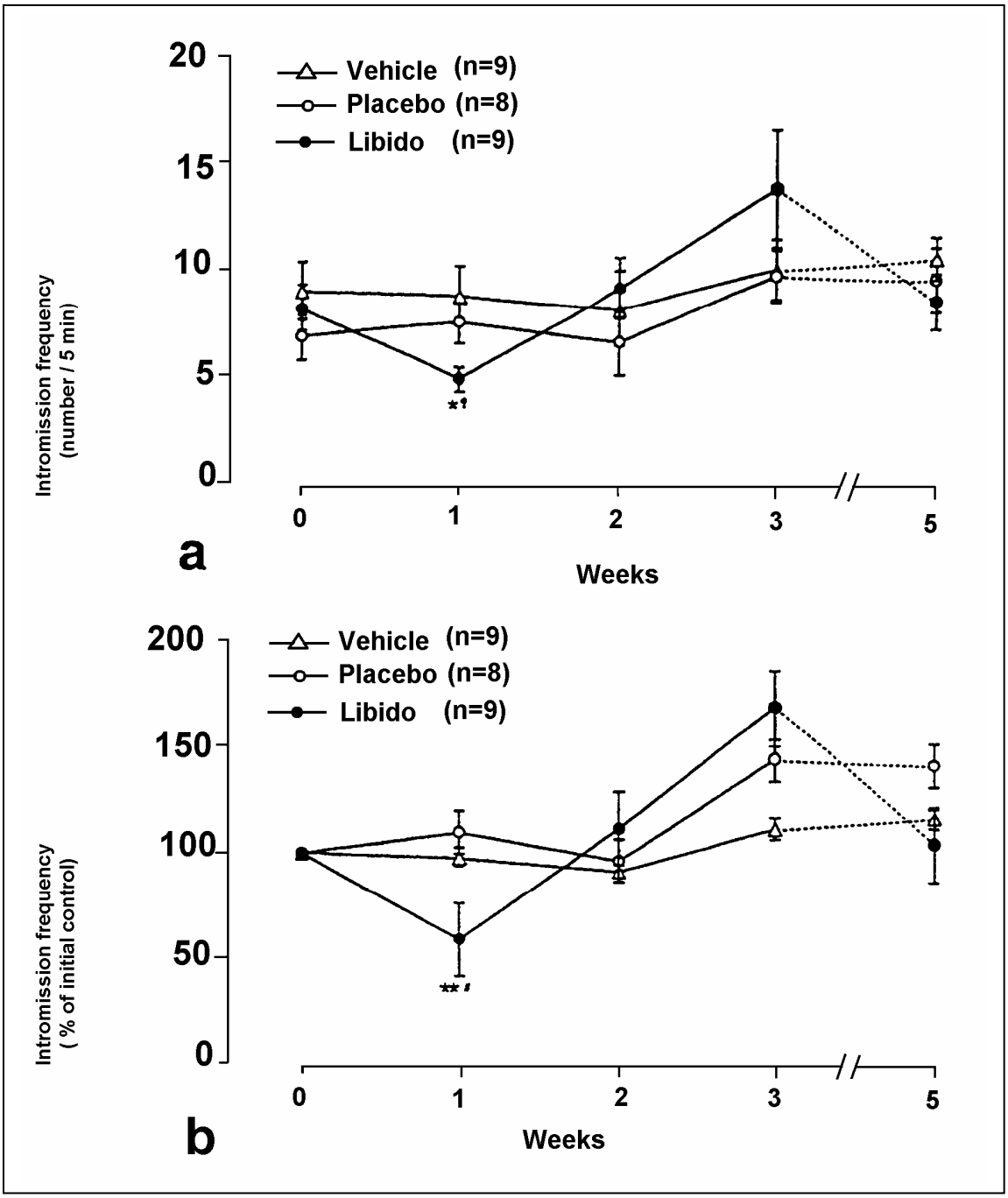


Fig.3. Effect of Libido on intromission frequency.
A) number per 5 minutes, B) % of initial control.
……. no treatment during 3-5 weeks.

* p<0,05 (compared with placebo, t-test).
** p<0,01 (compared with placebo, t-test).
Ŧ p<0,05 (compared with vehicle ,U-test).
\# p<0,05 (compared with vehicle, t-test).

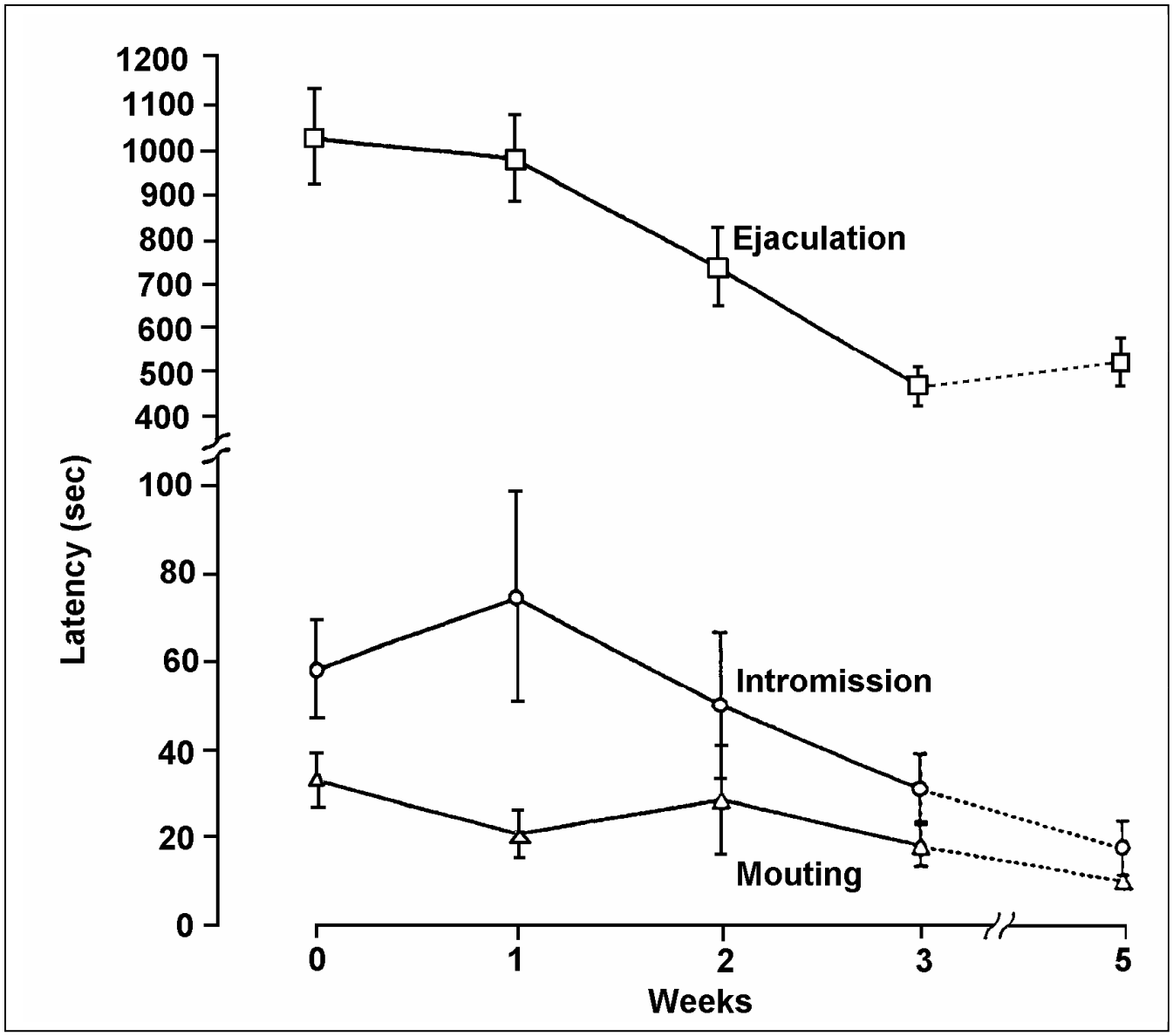


Fig 4

A) number per 5 minutes, B) % of initial control.

…… no treatment during 3-5 weeks

* p<0,05 (compared with placebo, t-test).

p<0,01 (compared with vehicle, t-test).

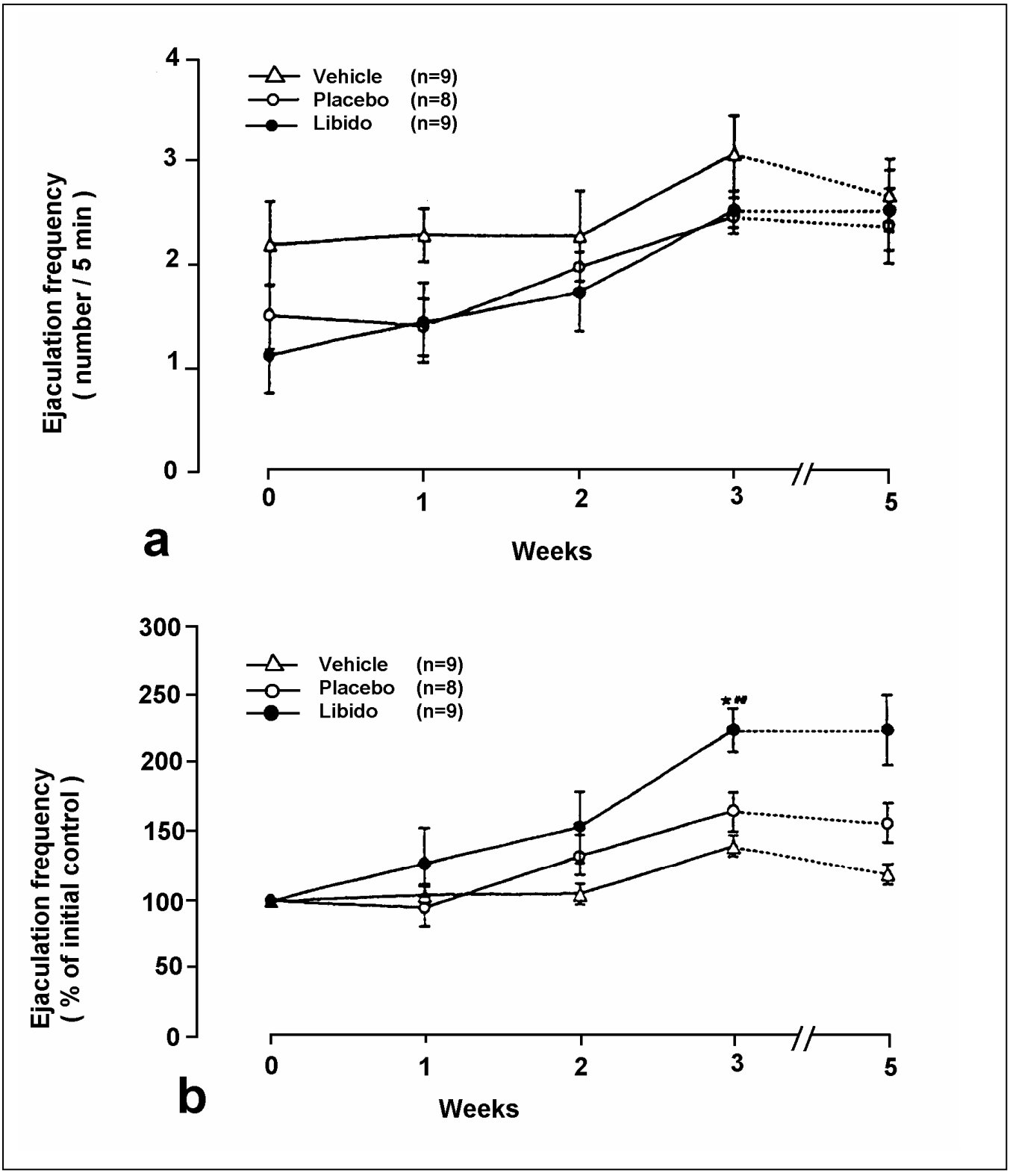


Fig 5

Mounting, intromission and ejaculation latencies
Whole groups combined (n=26).
Treatment with Libido, placebo or vehicle during 0-3 weeks.
…… No treatment during 3-5 weeks.

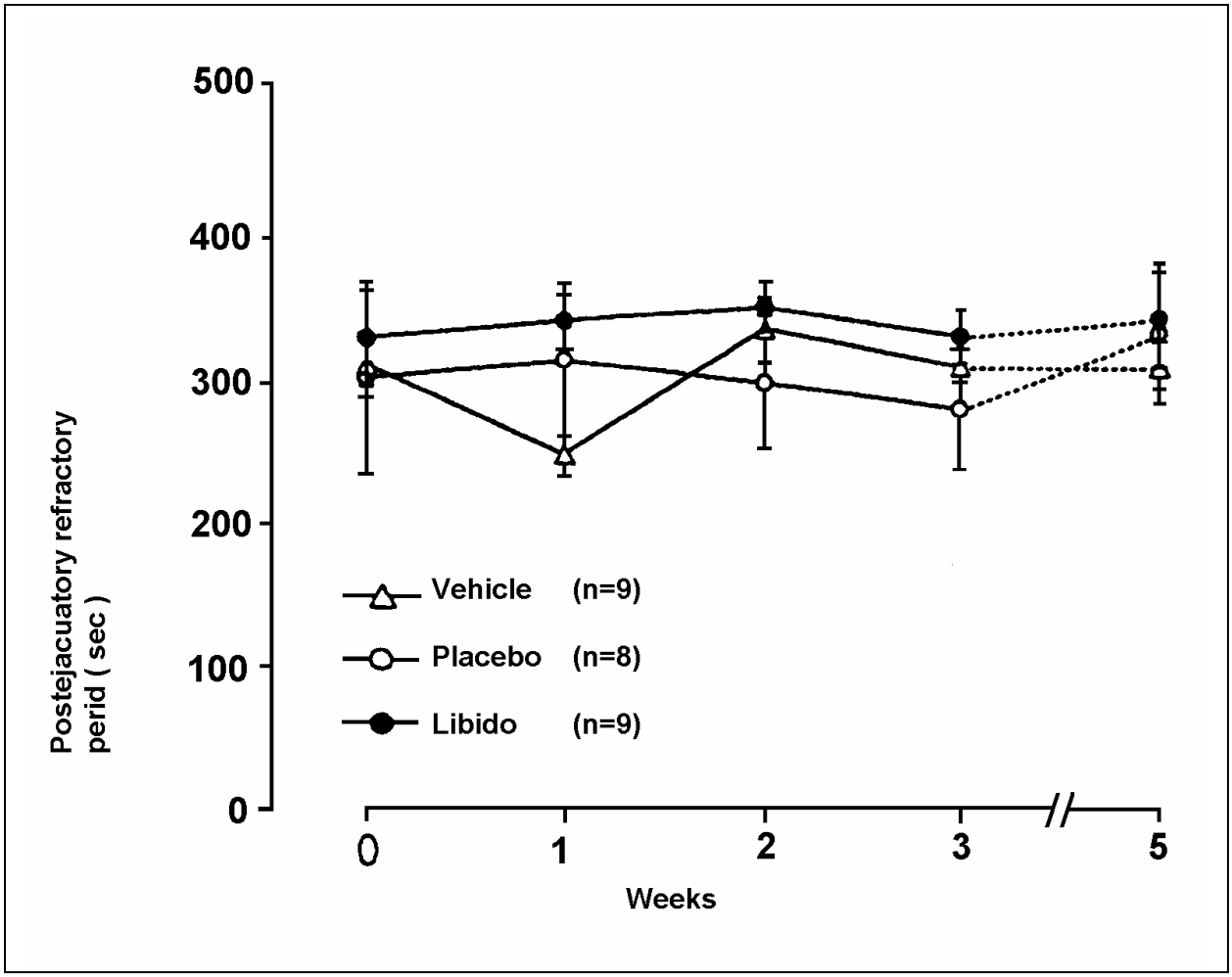


Fig 6

Effect of Libido on post ejaculatory refractory period.
…… No treatment during 3-5 weeks.

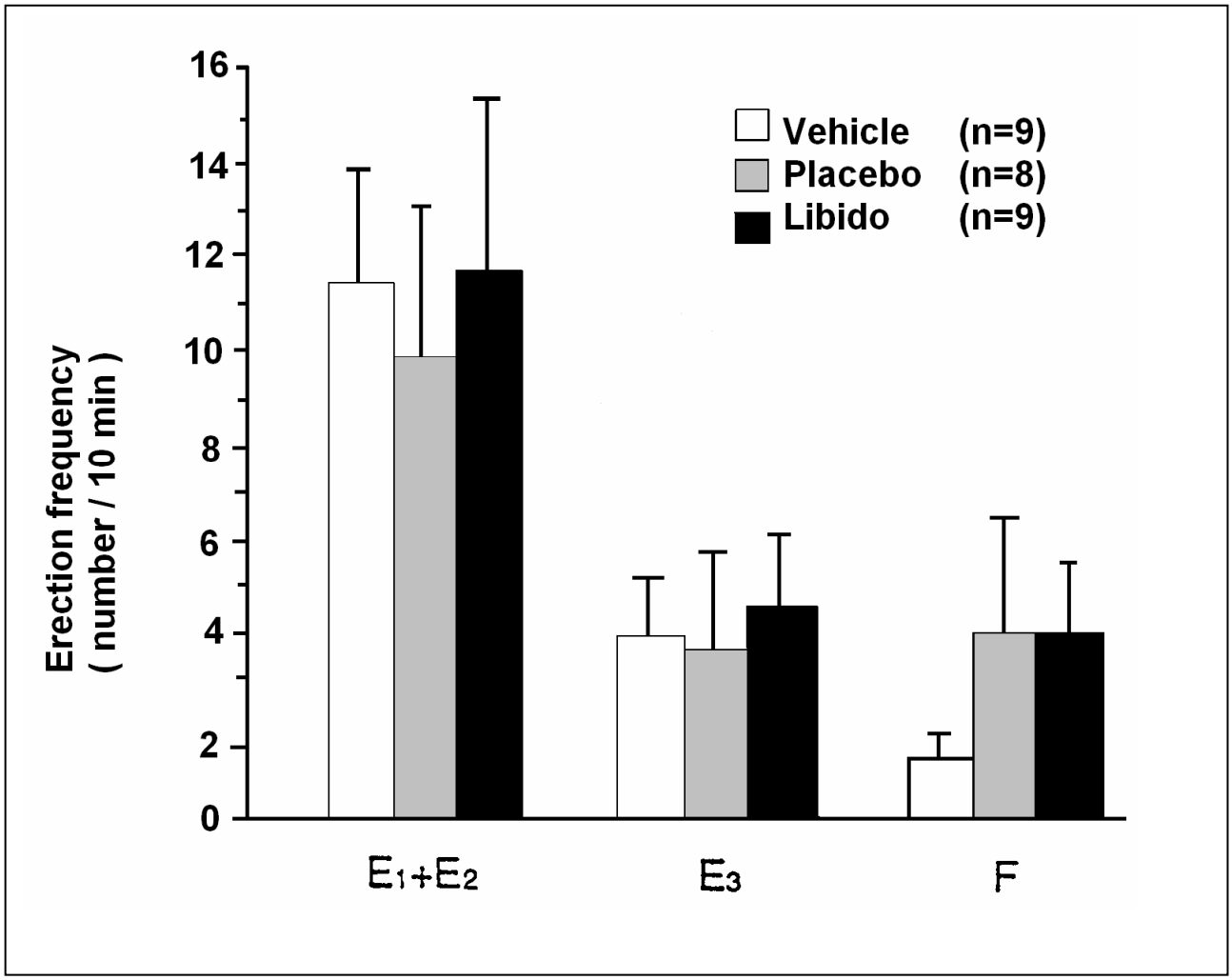


Fig 7

Effect of Libido on reflexive erection frequency after 3 weeks of treatment.

E1: Congestion of penis only, E2: Congestion and swelling of penis, E3: glans erection, F: Flip (penile body erection).

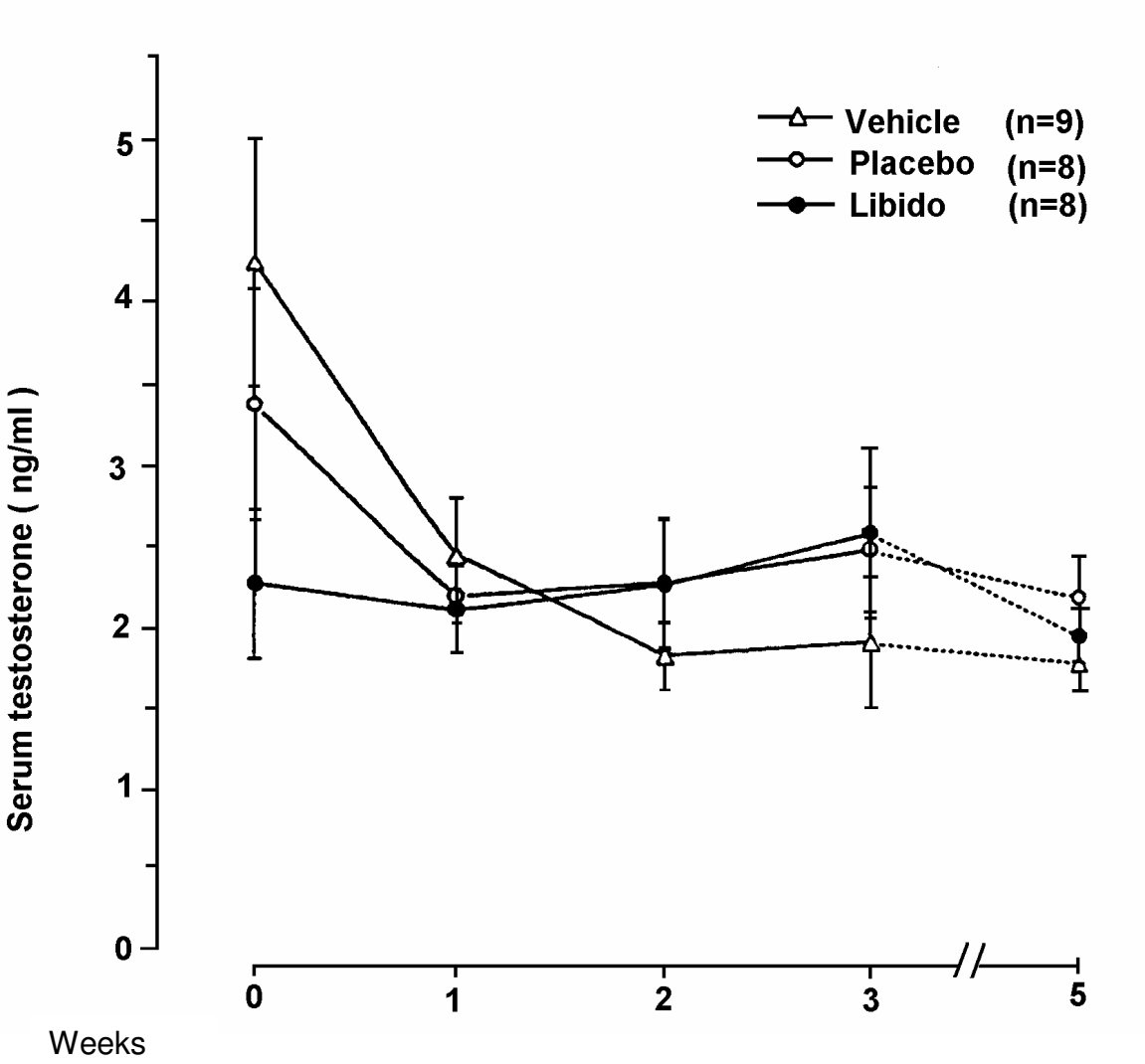


Fig 8

Effect of Libido on serum testosterone concentration.

Treatment with Libido, placebo or vehicle during 0.3 weeks.

……. No treatment during 3-5 weeks.

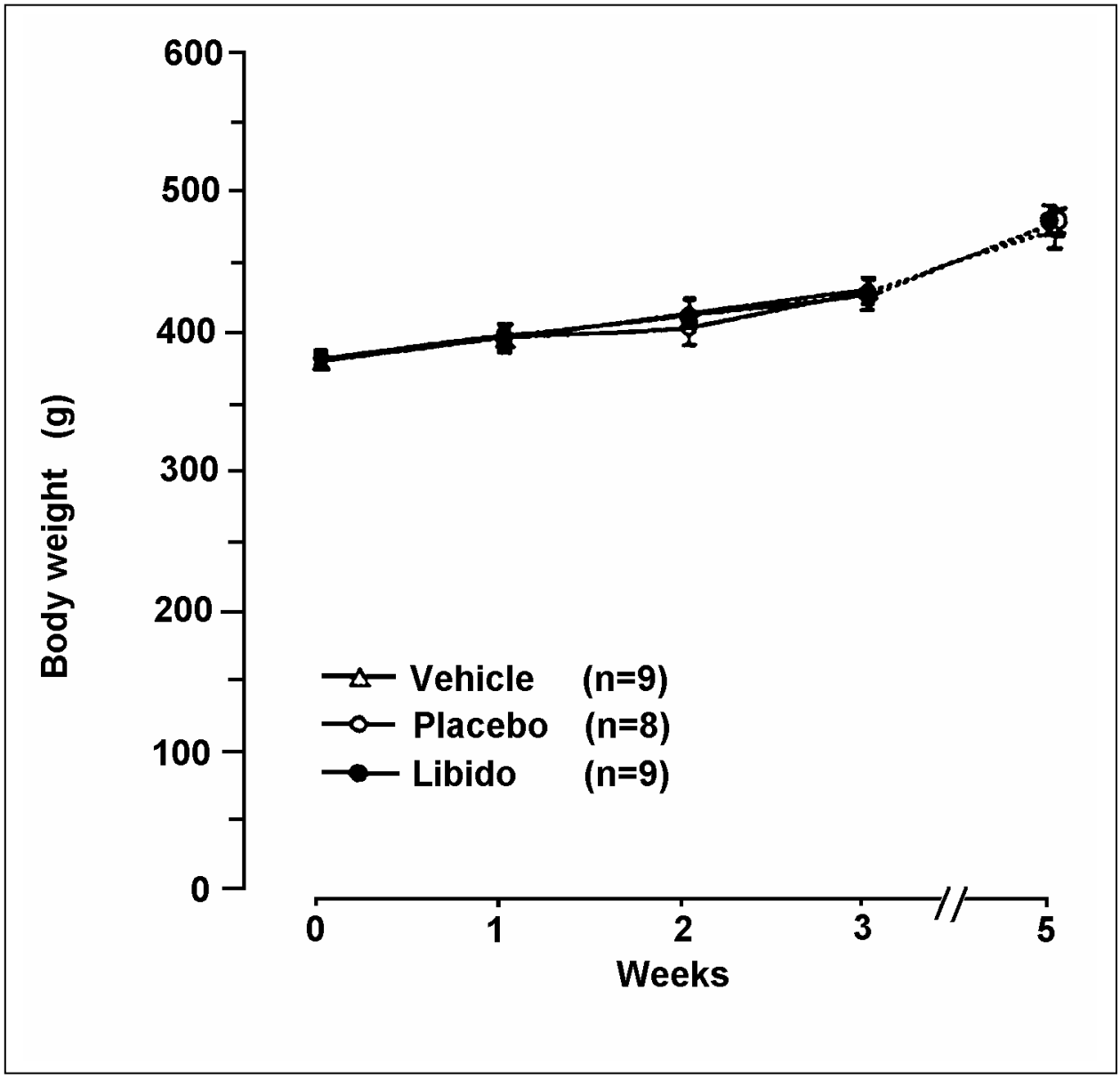


Fig 9

Changes of body weight.

Treatment with Libido, placebo or vehicle during 0.3 weeks.

……. No treatment during 3-5 weeks.

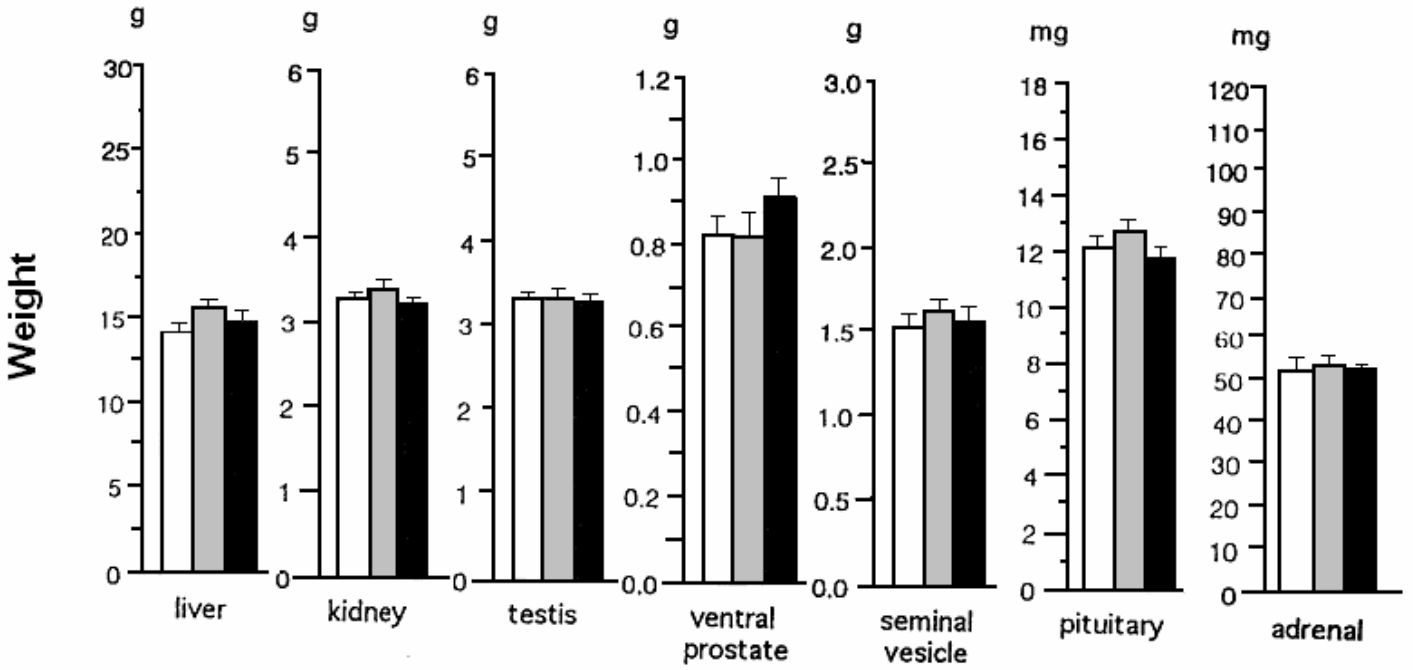


Fig. 10. Weights of some organs in rats 2 weeks after cessation of oral administration of Libido, placebo or vehicle for 3 weeks

□ Vehicle (n=9), ▒ Placebo (n=8), ■ Libido (n=9).