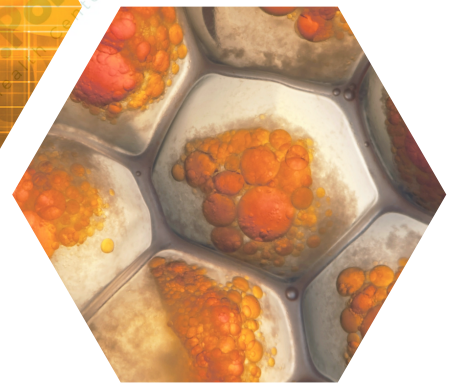


# 15

# RESEARCH PROPOSAL

## 1963 - NOW

www.pollitin.com



*No.1 pollen extract Global brand*

"Research is the key to unlocking new knowledge and advancing our understanding of the world."

## STEM CELL SUPPLEMENTS

Pollitin is a high quality natural extract. extracted from rye pollen under the production and research with technology The same standard as the production of drugs according to the requirements of the World Health Organization. therefore has been registered as "NUTRACEUTICAL" or "nutritional therapeutic nutrition" receiving the ORAC standard or the antioxidant concentration and the CAP-e Test or the ability to be absorbed into red blood cells at a very high level

The body receives almost 100% of the nutrients that are extracted from rye grass pollen. Sold to more than 50 countries on 6 continents around the world for more than 50 years, Swedish researchers have found that research studies. extracted from rye pollen contains Substances that are essential for the creation of new life in the plant family and are fundamental in the food chain. It is a natural anabolic steroid.

It has been proven by scientific laboratories that Contains a variety of nutrients including vitamins, minerals, phytosterols, carotenoids, flavonoids, nucleic acids, amino acids, substances necessary for the synthesis of RNA and DNA, antioxidant activity, enzymes, saturated fatty acids, precursors in the synthesis of prostaglandins.

So extracted from rye pollen Therefore, it is the ideal food for use in helping to make the body healthy and perfect holistic. Because there are nutrients that help to relieve fatigue, have antioxidants. The main culprit that causes many serious diseases to humans, contains important substances such as phytosterols that help boost immunity. keep the body healthy until able to cope with various illnesses caused by facing pollution and germs on a daily basis more effectively

## IN SCIENCE WE TRUST



### CELL REPAIRING

Research has confirmed that there are more than 300 types of nutrients, vitamins, minerals that are essential for the care of the body and cells.



### NUTRASCEUTICAL

Contains important substances that have antioxidant properties. Thus helping to slow down aging and help your skin look better.



### BODY IMMUNE DEFENCE

Research reports on efficacy that helps to inhibit prostatitis caused by hormones



### PHARMACEUTICAL FOOD

Contains nucleic acids and other important substances that stimulates the body to create interferon to stimulate white blood cells to work more efficiently better deal with germs

## GUARANTEED WORLD-CLASS PRODUCTION STANDARDS



## POLLITIN - EXCLUSIVE STEM CELL SUPPLEMENTS

Our premium natural extracts originate from meticulously selected flower pollen found in "Rye." These extracts undergo a unique proprietary production process crafted by Graminex L.L.C. in Ohio, United States. This exclusive process encompasses every stage, from cultivation and harvesting to the creation of high-quality natural extracts, specifically G60 and G63, derived from GBX flower pollen particles. Graminex holds the sole rights to this process and maintains adherence to strict pharmaceutical production standards in alignment with the World Health Organization's requirements.

Our extracts are renowned for their world-class production standards, boasting ORAC certification for exceptionally high antioxidant concentration and CAP-e Test accreditation, which signifies outstanding absorption into red blood cells. Over more than five decades, we have consistently refined and improved our product's efficacy.

Registered as a "NUTRACEUTICAL" or "nutritional therapy," Pollitin addresses issues at the cellular level, offering antibacterial properties and reinforcing immunity. By delivering essential nutrients tailored to various bodily systems, it equips the body to effectively combat abnormal cells. Our dedication to research is exemplified by over 150 certifications from medical and pharmaceutical institutions.

Moreover, Pollitin is not only a national achievement but a global triumph, available in over 50 countries. Our exclusive patented production process sets us apart as the sole producer of this unique formulation globally, rendering it impossible for anyone else to replicate our success in extracting and utilizing these flower pollen particles.

Pollitin - สารอาหารบำบัดเซลล์

สารสกัดธรรมชาติคุณภาพสูง สกัดจากเกสรดอกไม้ จาก "ข้าวไรย์" ที่มีสูตรลับเฉพาะของ บริษัท (Graminex L.L.C.) ที่รัฐโอไฮโอ ประเทศ สหรัฐอเมริกา ในการปลูก เก็บ และผลิตสกัด ธรรมชาติคุณภาพสูง G60, G63 จากอณูละอองเกสร ดอกไม้ GBX, Graminex® เอกสิทธิ์เฉพาะของบริษัท Graminex เท่านั้นที่ผลิตได้เพียงเจ้าเดียวในโลก อยู่ ภายใต้การควบคุมมาตรฐานการผลิตยา ตามข้อกำหนดขององค์การอนามัยโลก

จนเราได้รับการรับรองมาตรฐานการผลิตระดับโลก ระดับเดียวกับการผลิตยาเพราะ Pollitin ได้รับการทดสอบค่า ORAC หรือ ค่าระดับความเข้มข้นของ สารต้านอนุมูลอิสระที่สูงมาก และ CAP-e Test หรือ ค่าความสามารถในการดูดซึมเข้าสู่เม็ดเลือดแดงใน ระดับที่สูงจนได้รับ

การขึ้นทะเบียนเป็น "NUTRACEUTICAL" หรือ "โภชนเภสัช สารอาหารบำบัดระดับเซลล์" ที่สามารถ แก้ไขปัญหาฟื้นฟูได้ลึกถึงระดับเซลล์ มีฤทธิ์ฆ่าเชื้อ แบคทีเรีย และมีผลเสริมสร้างภูมิคุ้มกันต้านทานเมื่อเซลล์ ต่างๆ ได้รับสารอาหารที่เหมาะสมตามระบบต่างๆ ใน ร่างกาย ส่งผลให้ร่างกายสามารถต่อสู้กับ เซลล์ที่ผิดปกติภายในร่างกายได้ถึง 95% และยังคงได้รับรอง มาตรฐานการผลิตและประสิทธิภาพจากองค์กรต่างๆ มากมายระดับโลก รวมไปถึงยังได้รับรางวัลการันตีอีก มากมายจาก เอกสิทธิ์สูตรลับพิเศษเฉพาะของ Graminex ทำให้สินค้ามีคุณภาพและเกิดผลลัพธ์ที่ดี และน่าเชื่อถือ จนได้รับการยอมรับระดับสากลอีกด้วย

ตลอดระยะเวลากว่า 50 ปี เราได้มีการวิจัยพัฒนา ประสิทธิภาพอย่างต่อเนื่อง มีการวิจัยจากสถาบัน การแพทย์และเภสัชกรรมรับรองมากกว่า 150 การวิจัย เรามีความภูมิใจอย่างมากในการเป็นผู้ผลิต หนึ่งเดียวของโลกที่ได้ครอบครอง ถ้อยสิทธิ์ เอกสิทธิ์กระบวนการผลิตและสูตรเฉพาะ G60 และ G63 จากละอองเกสรดอกไม้ชนิด GBX ที่ไม่มีใคร สามารถทำได้ ส่งผลให้ Pollitin เป็นที่ยอมรับจากคน จำนวนมากใน 6 ทวีป 50 ประเทศทั่วโลก และได้รับผล ตอบรับที่ดีจากผู้บริโภคในการซื้อซ้ำสินค้าอย่างต่อเนื่อง มากกว่า 50 ปี

"Happy MPM: The exclusive importer and distributor of Pollitin in Thailand, Laos, Vietnam, Myanmar, and Malaysia for over two decades. our commitment to unparalleled reliability has touched the lives of over one billion consumers worldwide."



# TOPPIC

## Contents

- 1. สารสกัดจากเกสรดอกไม้ CERNITIN GBX VS CERNITIN T60
- 2. งานวิจัยเกี่ยวกับเกสรดอกไม้ต่อโรคมะเร็ง
- 3. งานวิจัยเรื่องโรคหัวใจ
- 4. งานวิจัยเกี่ยวกับโรคเบาหวาน
- 5. งานวิจัยเกี่ยวเรื่องพืชสุราเรื้อรัง
- 6. งานวิจัยเกี่ยวกับภาวะโรคอ้วน
- 7. งานวิจัยเกี่ยวกับโรคตับ
- 8. งานวิจัยเกี่ยวกับโรคที่เกิดจากเชื้อไวรัสต่างๆ
- 9. งานวิจัยเกี่ยวกับการสืบพันธุ์
- 10. ผลการวิจัยเกี่ยวกับความผิดปกติของหญิงวัยหมดประจำเดือน
- 11. งานวิจัยเกี่ยวกับโรคภูมิแพ้
- 12. งานวิจัยเกี่ยวกับเกสรดอกไม้และผลกระทบบอื่นๆ
- 13. งานวิจัยเกี่ยวกับเกสรดอกไม้และผลกระทบท่อภูมิคุ้มกัน
- 14. งานวิจัยเกี่ยวกับเกสรดอกไม้และผลต่อตับ
- 15. งานวิจัยเกี่ยวกับเกสรดอกไม้และผลต่อการปรับตัวของกล้ามเนื้อ
- 16. งานวิจัยเกี่ยวกับเกสรดอกไม้และ Saw Palmetto
- 17. งานวิจัยเกี่ยวกับเกสรดอกไม้และผลกระทบท่อมลูกหมาก
- 18. งานวิจัยเกี่ยวกับกระเพาะปัสสาวะ
- 19. งานวิจัยเกี่ยวกับการต้านอนุมูลอิสระ
- 20. งานวิจัยเกี่ยวกับกล้ามเนื้อและข้อต่อ
- 21. การวิจัยเกี่ยวกับหลอดเลือดและไขมัน



# 15

## งานวิจัย

เกสรดอกไม้และ  
ผลต่อการปรับตัว  
ของกล้ามเนื้อ

[www.pollitin.com](http://www.pollitin.com)



### Metabolic Adaptation of Muscles to Exercise, Vibration and Raised Temperature under the Influence of Cernitin™

Teresa J. Sawicka, Piotr I, Laszczyca, Bogdan Smylla and Zbigniew Jethon

Chair of Human Physiology and Ergonomics, Faculty of Biology, Silesian University, Katowice

Sawicka, T. J., Laszczyca, P., Smylla, B. and Jethon, Z.: Metabolic adaptation of muscles to exercise, vibration and raised temperature under the influence of Cernitin™. *Acta physiol. pol.*, 1984, 35(2), 141-150. Wistar rats were used to study the effects of Cernitin™, i.e. aqueous and oil extracts of pollens, on the metabolic adaptation of the soleus muscle to exercise, vibration and raised ambient temperature. The animals were exposed to selected combinations of these factors for 5 days during 1.5 hour daily. A part of the animals was given orally Cernitin™ in daily doses of 6 mg/mg of body weight for 10 days before the exposure. Among the adaptation changes studied in the soleus muscle, 24 hours after the last exposure, Cernitin™ caused: 1) a reduction of the amount of total protein, 2) an increase in the proportion of soluble proteins in the protein fraction, 3) an increase in the tissue oxygen consumption, 4) an increase of already elevated pyruvate kinase activity, 5) a further rise in ATP level, 6) an increase in lactic dehydrogenase activity, 7) a rise in the activity of cholinesterases. Moreover, it increased significantly the body weight and the weight of the studied soleus muscle. Cernitin™, in combination with certain types of exposure used in this experiment, exerted a catabolic action, increased the rate of anaerobic metabolism and enhanced adaptation to exercise, vibration and temperature. The direction of the adaptation changes depended on the type of exposure to environmental factors.

The adaptation of human organism to the living and working environment becomes often difficult, due to the influence of new noxious environmental factors. This is true particularly of extreme conditions, which exist in certain types of work places. The means used for facilitating the adaptation of man – operator are not always sufficient for reducing the intensity of the harmful effects of the environment, nevertheless they enhance the ability of carrying out work.

One of the factors enhancing adaptation processes are Cernitin™, components of extracts obtained from pollens of flowers. Their favourable effect has been demonstrated on the rate of weight gain [23], course of adaptation to increased ambient temperature [5], resistance to

ambient temperature antagonizes in this case the effects of vibration [9, 22].

The aim of the present study was to investigate the possibility of using Cernitin™ as an agent

infections [25], and alleviation of the intensity of inflammatory processes [20, 25]. Moreover Cernitin™ have been shown to exert a favourable effect on the capability of performing exercise [1, 11] and the magnitude of training effect [1]. Cernitin™ influence also favourably the well-being and the ability to carry out intellectual and operational work [5].

Exposure to a combination of high ambient temperature and vibration is not infrequent during occupational work. Physical effort of the worker may increase the harmful effects of these factors leading to a rapid decrease of his ability to work. A synergistic action of vibration and physical activity leading to development of vibration-induced lesions is known [9]. Raised

increasing the adaptation effect, that is maintenance of the ability to work under conditions of high ambient temperature and exposure to vibration. The studied problems were

limited to the metabolic effects of Cernitin™ in the light of the data known as yet on the mechanism of action of these substances, especially on protein and steroid metabolism [2, 25].

The effects of Cernitin™ were investigated on certain aspects of the exercise metabolism in rats subjected to swimming exercise, sinusoidal vibration and raised ambient temperature.

## Materials and Methods

The experiments were carried out on 48 male Wistar rats weighing 190-240 g. The animals were divided into 8 groups in the following way:

Cernilton® was given for 10 days (the rats in groups KC, PC, PWC, PWTC) orally in daily doses of 2 ml. This dose was equivalent to 6

mg/kg of body weight of Cernitin™ T60 (the water-soluble fraction) and 0.3 mg/kg of body weight of Cernitin™ GBX (the lipid-soluble

- K — control, sedentary group,
- KC — sedentary group receiving Cernilton®,
- P — group subjected to swimming until exhaustion,
- PC — group subjected to swimming and receiving Cernilton®,
- PW — group exposed to swimming effort and sinusoidal vibration,
- PWC — group treated as PW and receiving Cernilton®,
- PWT — group exposed to swimming effort, sinusoidal vibration and high ambient temperature of 37°C during exposure to vibration,
- PWTC — group exposed as the PWT group after pretreatment with Cernilton®.

fraction). In accordance with the declaration of the producer this dose corresponded after calculation for 1 kg of body weight daily to: 0.36-0.55 mg of free amino acids, about 0.2 mg of a mixture of various lipids, among them sterols 0.030 to 0.048 mg. The amount of vitamins received by the animals with Cernilton® was of the order of 10<sup>8</sup> ng for thiamin, riboflavin, pyridoxamine, pantothenic acid, folic acid and

inositol, 10<sup>8</sup> ng for niacin, 10<sup>4</sup> ng for ascorbic acid, 10<sup>0</sup> ng for tocopherol, 10<sup>0</sup> mIU for calciferol. Besides that, this dose contained carotens, 10<sup>1</sup> ng, xantophils 10<sup>8</sup> ng, and mineral components such as Ca, K, P, Cl, Na, Mg, Al, Fe, Si, Zn, Mn, Cu in amounts found in vegetable tissues ranging from 50 ng to 60 ug. The preparation used was Cernilton® produced by AB Cernelle, Vegeholm 6250, S 2620 Engelholm, Sweden. The rats in groups K, P, PW, PWT received during the same time 2 ml of a 0.9% NaCl solution orally, as placebo.

On the first day after completion of Cernilton® or placebo administration the animals were subjected to swimming effort, vibration and raised ambient temperature for 5 days.

The animals swam without load until exhaustion in water at 32°C, in tanks making impossible passive floating on water surface. Sinusoidal vibration was applied during 1.5 hour daily using a vibration table steered by a vibration generator, at a mean acceleration of 1.11 m/sec<sup>2</sup> and 4 Hz frequency [8].

During the exposure to vibration the animals in groups PW and PWC remained in an ambient temperature of 19-20°C, and the animals in groups PWT and PWTC were exposed to a temperature of 37°C. Twenty-four hours after the last exposure the animals were killed by decapitation. The soleus muscle was taken from the hindpaws. Muscle fragments were homogenized at 0°C in a proportion of 40 mg of muscle tissue for 1 ml of 0.9% NaCl solution. The following determinations were carried out in the homogenate:

- 1) concentrations of total protein and protein soluble in isotonic saline by the method of Lowry [21],
- 2) ATP content in the muscle using Eskalab test kit, (in HClO<sub>4</sub> homogenate),
- 3) pyruvate kinase (PK) activity using Eskalab test kit,

4) lactic dehydrogenase activity (LDH) using Eskalab LDH-UV kit,

5) muscle acetylcholinesterase activity (AChE) by Hestrin's method using acetylcholine as substrate [10].

A part of the obtained muscle mass was homogenized in Tyrode's solution until a final proportion of 100 mg of tissue per 1 ml of the solution for determining the intensity of tissue respiration by Warburg's method.

The results were subjected to statistical analysis by the generally accepted methods. The calculated results included the arithmetical mean and standard deviation. The differences were accepted as significant at  $p < 0.05$ .

## Results

K) during exercise and exercise combined with vibration (groups P and PW) by 19.2% and 9.3% respectively, while combined exposure to exercise, vibration and high ambient temperature decreased the rate of weight gain by 14.2%. The ratio of the soleus muscle weight to the total body weight decreased significantly as compared with controls: by 9.2% in the group subjected to exercise (P) and by 4.2% in the group subjected to exercise and vibration (PW). The effect of combined exercise, vibration and high ambient temperature appeared as a decrease of the content of total protein in the soleus muscle which was significant only in group P decreasing by about 17% in relation to controls. The concentration of soluble protein in the soleus muscle decreased significantly in all groups in relation to controls: P – 17.9%, PW – 25.0%, PWT – 23.1%. The ratio of the content of soluble protein to that of total protein decreased significantly by 17.5% only in group PW.

**Table 1.** Metabolic adaptation of soleus muscle to exercise, vibration and high ambient temperature after treatment with Cernitin™.

		K	KC	P	PC	PW	PWC	PWT	PWTC
AM	g/24h	1.40	2.36	1.67	1.68	1.53	1.52	1.20	1.80
M <sub>m</sub>	mg/g b.m.	0.418 0.024	0.375 0.017	0.380 0.021	0.397 0.007	0.400 0.012	0.393 0.025	0.441 0.019	0.401 0.027
PrT	mg/g t.	101.0 11.7	73.6 14.6	83.6 10.5	72.3 14.2	91.9 15.4	56.8 4.1	79.3 34.3	69.0 12.0
PrS	mg/g t.	15.6 2.4	15.2 1.4	12.8 0.9	14.3 2.2	11.7 2.0	13.8 2.2	12.0 1.7	24.2 4.8
VO <sub>2</sub>	Nmol O <sub>2</sub>	19.7	34.2	40.2	33.2	25.6	64.1	54.6	41.8
	min mg prot.	5.9	12.3	9.3	20.9	10.1	12.1	25.5	15.4
ATP	mg/g prot.	16.8 3.3	39.4 7.2	17.3 4.3	22.7 14.6	45.9 30.6	47.4 6.9	32.7 25.4	35.2 10.4
LDH	IU/g prot.	438 78	777 125	444 49	743 156	437 74	1346 146	496 235	1005 162
PK	IU/ g Prot.	6.6 0.6	13.8 0.8	16.8 0.4	14.2 0.6	9.6 0.4	20.7 0.3	10.1 0.5	15.7 0.5
ChE	IU/ mg prot.	21.53 1.69	23.54 2.24	21.03 2.06	23.52 1.99	20.84 2.44	25.03 1.42	19.65 1.90	24.93 2.65

Designations: K – control group, P – swimming, T – high temperature, C – Cernitin™ administration, AM – weight gain, m<sub>m</sub> – proportion of muscle mass to body mass, PrT and PrS – total and soluble protein levels in the muscle, ATP – ATP level, LDH, PK, ChE – activity of: lactic dehydrogenase, pyruvate kinase and cholinesterase

The rate of weight gain in the rats not receiving Cernilton® was higher than in the controls (group

The activity of tissue metabolism expressed as oxygen consumption by tissue homogenate



showed an increasing tendency during the exposure to these external factors by 104.5% in group P and 179.0% in group PWT ( $p < 0.01$ ). The increase of kinase activity in the studied muscle was significant in all groups in relation to controls and it was 154.5% in group P, 45.5% in group PW and 53.1% in group PWT. Attention is called to the agreement between the directions of changes in tissue respiratory activity and pyruvate kinase activity. The ATP content of the muscle calculated for one unit of total protein was 173% above the control level in group PW ( $p < 0.05$ ). In the remaining groups the rise was statistically not significant. The activity of LDH and ChE showed no significant changes in the groups of rats not subjected to treatment with Cernitin™, independently of the action of other factors.

A particularly evident effect of Cernitin™ on the weight gain rate was observed in the group of sedentary rats (KC) as compared with group K (a 68.2% increase) and in group PWTC as compared with PWT group (a 50.0% increase). In the remaining groups changes in relation to the control group and between the corresponding groups were below 20%. The ratio of the soleus muscle weight to the total body weight was not significantly changed. The only exceptions were: a decrease of this index by 9.4% in group KC as compared with group K, and a decrease by 9.1% in group PWTC as compared with PWT.

The concentration of total protein in the soleus muscle was decreased by Cernitin™ in all groups amounting in the case of group pairs to the following values: KC/K – 27.2%, PC/P – 13.5%, PWC/PW – 38.2% and PWTC/PWT – 13%.

A reverse relationship is observed in the concentration of soluble protein since Cernitin™ raised this concentration. However, only a rise by 100% between PWTC/PWT groups was significant. This increased the proportion of enzymatic soluble proteins in the total protein pool by 34.3% for KC/K, 29.4% for PC/P, 91% for PWC/PW and 131.0% for PWTC/PWT.

The oxygen consumption by the homogenates of muscles from the animals treated with Cernitin™ was always higher than in controls independently

of the exposure, and in the rats in groups KC and PWC Cernitin™ caused a significant increase in tissue respiration by 73.7% for KC/K and 150.0% for PWC/PW. The remaining changes were not significant.

Significant differences in pyruvate kinase activity were observed only between groups KC and K, and PWC and PW. In both these cases this increase was 115.5% and 115.4% respectively.

The ATP content showed an increasing tendency during treatment with Cernitin™, but this increase was significant only for KC/K, being 111.0%.

LDH activity increased in all groups treated with Cernitin™: 77.5% for KC/K, 67.3% for PC/P, 207.2% for PWC/PW, 102.5% for PWTC/PWT.

ChE activity increased significantly in all groups treated with Cernitin™ with the exception of the sedentary group: 9.2% for PC/P, 11.8% for PWC/PW, and 27.0% for PWTC/PWT.

## Discussion

Physical exercise, vibration and high ambient temperature produced disturbances in the protein metabolism in the soleus muscle manifesting themselves as a decrease in the amounts of total and soluble proteins and of the ratio of the soleus weight to the total body weight. These changes were associated with increased intensity of tissue respiration and pyruvate kinase activity. The rate of weight gain decreased or increased in different groups without an unequivocal correlation with the metabolism of the studied muscle. This absence of correlation might be explained as due to brief exposure time or to considerable metabolic, functional differences between various muscles and tissues. The muscle fibres of the ST type prevailing in the soleus respond differently than the FT muscle fibres prevailing in other muscles. Moreover, these responses are specific with respect to the stimulus and hormonal regulation [23, 29]. Metabolic changes observed by other authors induced by exercise [15, 26, 30], raised ambient temperature [28] and vibration [9, 22] were similar to those observed by us. The character of these changes resembled the

metabolic changes observed during stress reaction. Less data are found on the combined effect of several stress-inducing factors on the organism. The effect of exercise combined with vibration and the effect of vibration and high temperature studied by us suggested that under these conditions the reaction of the organism was changed. The most characteristic finding was the change in the equilibrium of the anabolic and catabolic processes. The change was manifested as a decrease in protein concentration and an increase of tissue respiration, as well as an increase in ATP level, which was particularly evident after exposure to vibration (PW group). Raised ambient temperature seemed to exert a protective effect on the equilibrium between catabolism and anabolism. The damaging effect of low ambient temperature on humans exposed to vibration has been reported in the literature [9, 22].

The observed absence of changes in LDH activity after the exposures used in this experiment indicates that their intensity was too small to cause mobilization of anaerobic metabolism [31, 34].

In our experiment Cernitin™ were given before exposing the rats to exercise, vibration and high ambient temperature. Thus the effects observed after Cernitin™ administration were due either to metabolic changes caused by them prior to exposure or to the action of tissue deposits of Cernitin™ or their derivatives mobilized by exposure to stress-inducing factors. The rise in the requirements for amino acids, vitamins, steroids, trace elements, and energy in animals subjected to stress-inducing exposure is known [12, 13, 14, 18, 32]. The five-day exposure to stress-inducing factors in this experiment failed probably to exhaust the stores of these substances in the organism, since the work of Karvonen [14] shows that they can cover much longer time periods.

Administration of Cernitin™ to rats caused in all groups an increase in the proportion of the soluble protein fraction and in the activity of catabolic enzymes (PK, LDH, ChE) in the soleus

muscle. Cernitin™ potentated also tissue respiration during exercise, vibration and high ambient temperature. Similarly also, a further rise of ATP was observed in the muscle. The increase of the catabolic activity induced with administration of Cernitin™ manifesting itself as a better utilization of the energy of food components [1, 6], enhanced training effect and effort tolerance has been already described in man and rats [1, 25]. Cernitin™ raise also intestinal absorption of food components [33] and in this field their action is contrary to that of vibration which decreases absorption [27]. Increased LDH activity and reduction of post-exercise blood lactate concentration following intake of Cernitin™ have been described by Jethon [13] and Dabrowski [5]. This effect was observed also in our experiment.

The effect of Cernitin™ on protein metabolism in the soleus muscle manifested itself as an increase in the proportion of the soluble fraction of cell proteins with a decrease in the total protein concentration. The suggested intensification of protein catabolism at the expense of protein anabolism, and intensification by Cernitin™ of changes induced by stressors. These results disagree with those obtained by other authors who found decreased catabolism of amino acids and proteins in humans receiving Cernitin™, with decreased loss of nitrogen in the form of urea [11, 13]. The above discussed differences in the metabolic characteristics of muscles [23, 29] and acceleration, or at least stabilization, by Cernitin™ of the weight gain of rats suggest that in muscles belonging to other metabolic type than the soleus muscle, or in other tissues Cernitin™ stimulated the anabolic processes. This supposition is supported by observations of other authors that Cernitin™ enhanced protein synthesis during healing of wounds or fractures [20, 25] and increased the rate of weight gain [6, 25, 29].

Increased activity of muscle cholinesterases observed after administration of Cernitin™ may be due to the action of these substances on the nervous system. Cernitin™ are known to increase the psychotechnical performance and

intellectual ability as well as the sense of well-being [5, 7, 20, 25]. The possibility of intensification of the tropic action of the nerves on the muscles is not ruled out and the activity of acetylcholinesterase (AChE) depends on this action [3, 4, 35]. Another possibility is increased production or change of the turnover half time of enzymatic proteins similar to the hepatic fraction of secretory enzymes, such as pseudocholinesterase. This supposition is confirmed by the parallelism between increasing ChE activity and the proportion of soluble protein fraction.

The mechanism of the action of Cernitin™ on the effects of the tested exposures depends, probably on a synergistic action of the various components of this mixture of substances [2]. This mechanism may be connected with increased availability (through better absorption) of vitamins as precursors of coenzymes and trace elements as activators. Among the literature reports attention is called to the fact that Cernitin™ raise the amount of 17-ketosteroids and 17-hydroxysteroids excreted with urine [7, 25] and cause vacuolization of the fascicular zone of the adrenal cortex [25], without concomitant signs of adrenal cortical hypertrophy [2, 6]. The possibility cannot be ruled out that 27-29-carbon steroids of Cernitin™ undergo metabolic changes in the organism. The possibility of increased production of 21-carbon corticosteroids is suggested by the investigations described by Oudot [25], and mobilization of the production of 18 and 19-carbox sex steroids is suggested by the results of the investigations of Diczfalusy [6]. Chemical analysis carried out by Kvantá [17, 19] showed a similarity between Cernitin™ steroids and certain oestrogens. Thus the changes observed in our experiment may be due to "facilitation" of the hormonal regulation considering the interaction between steroids, and differences in the sensitivity of the metabolism of various types of muscles and tissues to their action [16, 23, 29].

In summary, the administration of Cernitin™ during exposure of the organism to combined exercise-vibration-thermal stress produces multi-

directional changes not always favorable for such metabolic indices as: muscle mass, level of muscle protein or weight gain. Administration of Cernitin™ seems to be advantageous in individuals not exposed to physical work (KC rats) and subjected to thermal stress (PWTC rats) in view of the necessity of maintenance of homeostasis in protein metabolism and growth processes.

However, during exercise and vibration stress Cernitin™ increases the metabolic fitness (PC and PWC rats) which is unquestionably and advantageous effect enhancing adaptation to working environment.

The always-present Cernitin™ effect increasing LDH activity with increased aerobic metabolism suggests a greater anaerobic potential and tolerance.

## Conclusions

1. Cernilton® shows an action stimulating the cellular metabolism and when administered to rats exposed to stress it increases the intensity of catabolic processes.
2. Significantly raised LDH activity after Cernilton® suggests intensification of aerobic metabolism, and increased potential and tolerance of anaerobic metabolism.
3. Cernilton® increases also the energy stores of the phosphagen pool of the muscles at rest during exercise stress.
4. The anabolism of proteins in muscles with prevalence of ST fibers (soleus muscle) in animals exposed to stress was increased much less than the overall catabolism of protein. Metabolic changes induced by Cernilton®, particularly those of protein anabolism, are probably, quite different, in various tissues, and probably the ST muscles respond to this agent differently than FT muscles and various non-muscular tissues.
5. Cernilton® effect differed significantly during various types of stress to which the rats were exposed:
  - during vibration stress the drug caused mobilization of catabolism at the expense of anabolism,

- during exercise stress it caused no drastic changes in the anabolism-catabolism equilibrium,
  - the exposure to high ambient temperature and other stress types applied in this experiment increased anabolism above the level observed at room temperature, although catabolism activation by Cernilton® was still significant.
6. Changes in the activity of enzymes and weight gain in the rats exposed to thermal stress point to a favorable effect of Cernilton® on thermal adaptation.
  7. Changes in cholinesterase activity in the soleus muscle caused by Cernilton® indicate that its effects either the production of proteins similar to hepatic secretory fraction or the tropic action of motor neurons on the muscle and on the metabolism of muscle proteins.
  8. In the potential mechanism of Cernilton®, besides the importance of vitamin and amino acid supplements, the effect of this drug should be analyzed on steroid metabolism and the related effects on hormonal regulation which are probably of essential importance in the development of the observed changes.

## References

1. *Asplund, B.*: Proc.Med.Pharm. Symposium, Florence 29.09.1978, pp. 55-59.
2. Cernilton® AB Cernelle, Vegeholm 6250, 2-26200 Engelholm, Sweden, p. 1-16.
3. *Dahistrom, A., Heiwal, p o., BOOJ, S., Dachlof, A. G.*: Acta physiol. Scand., 1978, 103/3, 308.
4. *Davey, B., Younkin, L H., Younkin, S. G.*: J. Phys. Lond., 1979, 289, 501.
5. *Dąbrowski, J.*: Thesis for the degree of doctor of physical education. WHIE Warszawa, AWF Krakow 1980
6. *Diczfalvsi, J.*: Effect of Cernitin™ on rat physiology endocrinologic test. In: "Cernilton®" AB Cernelle, Sweden.
7. *Dubrisay, J.*: Clinical trial of the proprietary product C.P. (Amplamil). In: "Cernilton®" AB Cernelle, Sweden.
8. *Dutkiewicz, J., Jethon, Z.*: Egonomia, 1980, 3/1, 13.
9. *Grzegorzcy K., L., Walaszek, H.*: Vibration and its effect on the human organism In Polish. PZWL., Warszawa 1972.
10. *Hestrin, S. J.*: Biol. Chem., 1949, 180, 249.
11. *Jethon, Z.*: The influence of the application of Pollisport™ and Pollen Stark™ on the physical efficiency of weight lifters. In: Symp. For Sportsmen, Helsingborg 1972, AB Cernelle, Sweden.
12. *Jethon, Z.*: Sport Wyczynowy, 1976, 3-4, 13.
13. *Jethon, Z.*: Effects of the administration of minerals and vitamin additives upon the physical capacity of athletes. In: Proc. Med. Pharm. Symp., Florence. 1978, p. 52.
14. *Karvonen, J.*: Sport Wyczynowy, 1976, 3-4, 18.
15. *Kawka-Serwecinsko, E.*: Effect of certain types of exercise on amino acid levels in the blood, liver and muscle of rats. Doctoral thesis. Silesian University, Katowice 1982.
16. *Krotkiewski, H., Kral, J. G., Karlsson, J.*: Acta physiol. Scand., 1980, 109/3, 233.
17. *Kvanta, E.*, Acta chem. scand. 1968, 22, 2161.
18. *Kvanta, E.*,: Sport Wyczynowy, 1976, 3—1, 36.
19. *Kvanta, E.*,: Determination of beta steroids in Pollen tablets. In "Cernilton®" AB Cernelle, Sweden.
20. *Lindahl, O.*: Medicinal effect of pollen-based preparations. In: Proc. Med. Pharm. Symp., Florence 1978.
21. *Lowry, J. O. H., Rosenbrough, N. J., Farr, R. L., Randall, R. J.*: J. Biol Chem.. 1951. 193, 265.
22. *Mariewicz, L.*: Vibration. In Polish. IW CRZZ, Warszawa 1980.
23. *Martin, W. P.*... Avaiat. Space Environ. Med, 1980, 51/5, 473.
24. *Noyes, CH. E. Jr.*: The use of Cernitin™: pollen an extract of organic pollen, to increase body weight and to increase resistance against infections. In: Symp. For Sportsmen, Helsingborg 1972, AB Cernelle, Sweden.
25. *Oudot, P.*: Amphamil capsules. In: Symp. For Sportsmen 1972, AB Cernelle, Sweden.
26. *Poortmans, J. R.*: Sport Wyczynowy, 1976, 3-4, 104.
27. *Popow, B., Hadjieva, N.*: Eksp. Med. Morfol.(Sofia). 1977, 16/2, 85.
28. *Rakchimow, K., Aleksandrova, N. N., Demidova, A. J.*: Fizjol. Zh. ZSRR in. J. M. Sechenova, 1979, 65/7, 1024.
29. *Rannels, S. R., Jellerson, L. S.*: Amer. J. Physial.. 1980, 1/6, # 564..
30. *Sawicka, T.*: Biochemical changes in skeletal muscle during endurance: training and electrostimulation. Doctoral thesis. Warszawa 1979.
31. *Shepherd, R. B., Golnick, P.D.*: Pflvg. Arch., 1976, 362/3. 219.
32. *Szejtwanow, P.*: Sport Wyczynowy, 1976, 3-4, 124.
33. *Syrmanski, A.*: Cernilton® effect on intestinal absorption of amino acids (unpublished). WHIE, Warszawa 1980.
34. *Wojciechowska, F.*: Role of lactic dehydrogenase (LDH) of skeletal muscles and liver in the processes of adaptation to exercise. Doctoral thesis. Medical Academy, Poznan 1971.

35. Wooten, G. F., Cheng, C.H.: *J. Neurochem*, 1980, 3-4/2, 359.

Dr. Pollitincells  
Health Center

Dr. Pollitincells  
Health Center

Dr. Pollitincells  
Health Center

Dr. Pollitincells  
Health Center

Dr. Pollitincells  
Health Center

Dr. Pollitincells  
Health Center

Dr. Pollitincells  
Health Center

Dr. Pollitincells  
Health Center



### Physical performance by weightlifters after consumption of nutritive preparations

Jethon, Z., Luczak-Szcurek, A. & Put, A.

Institute of Hygiene and Epidemiology, Warsaw, Poland

In order to increase performance and maintain health, it is common for sportsmen to consume various nutritive preparations containing, for example, vitamins, amino acids, proteins and minerals, particularly iron. It is often considered that the intense physical activity of sportsmen requires an increased intake of such substances, compared with the requirements of those who lead physically less active lives. Systematic evaluation of the effects of these substances has been rather rare. On the contrary, any effects have often been judged subjectively.

The purpose of this experiment is to endeavor to assess how consumption of various nutritional preparations during training affects weightlifters.

#### Test subjects and procedure

42 weight lifters (aged between 18 and 24) of good but not international standard were divided at random into 6 groups each consisting of 7 persons. One control group was not given nutritive supplements, while each of the other groups received various preparations, as shown in Table 1.

Table 1

Nutritive preparations used:

1. Multivitamin preparations (Polfa®) (A<sub>1</sub>, B<sub>1</sub>, B<sub>2</sub>, nicotinamide, pyridoxine, Ca pantothenate, B<sub>12</sub>, C, D, E) in approximately double the recommended daily doses.
2. Hemoglobin preparation (Hemoglobin-caps®), 2 g/d
3. Multivitamin preparation (Vital®) A<sub>1</sub>, B<sub>1</sub>, B<sub>2</sub>, pyrodoxin, C, D, approximately normal recommended daily dose.
4. Pollen preparation (Pollitabs sport®), Cernelle – 4 tablets daily, pollen extract, Cernitin T60 – 50 mg, Cernitin GBX – 1 mg per tablet.
5. Pollen/ amino acid preparation (“Stark-protein®” – 8 capsules daily, Pollitabs® - 4 tablets daily, Cernelle). Pollen extract,

Cernitin T60-50 mg. Cernitin GBX – 1 mg./1 tablet. Amino acid concentrates containing 18 free amino acids, including all the essential ones: 350 amino acids per capsule.

6. No nutritive preparations.

The work capacity of the participants, measured with a standard bicycle ergometer at the beginning of the experiment and then again after 6 weeks at the training camp, at which all the participants undertook approximately the same type of training. The increase in the blood's lactic acid level after 10 minutes of cycling, with a load of 2 watts per kg of body weight was measured before and after 6 weeks of training. The results were processed according to normal statistical procedures and the Student “t-test.”

#### Results

Work capacity, expressed in terms of oxygen consumption per kg of body weight, increased in all participants during the training period. There were, however, considerable differences among the groups; differences which, for three of the nutritive

preparations, were significant in comparison with the control group (see Table 2).

The increase in lactic acid concentration in the blood after 10 minutes of cycling showed

a decline after 6 weeks of training in all groups. However, the decline differed from group to group and diverged significantly from the control group in two of the groups given nutritional supplements (Table 3).

**Table 2** Average increase of work capacity after 6 weeks of training.

	Increase %	Significant level compared to control group
1. Multivitamins	93	5%
2. Hemoglobin preparation	84	5%
3. Multivitamins	48	no change
4. Pollen extract	70	no change
5. Pollen extract + amino acids	123	1%
6. Control group	31	--

**Table 3** Average decrease in lactate after exercise and after 6 weeks of training.

	mM/1	Significant level compared to control group
1. Multivitamins	2.3	5%
2. Hemoglobin preparation	1.9	5%
3. Multivitamins	1.5	no change
4. Pollen extract	1.7	no change
5. Pollen extract + amino acids	2.6	1%
6. Control group	1.4	--

### **Summary:**

The work capacity of weightlifters, and the formation of lactate in their blood, during a 6-week training period were significantly affected by the administration of various nutritional substances. A multivitamin preparation and a combination of amino acids and pollen show the greatest effect.

### **Discussion:**

The fact that the increase in lactic acid after exercise decreases and work capacity increases in connection with training is, of course, well-known and self-evident. The differences between the changes noted among the groups must reasonably be attributed to the administration of different nutritional substances. The greatest changes in both variables were noted in the group given the combination of Pollitabs and "Stark-protein" ("Strong" or "concentrated" protein), while Pollitabs alone did not produce any significant difference compared to the control group. The protein administered in the form of "Stark-protein" corresponded to only about 2.8 g of protein per day and could not reasonably have affected in any way the protein balance. The

overall effect must therefore be caused by some form of synergism between the amino acids in the "Stark-protein" and the pollen extract.

Of course, on the basis of this relatively small experiment, it is not possible to analyse with certainty the relative importance of the various factors. Nevertheless, the experiment does support the notion that various nutritional additives – particularly the combination of pollen extract and amino acids – can lead to a distinct improvement in performance in connection with training.

In discussions about the importance of vitamins to physical performance, it has sometimes been said that the benefits of vitamins observed among Eastern Bloc performers – as distinct from the results of experiments in the West – could be attributable to a lower vitamin "status" in the Eastern Bloc. All of the weightlifters participating in the experiment enjoyed a balanced, high-vitamin diet. Thus, latter interpretation is not confirmed by the present experiment.





### Prevention of muscle soreness by pretreatment with antioxidants

M. Krotkiewski<sup>1</sup>, Z. Brzezinska<sup>4</sup>, B. Liu<sup>2</sup>, G. Grimby<sup>1</sup>, S. Palm<sup>3</sup>

<sup>1</sup>Department of Rehabilitation Medicine and <sup>2</sup>Department of Cardiothoracic Surgery, Sahlgrenska Hospital, University of Göteborg, Sweden, <sup>3</sup>Panafarma Med, Sweden, <sup>4</sup>Department of Applied Physiology, Medical Research Center, Polish Academy of Sciences, Warsaw, Poland

A free radical-scavenging preparation (pollen extract) or the corresponding placebo was given to 50 volunteers over a period of 4 weeks to test the hypothesis that muscle soreness is associated with the generation of free radicals. The increase in malonyldialdehyde and lactate immediately after exercise both in blood and in muscle tissue was significantly lower after treatment with the scavenging preparation. The same was true for the prolonged post-exercise increase in creatine kinase over a 5-day period. The post-exercise glycogen content of muscle was higher in the pollen extract group, as were the subjective feelings of pain, oedema, discomfort and tension in the working muscle. We conclude that the beneficial preventive effect of pollen extract on post-exercise muscle soreness and lowering of the concentration of lipid peroxides indicate that free radicals are probably involved in the development of muscle soreness.

**Key words:** free radicals; muscle soreness; MDA; exercise; creatine kinase; antioxidant

Free radicals are ubiquitous in biological systems and have been implicated as factors in cellular differentiation (1), aging (2), mutagenesis (3) and carcinogenesis (4). Furthermore, the actions of free radicals have been implicated in the pathophysiology of many diseases, including ischaemia reperfusion injury involving the brain (5), heart (6), skin (7), intestines (5, 7), pancreas (4, 5, 7), liver (4, 5, 7), muscles (8), kidneys (9) and lungs (4).

The realization that free radicals might be injurious to organisms has been slow, perhaps because of the difficulties encountered in detecting these short-lived moieties in biological tissues. Nevertheless, the many animal and human studies claiming that modification of free radical production attenuates or eliminates tissue destruction have forced the medical community, and particularly cardiologists, to consider a specific therapy against free radicals as a realistic approach to disease after unaccustomed physical exercise. Muscle soreness is common and is observed to reach peak intensity 1-5 days later (10,11). The subjective sensation of soreness is accompanied by evidence of damage

to the affected muscles. Ultrastructural examination of muscle samples has shown extensive degenerative changes affecting a large part of muscle (10, 12). Evidence of an inflammatory response is also present, necrotic tissue being invaded by macrophages (13-15) and increased local levels of lysosomal enzymes (16). Related to these changes is the release into the lymph, and subsequently into plasma, of cytoplasmic enzymes, which are normally unable to cross the cell membrane. Large increases in the serum levels of creatine kinase, lactate dehydrogenase (LDH) and other enzymes of muscular origin occur, peak activities being observed at varying times from a few hours to several days after exercise (17). This enzyme efflux is considered to reflect a change in normal membrane structure, such that permeability to large protein molecules is increased. Although the mechanisms underlying these changes in muscle after exercise are not clear at present, there are striking similarities between the observed response and the tissue changes induced by an increased production of free radicals (15).

If the post-training syndrome and muscle soreness with enzyme leakage, pain, discomfort, oedema and tenderness of working muscles are due to the generation of free radicals and the reperfusion injury of skeletal muscles, supplementation with free radical scavengers should prevent or ameliorate the condition.

The aim of this study was to test the effect of a pollen extract preparation, known to be rich in SOD (superoxide dismutase) mimics, on the biochemical, morphological and clinical signs of muscle soreness.

### **Pollen-pistil extract with antioxidative activity**

The preparation used in this investigation is unique as to its composition, method of production, source and high SOD activity. The source is freshly harvested pollen grains and pistils from the family *Gramineae* spp. The pollen grains and the pistils are collected separately by machines specially designed for this purpose. After collection, they are thoroughly analysed for purity and specificity (18). The SOD active base material is produced in a reactor where pollen grains and pistils are allowed to react under very specific and well-defined conditions. The reactant solution is partly evaporated to concentrate the solution and increase the activity. The hypothesis regarding what substances are obtained in this reaction is still under investigation (19), but the following substances are probably present in the reactant solution:

1. SOD mimics, such as flavonoids, tannins and polyphenols. These are low-molecular-weight substances and are therefore absorbed through the intestinal wall (20, 21).
2. Maillard reactants, formed mainly from the reaction between certain amino acids and sugars via the Maillard reaction. This yields small amounts of antioxidative substances. These phenomena have been studied for many years and are not yet fully understood (22, 23).
3. SOD enzyme, released from disrupted mitochondria and pollen tubes.

This pollen-pistil extract exhibits an SOD activity of approximately 30,000 units<sup>a</sup> per gram of

substance (24). When adsorbed and complexed to a defined mixture of proteins, it gives an SOD activity of 4000-6000 F per gram of extract (19). The test preparation, pollen extract (Polbax<sup>®</sup>) (Allergon, Sweden), is manufactured from this extract (20). The contribution of SOD activity from the 3 possible sources mentioned above has not yet been fully established.

With reference to the results achieved in clinical trials, it seems likely that low-molecular-weight substances, which are easily absorbed from the gastrointestinal tract, contribute a major part of the antioxidative activity. Only a minor part can theoretically come from native SOD enzyme, which might be absorbed via the endocytosis-exocytosis mechanism (25). This mechanism has been shown in rats (26, 27). However, direct absorption of SOD – a rather large protein molecule – has not been verified in humans and should be considered a rather unlikely possibility. On the contrary, anthocyanidins, pyknogenols, other polyphenols and tannins present in pollen extract have been found to be fully absorbable, and their antioxidant activity is several times higher than that of vitamin E (28-30).

### **Material and methods**

Fifty male volunteers were recruited to the study via a local daily newspaper. Before admission to the study, all participants were informed about the aims, methods, anticipated benefits and potential hazards of the study, and verbal consent to inclusion was obtained from each of them. The study protocol was approved by the Ethics Committee of the University of Göteborg. The inclusion criteria were absence of hypertension, diabetes, cardiovascular disease, organic brain disease, alcohol or drug dependence and any other deviation from good health, no physical training on a permanent or intensive basis and the lack of any ongoing medication. All volunteers were explicitly asked to follow their habitual style of life, particularly with regard to diet and level of physical activity. All patients were allocated to one of two groups (36 to the pollen extract and 14 to the placebo group). The analysis of variance did not reveal any significant difference between

the groups with respect to any variable shown in Table 1.

#### General design

After the first selection, each participant was allocated to the first pretreatment period. During this period, participants were asked to report their daily food intake while keeping their body weight stable to within  $\pm 200$  g. At the end of this period (1 week), blood samples were drawn from the antecubital vein after an overnight fast. Body weight and anthropometric variables (waist to hip circumferences) were measured on the same day. The next day, participants arrived at the laboratory at 0800. After the initial blood sampling, a short venous catheter was inserted into an antecubital vein and the volunteer started to perform the exercise programme.

#### Acute exercise test

After 10 min of rest, participants started to perform the following exercise:

- 10 min on the step test (stepping up and down a 45-cm foot-stool 15 times per min);
- 30 min cycling at 70% of  $V_{O_{2max}}$  followed by 10 min on the step test;

#### Determination of $V_{O_{2max}}$

Participants started cycling on the electrically braked ergometer bicycle (Monark, Varberg, Sweden). Blood pressure and heart rate were measured during the last minute of the 6-min steady-state period at the submaximal working capacity (i.e., oxygen uptake) was then calculated according to Astrand (31).

Immediately after the acute exercise test, muscle biopsies were taken with an alligator forceps (32) from the lateral vastus. The muscle specimens were divided into 2 parts: one was frozen immediately in liquid nitrogen and used for analysis of enzymatic activities; the other part was trimmed, mounted and frozen in cooled isopentane ( $-160^{\circ}\text{C}$ ) and used to histochemical analysis. Both parts were stored at  $-80^{\circ}\text{C}$  until analysed. In the histochemical analysis, the myofibrillar adenosine triphosphatase (ATPase) method was used for muscle fibre classification (33, 34). Amylase-periodic acid-Schiff staining was used to visualize capillaries (35), and the number of capillaries per fibre and the fibre area

per capillary were calculated for the different fibre types. Glycogen synthase activity in the lateral vastus muscle was measured according to previous methods (36, 37), malonyldialdehyde (MDA) by the thiobarbituric acid method (38),

Table 1. General characteristics of participants (body composition, anthropometry and maximum oxygen uptake) (means  $\pm$  SEM)

	Pollen extract group		Placebo group	
	Before treatment	After treatment	Before treatment	After treatment
Age (years)	35.1 $\pm$ 2.62		35.1 $\pm$ 2.62	
Height (cm)	183.6 $\pm$ 2.26		180.7 $\pm$ 1.73	
Waist (cm)	85.9 $\pm$ 1.35	85.8 $\pm$ 1.40	85.0 $\pm$ 1.13	85.2 $\pm$ 1.30
Hip (cm)	98.5 $\pm$ 1.33	98.7 $\pm$ 1.37	97.1 $\pm$ 0.84	98.9 $\pm$ 1.10
Waist-to-hip ratio	0.87 $\pm$ 0.01	0.87 $\pm$ 0.01	0.88 $\pm$ 0.01	0.88 $\pm$ 0.01
Weight (kg)	80.6 $\pm$ 1.98	80.6 $\pm$ 2.10	76.5 $\pm$ 2.15	76.7 $\pm$ 2.13
Body $K^{+1}$ (mmol)	4493 $\pm$ 136	4525 $\pm$ 132	4296 $\pm$ 146	4291 $\pm$ 155
Body fat (kg)	14.6 $\pm$ 1.93	14.2 $\pm$ 1.97	13.5 $\pm$ 1.96	13.7 $\pm$ 1.91
Fat free mass (kg)	66.0 $\pm$ 2.01	66.4 $\pm$ 1.94	63.1 $\pm$ 2.15	63.0 $\pm$ 2.27
$V_{O_{2max}}$ (l/min)	3.35 $\pm$ 0.19	3.45 $\pm$ 0.19	3.36 $\pm$ 0.11	3.41 $\pm$ 0.15

- 30 min cycling at 60% of  $V_{O_{2max}}$  followed by 10 min on the step test;
- 30 min cycling at 60%  $V_{O_{2max}}$ ;
- 10 min on the step test;
- 30 min cycling at 60%  $V_{O_{2max}}$ .

vitamin E and ceruloplasmin according to Storer et al. (39) and body composition by calculating naturally occurring  $^{40}\text{K}$  (40). Other enzymes (ALAT, ASAT, alkaline phosphatase and creatine kinase) and other metabolic variables were

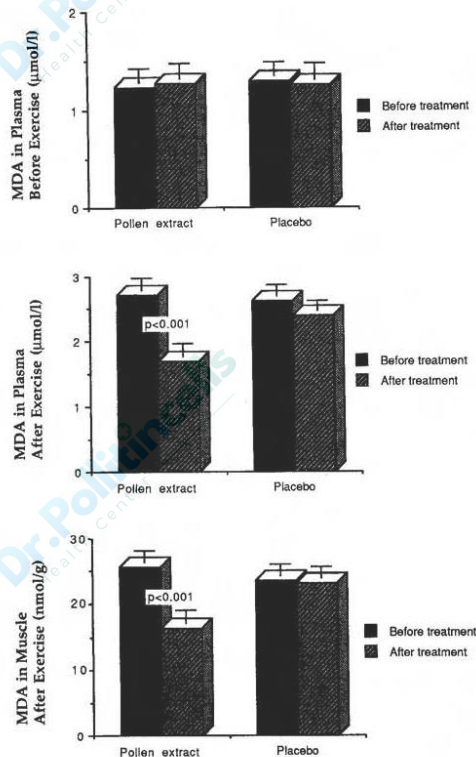
determined according to routine automated hospital methods. Muscles enzyme activities were measured according to Lowry et al. (41, 42) and lactate concentration according to Karlsson (43).

One hour after the exercise test, and then at the same time on the second, third, fourth and fifth days, the participants rated their pain, oedema and discomfort in the working muscles on a 10-cm visual analogue scale ranging from no pain

As mentioned before, all participants were allocated either to the placebo group or to treatment with the pollen extract with antioxidant effect. The pollen extract group received 2 tablets 3 times a day, and the placebo group received the same number of identical placebo tablets. The tablets were given in a double-blind manner. All tests and measurements were performed in an identical manner before and 4 weeks after treatment. Results were calculated by 2-way analysis of variance by means of Macintosh StartView statistical program.

## Results

Four weeks of treatment with pollen extract or placebo did not induce any changes in



and

Fig. 1. The concentration of malonyldialdehyde (MDA) in plasma before and immediately after strenuous exercise and in the lateral vastus muscle immediately after strenuous exercise (mean+SEM) and before and after 4 weeks of treatment with either pollen extract (n=36) or placebo (n=14).

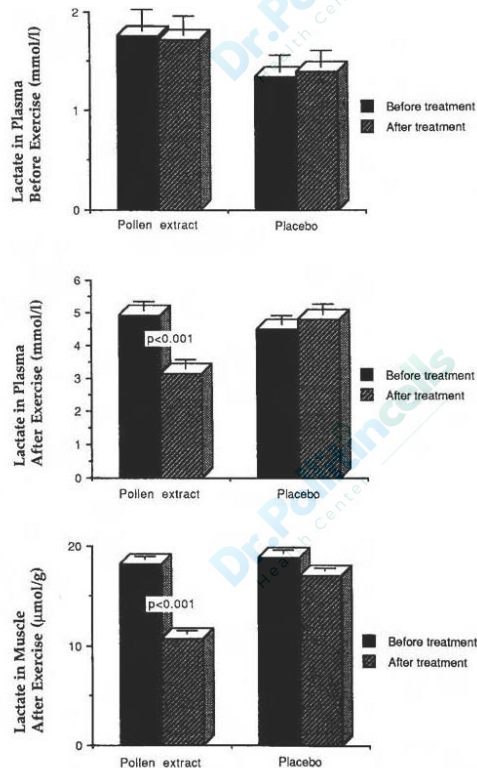


Fig. 2. The concentration of lactate in plasma before and after strenuous exercise and in muscle immediately after strenuous exercise (mean+SEM), and before and after treatment with either pollen extract (n=36) or placebo (n=14).

discomfort at the far left to intense pain and discomfort at the far right. Visual analogue scales were used in a similar way for the questions related to oedema and tenderness and the feeling of muscle tension.

body weight or body composition (Table 1). There was no difference in the concentration of MDA in plasma before exercise between the pollen extract and placebo groups. Immediately after exercise, the concentration of MDA increase significantly ( $P < 0.001$ ) in both groups. However, after pollen extract treatment increase in MDA level (Fig. 1) immediately after exercise was significantly lower than in the placebo group, in which placebo had no effect on the post-exercise MDA levels. The concentration of MDA in muscle, measured immediately after exercise, decreased significantly after treatment with pollen extract but remained unchanged in the placebo group (Fig. 1). The decreases in plasma and muscle MDA levels at the end of the acute exercise test in the pollen extract group were 30% and 50%, respectively. The concentration of MDA 5 min and 24 and 48 h after the termination of exercise did not change after the pollen extract treatment (data not shown). The concentration of lactate in plasma before exercise was very similar in the placebo and the pollen extract groups and did not change after treatment (Fig. 2). In contrast, plasma lactate concentration immediately after exercise increased significantly. Treatment with pollen extract resulted in a significant diminution

of this increase, whereas the placebo treatment appeared to have no detectable effect on the exercise-induced increase in lactate concentration. When measured immediately after exercise, muscle lactate content showed a significant decrease after 4 weeks of treatment with pollen extract. There was no difference in muscle lactate content after the corresponding placebo treatment (Fig.2). The decrease in the post-exercise lactate concentration in plasma was found to be 28% and 39% in muscle. In the group treated with pollen extract, the percentage decrease in lactate concentration in muscle after exercise was positively correlated with the percentage decrease in muscle MDA concentrations (Fig. 3). In comparison with

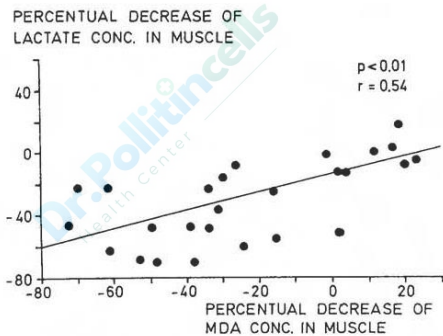


Fig. 3. The relationship between the percent decrease of the concentration of lactate and the percentage decrease of the malonyldialdehyde (MDA) concentration in the lateral vastus muscle after 4 weeks of treatment with pollen extract.  $P < 0.01$ ,  $r = 0.54$ .

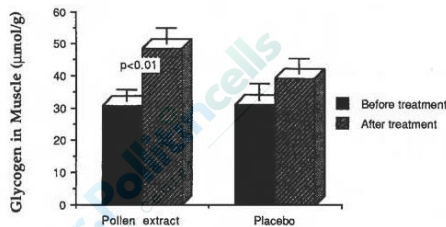


Fig. 4. The concentration of glycogen (mean+SEM) in the lateral vastus muscle before and after treatment with pollen extract or placebo.

#### Preventing muscle soreness with antioxidants

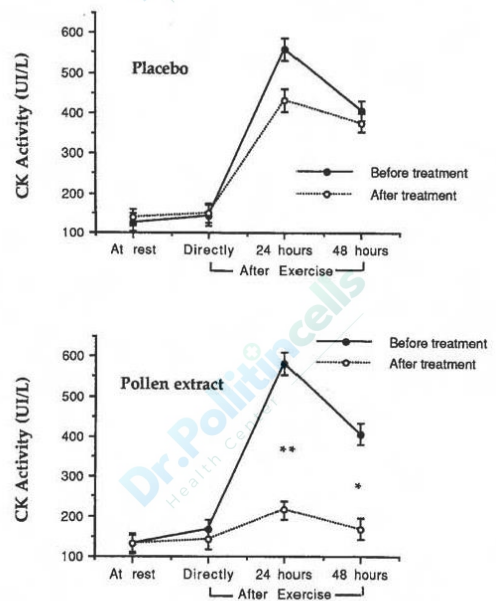


Fig. 5. The activity of creatine kinase (CK) (mean+SEM) before strenuous exercise and immediately and 24 and 48 h after exercise, before and after 4 weeks treatment with either pollen extract or placebo. \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

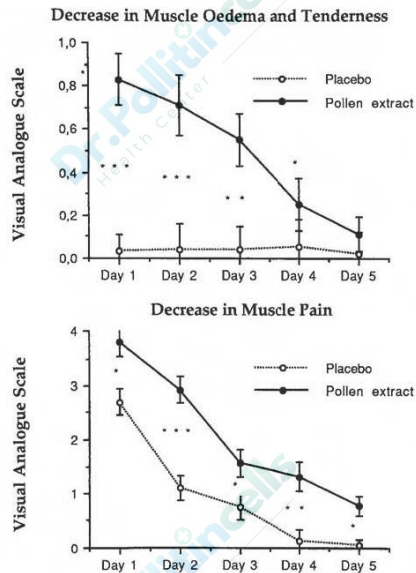


Fig. 6. Decrease in muscle oedema, tenderness and pain as evaluated by the participants on visual analogue scales on 5 consecutive days after strenuous exercise, after the treatment with either pollen extract or placebo. The decrease is calculated as a difference between the starting values (i.e., rated after strenuous exercise before the treatment) and the values rated after the treatment. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

the pretreatment values, there was a significant increase in the concentration of glycogen in the lateral vastus muscle after the treatment with pollen extract, whereas no significant change in muscle glycogen concentration was found after placebo treatment (Fig. 4). Creatine kinase activity increased significantly 24 and 48 h after strenuous exercise. The increase was found to be almost completely eliminated in the pollen extract-treated group but not in the placebo-treated group (Fig. 5). When using the visual analogue scales, the participants reported a significant decrease in muscle oedema and tenderness (Fig. 6), muscle pain (Fig. 6) and feeling of tension in muscles after the treatment with pollen extract. These decreased remained significant for all the 5 days, during which the participants were asked to record their subjective feeling on visual analogue scales. The differences between the two groups decreased gradually from day 1 to day 5 (Fig. 6).

Heart rate at rest and the different work loads on the ergometer bicycle, as well as blood pressure,

remained uninfluenced by both pollen extract and placebo treatment (Table 2).

No difference in either the peak isokinetic strength or the average value for the whole range of movement after the treatment with pollen extract or placebo was observed. Strength was tested on the KinCom dynamometer for knee extension at 60°/s angular velocity. The same (no change after treatment) was true of isometric strength measured by knee extension at a 60° knee angle and for the eccentric strength at the 60°/s angular velocity.

Neither pollen extract and placebo treatment nor the exercise itself has been shown to have any significant effect on the activities of aminotransferases (ASAT, ALAT) and alkaline phosphatase on the bilirubin concentration.

The vitamin E concentration in plasma increased slightly in the group treated with pollen extract 5 min after the exercise but remained unchanged 24 and 48 h after the exercise.

The 4-week course of treatment with pollen extract did not evoke any significant effect on the concentration of ceruloplasmin in plasma.

No differences in the concentration of plasma triglycerides 5 min or 24 and 48 h after exercise were noted after the pollen extract treatment. The same was true for the plasma cholesterol level.

The activities of different enzymes in the lateral vastus muscle (oxidative: hydroxy-acyl dehydrogenase, citrate synthase, glycolytic: hexokinase and LDH), expressed per gram of muscle wet weight or per gram of protein, did not change after either pollen extract or placebo treatment. The activity of muscle glycogen synthase, both its  $V_{max}$  and its fractional velocity, did not change significantly after either form of treatment.

Table 2. Heart rate during cycling (means±SEM)

	Pollen extract group		Placebo group	
	Before treatment	After treatment	Before treatment	After treatment
Heart rate (beats/min)				
At rest	63.4±2.38	65.6±2.22	65.5±2.51	62.8±3.01
After cycling 50 W	84.7±2.90	87.3±2.29	84.1±2.96	84.7±3.64
After cycling 100 W	110.4±3.77	113.6±2.96	109.0±3.40	109.4±3.32
After cycling 150 W	139.9±5.00	136.1±3.64	137.1±3.42	136.3±4.22
After cycling 200 W	171.1±5.60	168.9±5.31	177.5±6.96	169.8±5.45

There was no difference in the relative percentage distribution of the different types of muscle fibres between the two groups. No changes were found after pollen extract or placebo treatment. The number of capillaries in contact with different types of fibres did not change after either of the treatments. The number of capillaries around type IIB fibres was found to be lower than those supplying type I and type IIA muscle fibres ( $P<0.05$ ). The mean muscle fibre cross-sectional area did not change after either of the treatments.

## Discussion

The main findings of the study suggest that muscle soreness is at least to some extent caused by the creation of free radicals and lipid peroxides and secondary damage to muscle membranes followed by the leakage of muscle enzymes. Factors that may be implicated in the generation of free radicals and lipid peroxidation include substrate depletion and disturbances in the oxidation and reduction status of the cell. Lovlin et al. (44) found a similar parallel increase of lactate and MDA in blood as we observed in our study. The authors discuss these findings, quoting the available literature, and argue that exercise intensity influences both lactate production and removal, and secondarily the concentration of reducing equivalents NADH/NADPH and the production of free radicals and lipid peroxidation. Although we did not measure lactate uptake, our findings of a direct correlation between blood lactate and plasma MDA, and muscle lactate and muscle MDA, as well as the correlation between the differences in

MDA and lactate concentrations after treatment, support such a hypothesis. As pointed out by Kappus & Sies (45), any stress on the system, such as hypoxic tissues, that results in depletion of glycolytic substrates, may cause a decrease in the generation of NADH and NADPH.

Although it seems theoretically possible that pollen extract could influence the system and have some glycogen-sparing effect ameliorating the glycogen depletion and lactate production, it can hardly influence the NADPH concentration to a degree directly affecting the production of free radicals. The concentration of NADPH is, on the other hand, involved in the glutathione-dependent oxido-reductive protecting system (46-48).

Submaximal exercise has been reported to deplete the concentration of glutathione, and NADPH may be regarded as a natural reducing agent to restore the concentration of glutathione. Although glutathione depletion is likely to occur, the involvement of NADPH, in association with the lactate production, seems less probable.

On the other hand, it seems probable that pollen extract could influence the generation of ATP. During the exhaustive exercise, regeneration of ATP is insufficient due to the high rate of ATP turnover. The concomitant accumulation of AMP, causing increasing amounts of hypoxanthine and the conversion of xanthine dehydrogenase to xanthine oxidase (as described during ischaemia-reperfusion injury), causes the creation of free radicals (46, 49). The concentration of lactate is an indirect measure of

the relative ischaemia, insufficient regeneration of ATP, build-up of hypoxanthine, and the creation of free radicals and lipid peroxide. This concept explains the correlation between the concentration of lactate and MDA. It is possible that this coincidence (between the lowering of both MDA and lactate post-exercise concentrations) also indicates some other kind of indirect effect on the muscle cell metabolism. Pollen extract can act by accelerating the restoration of ATP-yielding metabolism or by some mechanisms preventing or delaying the shift from aerobic to anaerobic metabolism. Similarly, any influence on the phosphofructokinase or any other mechanism preventing the depletion of glycolytic substrate can prevent or delay the decrease in the generation of NADH and NADPH. As discussed above, the decreased concentration of these reducing compounds can lead to higher generation of free radicals and lipid peroxides. Thus, the mechanism of action of pollen extract could depend on the effect on the metabolic pathways determining both the generation and regeneration of ATP and the production of lactate. No data are so far available explaining the exact influence of pollen extract on the intracellular muscle metabolism, and further studies are required to elucidate the parallel changes in MDA and lactate concentration both in blood and in muscle tissue.

An increase of hypoxanthine 10 min after termination of exercise, together with a significant arteriovenous difference, and together with the concomitant increase of the blood and plasma concentration of glutathione (49), has recently been reported. The same authors could not find any post-exercise increase of MDA. The obvious discrepancy between this finding and several other reports on the post-exercise MDA increase in animals and humans has been explained as dependent on differences in method (49). However, our findings are very consistent and, in accordance with other reports on the MDA increase, they also show that the increase of MDA is very transitory and depends very much on the time of sampling and intensity of exercise.

Our study has shown that the decrease in muscle soreness parallels the decrease in the concentration of lipid peroxides and the decrease in lactate production. The improvement of the symptoms (muscle pain, tenderness and oedema) occurred after a time period of sufficient to eliminate any influence of familiarization with the test situation and learning, both possibly improving the efficiency of the performed exercise. This was also suggested by the observations that the level of physical fitness and increases of heart rate and blood pressure were not different before and after treatment. The degree of oedema was verified by the objective blind measurements by the non-involved observer and confirmed to be significantly different before and after the treatment with the antioxidant but not after the treatment with placebo. Thus, at least this variable was in harmony with the results obtained by means of visual analogue scales. These scales are otherwise commonly used (also by us for measurements of pain, hunger feelings, etc.) and generally accepted as a good measure of subjective feelings.

The addition of pollen extract to the red blood cell incubation medium, with and without a free radical-generating system, caused a significant reduction of the concentration of MDA. The decrease of MDA was parallel and significantly correlated to the improvement of the erythrocyte deformability (50). A similar improvement in blood rheology has also been observed in volunteers after 3 weeks of oral administration of pollen extract (50).

Whatever the underlying mechanism, the improved blood rheological properties could influence the availability of the oxygen derived from the red blood cells and, in that way, ameliorate ischaemia and lactate production in the working muscle. It seems probable that the administration of pollen extract does not influence the production of lipid peroxides or the concentration of MDA during the late reperfusion period during recovery but acts only during strenuous exercise and possibly directly after its termination.



The concentration of MDA was already normal within 5 min after terminating exercise. This is in accordance with the findings of Lovlin et al. (44), who also stressed the dependence of MDA production on the intensity of exercise. The rapid decrease in MDA concentration most probably indicates that the cytosolic NADH increases and the formation of free radicals decreases very soon after the termination of exercise, due to an increased uptake of lactate and increased activity of enzymes that inhibit peroxidative processes. The observation that lactate production was diminished and the glycogen content spared (Fig. 2, 4) suggests that the mechanism of action of pollen extract is the facilitation of the aerobic metabolism in muscles with a glycogen-sparing and lactate production-diminishing effect.

In summary, the administration of pollen extract, the antioxidant-containing preparation, effectively ameliorated the subjective symptoms of muscle soreness parallel to both the decrease in enzyme leakage and decrease in MDA and lactate concentrations in the muscle tissue. The results of the study strongly suggest the involvement of lipid peroxides in the pathogenesis of muscle soreness and indicate the potential of free radical scavengers in its prophylaxis. Further studies are needed to confirm the usefulness of the present study design as a model for reperfusion injury.

#### Acknowledgements

This study was supported by the Swedish Sports Council and the Askers Foundation.

#### References

1. Sohal RS, Allen RG, Nations C. Oxygen free radicals play a role in cellular differentiation: a hypothesis. *J Free Radicals Biol Med* 1986; 2: 175-181.
2. Sohal RS, Allen RG. Relationship between metabolic rate, free radicals, differentiation and again: a unified theory. *Basic Life Sci* 1985; 35: 75-104.
3. Knuutila S. Role of free radicals in genetic damage (mutation). *Med Biol* 1984; 62: 110-114.
4. Southorn PA, Powis G. Free radicals in medicine. 11. Involvement in human disease. *May Clin Proc* 1988; 63: 390-408.
5. Cohen M. Free radicals in ischemic and reperfusion myocardial injury: is this the time for clinical trials? *Intern Med* 1989; 111: 918-931.
6. Hearse DJ, Humphery SM, Nayler WG, Slade A, Border D. Ultrastructural damage associated with reoxygenation of the anoxic myocardium. *J Mol Cell Cardiol* 1975; 7: 315-324.
7. Bulkeley GB. Free radical-mediated reperfusion injury: a selective review. *Br J Cancer* 1987; 55 (suppl 8): 66-73.
8. Davies KJ, Quintanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* 1982; 107: 1198-1205.
9. Faedda R, Satta A, Branca GF, Turrini F, Contu B, Bartoli E. Superoxide radicals (SR) in the pathophysiology of ischemic acute renal failure (ARF). *Adv Exp Med Biol* 1987; 212: 69-74.
10. Sjöström M, Friden J. Muscle soreness and muscle structure. *Med Sport Sci* 1984; 17: 169-186.
11. Maughan RJ, Donnelly AE, Gleeson M, Whiting PH, Walker KA, Clough PJ. Delayed-onset muscle damage and lipid peroxidation in man after a downhill run. *Muscle Nerve* 1989; 12: 332-336.
12. Warhole MJ, Siegel AJ, Evans WJ, Silverman LM. Skeletal muscle injury and repair in marathon runner after competition. *Am J Pathol* 1985; 118: 331-339.
13. Jones DA, Newham DJ, Round JM, Tolfree SEJ. Experimental human muscle damage: morphological changes in relation to other indices of damage. *J Physiol* 1986; 375: 435-448.
14. Smith JK, Grisham MB, Granger DN, Nortinus J. Free radical defense mechanism and neutrophil infiltration in postischemic skeletal muscle. *Am J Physiol* 1989; 256: H789-H793.
15. Reiker AO, Ytrehus K. Oxygen radicals and scavenger enzymes in ischemia reperfusion injury of skeletal muscle. *Scand J Clin Lab Invest* 1992; 52: 113-118.

16. Salminen A. Lysosomal changes in skeletal muscles during the repair of exercise injuries in muscle fibres. *Acta Physiol Scand* 1985: 539 (suppl): 1-31.
17. Newham DJ, Jones DA, Edwards RHT. Large delayed plasma creatine kinase changes after stepping exercise. *Muscle Nerve* 1983; 6: 380-385.
18. Dreborg S, Einarsson R, Longbottom JL. The chemistry and standardization of allergens. In: Wier DM, ed. *Handbook of experimental immunology*. Vol 1: Oxford: Blackwell Scientific Publishers, 1986: 10.1-10.28.
19. Marklund SL. Direct assay with potassium superoxide. In: Greenwald RA, ed. *Handbook of methods of oxygen radical research*. Boca Raton, FL: CRC Press, 1984: 249-255/
20. Oden PC, Karlsson G, Einarsson R. Demonstration of superoxide dismutase enzymes in extracts of pollen and anther of *Zea mays* and in two products, Baxtin® and Polbax®. *Grana* 1992: 31: 76-80.
21. Cheng Y, Li X, Zhao B. [Superoxide and hydroxyl radical scavenging activities of routine and other natural products studied by ESR] (in Chinese). *Acta Biophys Sin* 1989: 5: 253-240.
22. Monnier VM. Non-enzymatic glycosylation, the Maillard reaction and the aging process. *Gerontol* 1990: 45: 105-111.
23. Schuler P. Natural antioxidants exploited commercially. In: Hudson BF, ed. *Food antioxidants*. Amsterdam: Elsevier, 1990: 135-136.
24. Kozi A, Kanematsu S. Distribution of Cu/Zn, Mn and Fe superoxide dismutases in plants and fungi, an evolutionary aspect. *Proc Evol Protein Mol* 1978: 77: 361-372.
25. Undall JN, Walker WA. The physiologic and pathologic basis for the transport of macromolecules across the intestinal tract. *J Pediatr Gastroenterol Nutr* 1982: 1: 295-301.
26. Steffen C, Menzel J. Grundlagenuntersuchung zue Enzymtherapie bei Immunkomplkrankheiten. *Wiener Klin Wochenschr* 1985: 97: 376-384.
27. Walker WA. Antigen uptake in the gut: Immunologic implications. *Immunol Today* 1981; 2: 30-34.
28. Fragl GC, Marino VS, Ferraro GE et al. Flavonoids as antioxidants evaluated by *in vitro* and *in situ* live chemiluminescence. *Biochem Pharmacol* 1987: 36: 717-720.
29. Huguet AL, Mánez S, Alcaraz MU. Superoxide scavenging properties of flavonoids in a non-enzymatic system. *Z Naturforsch* 1990: 45: 19-23.
30. Chen YT, Zheng RL, Jia ZJ, Ju Y. Flavonoids as superoxide scavengers and antioxidants. *Free Radical Biol Med* 1990: 9: 19-31.
31. Astrand PO. *Experimental studies of physical working capacity in relation to sex and age*. Copenhagen: Munksgaard, 1982.
32. Herriksson KG. "Semi-open" muscle biopsy Technique. A simple outpatient procedure. *Acta Neurol Scan* 1979. 59: 317-323.
33. Brooke MH, Kaiser KK. Three "myosin ATPase" systems: the nature of their pH lability and sulfhydryl dependence. *J Histochem Cytochem* 1970: 18: 670-672.
34. Dubowitz V, ed. *Muscle biopsy, a practical approach*, 2<sup>nd</sup> edn. London: Bailliere Tindall, 1985.
35. Andersson P, Herriksson J. Capillary supply of the quadriceps femoris muscle in man: adaptive response to exercise. *J Physiol (Lond)* 1977: 270: 677-690.
36. Kochan RG, Lamb DR, Reimann EM, Schlender KK. Modified assay to detect activation of glycogen synthase following exercise. *Am J Physiol* 1981: 240: E197-E202.
37. Allenberg K, Nilsson M, Landin L, Lindgärde F. The glycogen and lactate synthetic pathways in human skeletal muscle in relation to obesity, weight reduction and physical training. *Eur J Clin Invest* 1988: 18: 250-255.
38. Plazer ZA, Cushman LL, Johansson BC. Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical systems. *Anal Biochem* 1966: 16: 359-367.

39. Storer JB. Fluorometric determination of tocopherol in sheep plasma. *Biochem Med* 1974; 11: 71-80.
40. Sköldbörn H, Arvidsson M. A new whole body monitoring laboratory. *Acta Radiol* 1972; 313 (suppl): 233-241.
41. Lowry OH, Rosebrough HJ, Farr AI, Randall RJ. Protein measurement with the Folin phenol reagent. *Biol Chem* 1951; 193: 265-275.
42. Lowry OH, Passonnan JV. A flexible system of enzymatic analysis. New York: Academic Press, 1972.
43. Karlsson J. Lactate and phosphagen concentration in working muscle of man. *Acta Physiol Scand* 1971; 358 (suppl): 19-32.
44. Lovlin R, Cottl W, Pyke J et al. Are indices of free radical damage related to exercise intensity? *Eur J Appl Physiol* 1987; 56: 313-316.
45. Kappus H, Siew H. Toxic drug effects associated with oxygen metabolism: redox cycling and lipid peroxidation. *EXperientia* 1981; 37: 1233-1241.
46. Sjödin B, Westing YH, Apple Fs. Biochemical mechanisms for oxygen radical formation during exercise. *Sports Med* 1990; 10: 236-254.
47. Ji LL, Fu R. Responses of glutathione system and antioxidant enzymes to exhaustive exercise and hydroperoxide. *J Appl Physiol* 1992; 72: 549-554.
48. Ji LL, Fu R, Mitchell EW. Glutathione and antioxidant enzymes in skeletal muscle: effects of fiber type and exercise intensity. *J Appl Physiol* 1992; 73: 1854-1859.
49. Sahlin K, Ekberg K, Cizinsky S. Changes in plasma hypoxanthine and free radical markers during exercise in man. *Acta Physiol Scand* 1991; 142: 275-281.
50. Krotkiewski M, Rashid M, Roberts DG, Palm S. The influence of SOD MIMICS (Polbax®) on the blood cells rheological properties and lipid peroxidation in an *in vitro* free radical generation system. Submitted.



## Flower Pollen Extract and its Effect on Metabolic Adaptation of Muscles

### The effects of pollen and protein extracts on selected blood factors and performance of athletes

Ralph E. Steben,<sup>1</sup> and Mr. Pete Boudreaux<sup>2</sup>

(from the <sup>1</sup> Associate Professor Health Physical Recreation Education, Louisiana State University, Baton Rouge, Louisiana, and <sup>2</sup> Coach Catholic High School, 855 Hearthstone Drive, Baton Rouge, Louisiana (U.S.A.)

The use of dietary supplements by athletes searching for the slight physiological edge over their opponents in competition is a fairly common practice. Pollen extract (PE), pure bee pollen (BP), and protein extract (PRE) preparations, extolled for their performance improvement characteristics, have recently been popularized by a number of world class athletes, their coaches, and trainers, and reported in several newspaper and magazine articles.<sup>3 4 5 19 20 21 22</sup> These articles report subjective opinion and heuristic observations and lack the credibility of scientific experimentation. While considerable evidence has been presented that PE is successful in the treatment of prostatitis,<sup>1 2 8 26</sup> bleeding stomach ulcers,<sup>10</sup> and increases resistance to colds and respiratory infections, little has been done experimentally to validate claims related to improvement of athletic performance. The daily dosage in the majority of the studies mentioned adhered to the manufacturer's labeled prescription (four to six tablets per day), with the length of the experiments ranging from three to 36 days,<sup>18 26</sup> eight to twelve weeks,<sup>9 18</sup> and as long as ten months.<sup>17</sup>

While Nuttala<sup>22</sup> claimed that the purpose of PE ingestion was to increase red blood cells in athletes and thus facilitate the transport of oxygen, Millar<sup>21</sup> reported that no direct correlation was established between PE ingestion and Hgb increase. However, Nuttala attributed the increase in Hgb concentration found among Finnish runners to the effects of PE and high protein diet.

Steben et al.<sup>27</sup> studied the effects of a BP on selected blood factors and performance of varsity college swimmers and found no significant differences in Hgb and Hct levels among the groups studied. Since evidence presented by Rose<sup>25</sup> indicated the possible incidence of the muscular weakness and lethargy of hypokalemia among collegiate varsity distance runners in spite of otherwise good aerobic fitness, it was conjectured that the addition of potassium (K<sup>+</sup>) to the diet in a palatable form would alleviate the condition. Steben found that BP did not significantly improve K<sup>+</sup> levels in the blood over other groups studied.

Fijalkowski<sup>9</sup> found a large, but non-significant, difference in the improvement of work capacity in weightlifters using PE. Steben<sup>27</sup> found that the use of PE did not result in any significant difference in performance of swimmers over that of the control group.

There are also conflicting ideas on the necessity of a PRE in the athlete's diet. Poortmans<sup>24</sup> indicated that protein is not used as a primary energy source when caloric supply is sufficient. It is a generally accepted fact that the major function of protein is to sustain cell growth and maintain various body tissues. Jormakka<sup>13</sup> indicated a PRE should be a supplement to the low protein diets that are normal in Third World countries. It seems that the presence of high quality protein foods, which contain all of the known amino acids, would be sufficient to maintain cell construction and would not be metabolized as an energy source unless an inadequate caloric intake was present.

The PRE used in this study (reported by the manufacturer A. B. Cernelle, to contain quick absorbable free amino acids and low molecular peptides) was also used in Fijalkowski's 14 day weight training study which attributed the increase Hgb content of the blood to the supplement. However, in his study the experimental group received both the PRE and PE-Pollitab in a dosage "according to the producers instruction" (sic),<sup>9</sup> either of which could have been responsible for the increase. No other studies have been found which compare the use of a protein supplement with changes in performance.

The purpose of this study was to validate and further investigate the question of whether normal training over a period of time rather than food supplements was primarily responsible for improved performance of endurance athletes who ingest normal diets. Specific biochemical parameters of blood serum K, Hgb, and Hct values were selected for investigation of their possible influence on prevention of the tiring effects of hypokalemia and improvement of oxygen carrying ability of the blood, respectively.

### **Procedure**

The placebo double blind experiment was undertaken at Catholic High School of Baton Rouge, Louisiana, with 18 male cross country runners for twelve weeks during the Fall Semester, 1977. The runners were randomly divided into three diet groups. Individuals in the first group orally ingested four PE capsules daily before breakfast. A similar procedure prescribing four placebo capsules was followed by members of group two, while the individuals of group three took four PRE capsules. The rationale for the prescription was based on recommended daily dosages suggested on the label of the product bottle and also found in the producer's monographs.

At the beginning and the conclusion of the experiment, blood samples were drawn from each individual for three consecutive days before practice. Serum K<sup>+</sup> levels were analyzed by an Instrument Lab flame photometer, with Hgb and

Hct levels determined by a Cotter S automatic Counter. All of the runners took their meals, except for lunch, at home and were advised to dine as normally accustomed.

The runners preceded their formal training with a voluntary summer program of slow, long mileage work. Formal training at the inception of the experiment consisted of 70 miles of over distance type work per week conducted on the road and cross country trails, gradually evolving into 100 miles per week of faster pace work. Near the end of the 12 week experiment, 40 percent of the mileage was negotiated with Fartlek style training and a limited amount of race pace interval type work. Prior to the State Meet, and the conclusion of the season and the experiment, the overall volume was reduced to 60 miles per week. Pre and post performance tests consisted of determining the average velocity for a three mile run conducted on the same surveyed and permanently marked cross country course. Split times were provided to assist the runners in the judgment of pace at the mile and two mile posts in both time trials and provided the information for change in performance.

The data which consisted of the serum K<sup>+</sup> mEq/L, Hgb gm, Hct %, and performance yds/ sec (velocity) for average three mile run performance were statistically treated with a split plot ANOVA with diet and pre-post measures as the main and split plot respectively. Probability was reported at the .05 and .01 levels of significance.

Analysis of samples of materials used in the study were reported to determine concentrations of minerals germane to the blood analysis. If PE and PRE is reputed to directly or synergistically help an individual become more enduring, then levels of K<sup>+</sup> and Fe, to assist in modifying the possible fatigue effects of hypokalemia and enhance the increased oxygen carrying characteristics of Hgb and Hct concentration respectively, should be found in relatively high concentration (Table 1).

TABLE 1.—Chemical analysis of diet treatments.

Sample	Fe ppm/capsule	K <sup>+</sup> ppm/capsule
PE, 365 mg*	100.0	2000.0
Placebo, 350 mg*	30.0	120.0
PRE, 360 mg*	4500.0	280.0

\*Mean of the contents of ten capsules.

## Findings

An ANOVA (Table 2) with diet and pre and post measures as the main and split plot respectively, found no significant differences among the diet groups in blood levels of K<sup>+</sup>, Hgb and Hct. Significant pre and post differences were found in blood levels of K<sup>+</sup> and Hgb. Pre versus post for performance was highly significant. The analysis in performance was nonsignificant and diet x pre and post measure interaction was nonsignificant for all variables.

While the ANOVA found significant pre and post differences in blood levels of K<sup>+</sup> and Hgb, they cannot be attributed to the diet supplements since the analysis found no significance among the diet groups in blood levels of K<sup>+</sup>, Hgb, and Hct. The difference in K<sup>+</sup> was negative yet within normal blood level limits (Table 3). An earlier study<sup>27</sup> conducted for eight weeks suggested that an extended experiment might sustain Rose's observation that hypokalemia could appear in endurance athletes at the conclusion of arduous season and might be moderated by the inclusion of K<sup>+</sup> bearing foods in the diet. The results of our study did not support the inclusion of pollen extracts or protein extract food supplements in normal diets for maintenance of K<sup>+</sup> levels and prevention of hypokalemia.

The fact that the analysis found a significant difference in Hgb and not in Hct is difficult to explain. Table 2 reveals that Hct just missed

being significant, even though once a rule of evidence is set up concerning significance, a nonsignificant result should not be considered important. An increase of hematocrit to optimal levels should be associated with an increased blood volume and hemoglobin concentration.<sup>7 14 23</sup> The increased hematocrit could then help

TABLE 2.—Split Plot ANOVA with diet as the main plot; Pre-post measure as the split plot.

Analysis of variance	DF	Mean square	F
<b>For K<sup>+</sup> mEq/L</b>			
Diet	2	.054	N.S.
Error A	15	.108	
Pre-post measure	1	.188	5.909*
Diet x pre post measure	2	.048	N.S.
Residual	15	.032	
Corrected total	35	.071	
<b>For Hgb gm</b>			
Diet	2	.093	N.S.
Error A	15	.573	
Pre-post measure	1	1.562	7.560*
Diet x pre post measure	2	.070	N.S.
Residual	15	.208	
Corrected total	35	.435	
<b>For Hct %</b>			
Diet	2	1.897	N.S.
Error A	15	5.436	
Pre-post measure	1	5.290	4.514 N.S.
Diet x pre-post measure	2	.041	N.S.
Residual	15	1.172	
Corrected total	35	3.094	
<b>For average velocity yds/sec</b>			
Diet	2	.080	N.S.
Error A	15	.752	
Pre-post measure	1	1.000	75.000**
Diet x pre post measure	2	.010	N.S.
Residual	15	.013	
Corrected total	35	.362	

\*P<sub>.05</sub> 4.45 C 1 and 15 df.  
\*\*P<sub>.01</sub> 6.20 C 1 and 15 df.

TABLE 3.—A summary of data for diet, blood, and performance measures.

Diet	Pre-post Measure	No.	Mean velocity (V) yds/sec	SD	K+ 3.3-5.5 mEq/L	SD	Hgb 14-18 gm	SD	Hct 41-42 %	SD
Pollen	Pre	6	4.700	.548	4.150	.234	13.233	.156	38.817	.987
	Post	6	5.067	.625	4.150	.485	13.783	.999	39.700	2.559
Placebo	Pre	6	4.650	.653	4.250	.234	13.367	.216	38.700	1.410
	Post	6	4.917	.725	4.050	.152	13.617	.426	39.350	1.249
Protein	Pre	6	4.767	.532	4.150	.197	13.750	.720	39.417	2.278
	Post	6	5.133	.609	3.917	.117	14.200	.576	40.183	1.888
Pre-post measure										
	Pre	18	4.706	.517	4.183	.209	13.450	.528	38.978	1.537
	Post	18	5.039	.605	4.039	.291	13.867	.693	39.744	1.833
Diet										
	Pollen	12	4.883	.567	4.150	.348	13.508	.776	39.258	1.824
	Placebo	12	4.793	.644	4.150	.206	13.491	.333	39.025	1.258
	Protein	12	4.950	.553	4.033	.188	13.975	.636	39.800	1.948
Overall means		36	4.872	.593	4.111	.263	13.658	.650	39.361	1.734

oxygen transport, and it is unlikely that any slight increase in blood viscosity would place any restriction on the efforts of cardiac output to assist oxygen uptake capabilities. Conversely, the combination of continued lysis of red blood cells due to vigorous exercise in any individual over a long period of time, even with a normal diet, could be reflected in decreased hemoglobin and hematocrit levels. An examination of Table 3 reveals, with the exception of the post mean values for the protein supplement diet, Hgb and Hct values remained slightly below normal levels. Whether this suggests that teen-age athletes may need to add protein or iron bearing foods to their normal diet is problematical since the study found similar values in subjects who did and did not use the protein supplement.

### Summary

R. E. STEBEN, P. BOUDREAUX

*The effect of pollen and protein extracts on selected blood factors and performance of athletes.*

Volunteer (18) male high school cross country runners were randomly subdivided into three diet groups for a twelve week, placebo, double blind design, nutrition-performance experiment. Diets 1, 2, and 3 supplemented normal diets with daily ingestion of four pollen extract, four placebo, and four protein extract capsules

respectively. Blood samples drawn from each individual before and after the experiment were analyzed for serum K, Hgb, and Hct levels. The mean velocity of a pre and post three mile run conducted on the same course was the performance measure. An ANOVA, with diet and pre and post measures as the main and split plot respectively, found no significant differences among the diet groups in blood levels of K, Hgb, and Hct. Significant pre and post differences were found in blood levels of K and Hgb. Pre versus post for performance was highly significant. The analysis to compare diets for differences in performance was nonsignificant, and diet x pre-post measure interaction was nonsignificant for all variables. The findings failed to uncover any advantage in taking pollen or protein extracts for improvement or maintenance of K, Hct, and Hgb blood levels or improvement in performance.

### References

1. Ask-Upmark E. E.: Treatment of Prostatitis. Z. Urol., 56, 113, March, 1963.
2. Ask-Upmark E. E.: Prostatitis and its Treatment. Acta Med. Scand., 181, 355, March 1967.
3. Bee Pollen from England. Track and Field News, 29, 7, December, 1976.
4. Bee Pollen: Wonder Drug or Humbug? New York Times, Section, 5, p. 1, February 6, 1977.
5. Bee Prepared. Track and Field News, 28, 48, July, 1975.
6. Binding C. J.: About Pollen. Thorsons Publishers Ltd., 1971.
7. Crowell J. W., Smith E. E.: Determinants of the Optimal Hematocrit. J. Appl. Physiol., 22, 501, 1967.

8. Denis L. J.: Chronic Prostatitis. *Acta Urol. Belg.*, 34, 49, January, 1966.
9. Fijalkowski A. et al.: Results of Studies of Effects of Taking "Pollitabs" and "Stark Protein" Drugs on Improvement of Working Capacity of no weightlifters. A. B. Cernelle Symposium of Sportsmen, London, November, 1973.
10. Georgieva E., Vasilex V.: Symposium on Use of Bee Products in Human and Veterinary Medicine. International Bee Keeping Congress, 23, Summer, 1971.
11. Glomme J.: The Effect of Cernilton on Upper Respiratory Tract Infections. A. B. Cernelle Symposium for Sportsmen, London, November, 1973.
12. Helander E.: Hay Fever and Pollen Tablets. *Grana Palynologia*, 2, 119, 1960.
13. Jormakka M.: An Athletes Diet. A. B. Cernelle Symposium for Sportsmen, London, November, 1973.
14. Kjellberg S., Rudhe U., Sjostrand T.: The Amount of Hemoglobin and the Blood Volume in Relation to the Pulse Rate and Cardiac Volume During Rest. *Acta Physiol. Scand.*, 19, 136, 1949.
15. Klapsch H.: Experiences of Fluaxin, an Anti-Influenza Medicine in Tablet Form. A. B. Cernelle Symposium for Sportsmen, Helsingborg, July, 1972.
16. Kvante E.: The Effects of Nutritive Supplement Substances on Athletes. A. B. Cernelle Symposium for Sportsmen, Helsingborg, July, 1972.
17. Leander G.: A Preliminary Investigation on the Therapeutic Effect of Cernilton in Chronic Prostatovesiculitis. *Svensk Lakartidn*, 59, 3296, 1962.
18. Malstrom S. et al.: Polen as a Prophylactic Against the Common Cold. A. B. Cernelle Symposium for Sportsmen, Helsingborg, July, 1972.
19. Marshall J.: Feeling Fit to Hurt a Lot of Feelings. *Sports Illustrated*, 46, 40, March 28, 1977.
20. Marshall J.: When Irish Guys are Miling. *Sports Illustrated*, 46, 14, February 7, 1977.
21. Millar S. L.: Flower Power Pills. *Track Technique*, 54, 1706, December, 1973.
22. Nuuttila S.: Nutrition Programme for Athletes. *London Sunday Times*, p. 21, August 5, 1973.
23. Oscai L., Williams B., Hertig B.: Effect of Exercise on Blood Volume. *J. Appl. Physiol.*, 24, 622, 1968.
24. Poortmans J. R.: Protein and Amino Acids Symposium on Sportsmen Nutrition, Waszawa, October, 1975.
25. Rose K.: Warning for Millions: Intense Exercise can Deplete Potassium. *The Physician and Sports Medicine*, 3, 67, May, 1975.
26. Saito Y.: Diagnosis and Treatment of Chronic Prostatitis. *Clin. Exp. Med.*, 44, 614, June, 1967.
27. Steben R., Wells J., Harless I.: The Effects of Bee Pollen Tablets on the Improvement of Certain Blood Factors and Performance of Male Collegiate Swimmers. *J. of the National Athletic Trainers Association*, 11, 124, Fall, 1976.





### Effect of Cernitin™ and Hydrolysed Protein on Adaptation to Physical Effort in Subtropical Conditions

**Igancy Dabrowski**

**Supervisor: Professor Zbigniew Jethon**

**Doc. Dr. Adam Klimek**

**Doc. Dr. Zenon Wazny**

**Doc. Dr. Erazm Wasilewski**

#### INTRODUCTION

The work on the effect of Cernitin™ and hydrolyzed protein in subtropical conditions was carried out in order to assist Polish servicemen serving in Polish Military Special units which are part of the United Nations Peacekeeping Force in the Near East. The men selected for the service in the Polish Military Special Unit were chosen on the basis of usefulness of their professional skills, high degree of discipline and awareness of their role in the Near East. It appeared that in practice not all the men selected for the Special Unit performed well under radically changed climatic conditions. This was mainly due to the fact that factors like physiological and fitness adaptation were not sufficiently considered in the selection of men for duties in subtropical conditions. The servicemen of the Polish Military Special Unit were transported to Egypt by air and consequently the process of acclimatization began abruptly on reaching the destination. The servicemen were allocated various tasks connected with their duties immediately on arrival. Under these conditions it seems important to find out whether the process of acclimatization could be speeded up by the use of some additional substances. Cernitin™, which is known as a substance increasing the nonspecific resistance of the organism as well as increasing physical fitness and capability, was chosen for this purpose (8). It is also known that the effect of Cernitin™ is enhanced by administration in conjunction with hydrolyzed protein which acts as a source of ergogenic amino acids (11).

The purpose of the investigations was as follows:

- Evaluation of changes in selected parameters of physical fitness and capability as well as in some psychological functions during the 20 weeks period of duty in subtropical conditions.
- Establishing which factors of physical fitness undergo the greatest changes in the first phase of stay in subtropical conditions.
- Observations of effect of Cernitin™ and hydrolyzed protein on parameters of physical fitness and capability as well as on psychological functions and subjective sense of well being.
- Establishing whether the use of Cernitin™ in conjunction with hydrolyzed protein facilitates return of physical fitness and capacity in subtropical conditions to their initial level.

#### METHODS AND ORGANIZATION OF THE INVESTIGATIONS

The investigations were carried out on three groups of soldiers all of whom were drivers of army vehicles aged 20-23 years old, average weight 66.28 kg and average height 170.9 cm. The soldiers belonged to three platoons engaged in the same tasks. Each platoon consisted of 30 men. Physiological examinations were carried out on 25 men from each platoon. All the men in the examined groups lived under similar conditions in regard to work, nutrition, and rest. Each group received different biologically active agents as follows:

- Group 1 did not receive any biologically active agents.
- Group 2 received Cernitin™ twice daily in the form of two tablets “Pollisport™”.
- Group 3 received twice daily: two tablets of “Pollisport™” and 1 g of hydrolyzed protein in the form of “Pollen Stark™ Protein” tablets.

These biologically active agents were given to Groups 2 and 3 after breakfast and dinner.

For the assessment of physical fitness the following test were used:

1. Pull-ups on the bar – measured strength.
2. Zigzag running – measured agility.
3. 100-m sprint – measured speed.
4. 1000 m runs – measuring endurance.
5. Long jump – measuring strength, speed, and agility.

Physiological testing on a bicycle ergometer used a load of 2 watt/kg of body weight maintained for 6 minutes at a speed of 60 revs/ min. The following parameters were measured before, during and at the end of the tests:

1. Heart rate – measured electrocardiogram.
2. Blood pressure – systolic and diastolic – measured by sphygmomanometer.
3. Cardiac stroke volume and minute volume – calculated from the Starr formula.
4. Oxygen uptake – calculated from Astrand tomogram.
5. Oxygen pulse.
6. Blood levels of: lactic acid, pyruvic acid, creatine phosphokinase (CPK), asparagine aminotransferase, and lactic acid dehydrogenase. All estimations were made using “Eskalab” reagents (Smith, Kline Instr. Inc.).

The values obtained for lactic acid and pyruvic acid were used for the calculation of lactate excess.

The following psychological tests were carried out in conditions of rest:

1. Speed and accuracy of observation – using Toulouse-Pieron Test.
2. Tempo of motor activity – using stroke test.
3. Speed and accuracy of mental activity – using Bourdon test.
4. Capacity of concentration – using Wiersma test.
5. Sense of well being – using 7 point self-assessment scale.

Preliminary investigations were carried out in Poland 2 weeks prior to the departure to the Near East and during the term of duty in weeks 1, 2, 3, 4, 6, 8, 12, 16, and 20. All the results were statistically analyzed using appropriate tests.

## RESULTS AND THEIR EVALUATION

Analyzing the general characteristics of the subjects it was evident that during a 20-week term of duty in the Near East the body weight was decreased but the change was not statistically significant. The height of subjects remained unchanged.

Tests showed that the stay in subtropical conditions impaired the subjects' physical fitness. The greatest changes were observed in the 1000-m run, long jump and 100 m sprint. The results of “pull-ups” on the bar and zigzag run also showed a decline but the differences were slight and not statistically significant.

In the groups given both Pollisport™, and hydrolyzed protein the level of strength remained almost the same and even slightly increased during the later period of the stay in the Near East. The results of the 100-m sprint in Group 3 (receiving both substances) showed at first a decline (similar to that in the other groups) followed by a return to initial values in the fourth week of the stay. In Group 2 receiving Pollisport™ only, this return to initial values took place in the 12th week of the stay, while in the control group not receiving any substances values did not return to baseline levels during the stay in Egypt.

In the 1000 m run the group receiving both substances showed a slight increase in time, noticeable during the first phase of the stay in Egypt. In the other two groups this increase was statistically significant and returned to initial values after 8-20 weeks (Fig. 1).

In the long jump the group receiving Pollisport™ and “Pollen Stark™ Protein” showed a decline of results as late as the fourth week of their stay, while in the other two groups this decline was still observed after 6-12 weeks (Fig. 2).

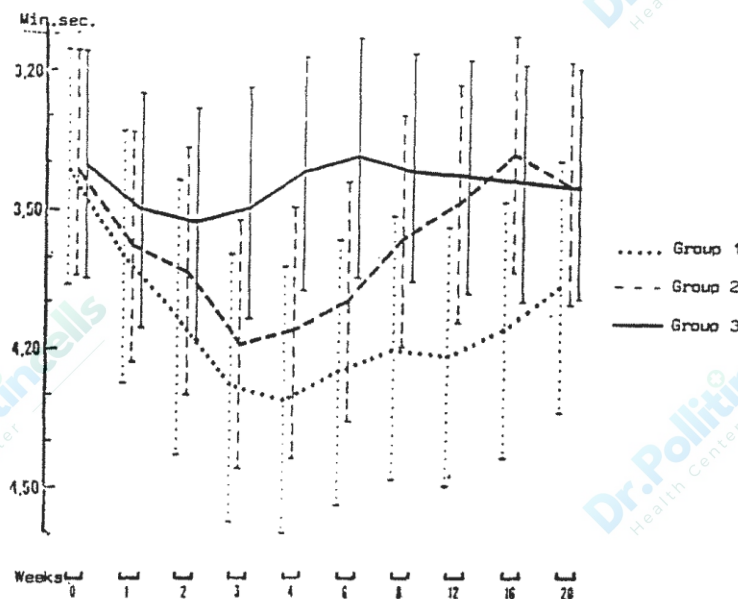


Fig. 1 Changes in results of 1000 m race before and during the stay in the Near East.

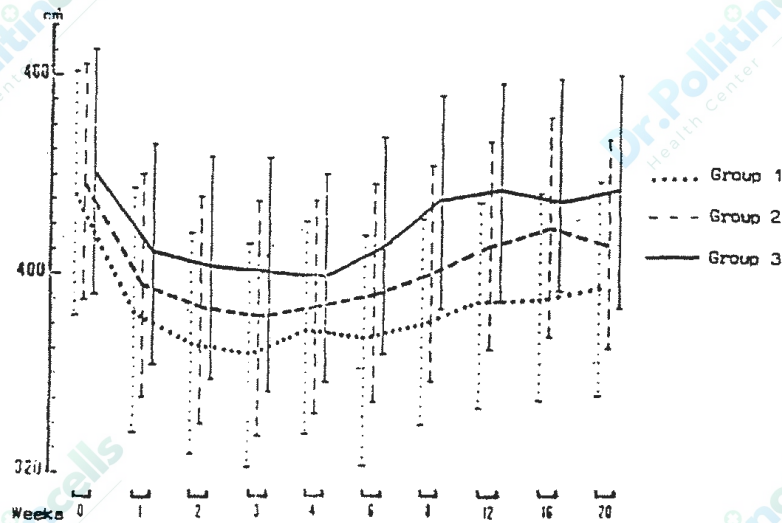


Fig. 2 Changes in running long jump results before and during the stay in the Near East.

The results of physiological test showed that changes in the chosen parameters at rest are slight and statistically insignificant. Nevertheless, the results suggest an increase in the activity of the parasympathetic nervous system; the heart rate, systolic blood pressure, cardiac minute volume, and enzyme levels (CPK and LDH) were lowered. The stay in the Near East had a particularly marked effect on measurements of physiological parameters during standard loading in the bicycle ergometer test. The changes in the physiological cost of effort were particularly noticeable in the first 3-4 weeks of stay in the Near East, returned later to the initial values obtained before the posting to the Near East.

The effect of Cernitin™ and hydrolyzed protein was observed in the rate at which the physiological cost of effort returned to the normal value. In Group 3 receiving both substances the phase of physiological acclimatization was shortened by about 2 weeks (from 6 to 4 weeks). This is shown by changes in minute volume (Fig. 3), lactate levels (Fig. 4) and aspartate aminotransferase levels (Fig. 5).

The results of psychological investigations indicate that in the Near East changed all the psychological parameters investigated, including the subjective sense of well being of the individuals tested. Only the tempo of motor activity did not show statistically significant changes. The greatest changes were found in concentration and observation ability. The remaining psychological functions were affected to a lesser degree.

The subjective sense of well being of the individuals investigated was impaired; this effect lasted a long time (up to 8-12 weeks in groups not receiving any substances) during the stay in the Near East (Fig. 6). Administration of Pollisport™ and hydrolyzed protein in the first phase slightly speeded up the correction of psychological disturbances. These substances were shown to be beneficial by improving the sense of well-being lessening disturbances and accelerating return to normal. Ability to concentrate was least adversely affected in the third group, given both Cernitin™ and hydrolyzed protein, and showed the fastest return to normal (Fig. 7).

All the physical fitness investigations were carried out using a set of tests routinely applied in the Polish Army (14). The tests were used according to general instructions for evaluating particular motor characteristics (3, 5, 18).

The physiological methods were based on the correlation between effort, capacity and post-exercise in the parameters analyzed. It is particularly relevant in the case of the haemodynamic parameters and maximum oxygen uptake – which form the basis of the evaluation of effort capacity (15).

In the psychological investigations use was made of methods tried previously in the Near East and developed by Galubinska et al (7).

Analyzing the results one observes that the work on the bicycle ergometer resulted in a rise of the heart rate to 170/ min. These figures are in excess of the norm allowed for the work in high temperatures as given by Brouh and Wenzel (2, 22). Other authors also report a decrease in capacity to work in a high temperature environment. The authors linked this fact with the inability of the thermo regulating mechanism to cope with excessive external temperature in addition to the heat generated during work (19, 23 and others).

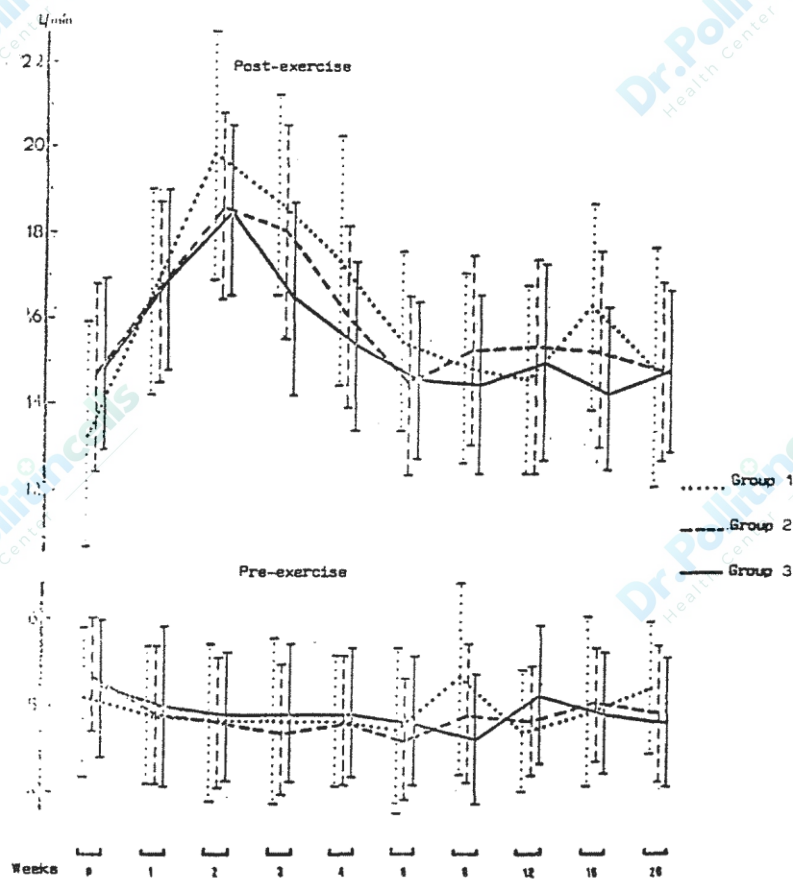


Fig. 3 Changes in cardiac minute volume at rest and post-exercise before and during the stay in the Near East.

The decline in physiological effort capacity, particularly in the early stages of the stay in Egypt is supported by the findings of many authors; e.g. Brouha et al (1) found that the length of the distance travelled per one heart contraction is decreased by a quarter in conditions where the temperature is 41°C and relative humidity 43%.

Our investigations showed that the most significant and long-lasting changes occurred in the biochemical parameters. Haemodynamic parameters were affected to a much lesser degree and differed slightly from the initial values obtained before the transfer to the Near East and returned to initial values more rapidly.

The beneficial effect of administration of Cernitin™ combined with minerals, vitamins and hydrolyzed protein on effort capacity was observed by many authors. The administration of these substances to sportsmen of various disciplines during training accelerated the achievement of peak form (4, 6, 11, 12, 13). Cernitin™ also increases tolerance to changes in climatic condition, and general nonspecific resistance of the body (17, 8, 9, 16, 20). It has been suggested that this effect is due to the presence of plant growth hormones in Cernitin, while vitamins and mineral salts are present in optimal proportions. Experiments carried out on animal receiving Pollisport™ tablets showed among other effects an increase of enzymatic protein in the muscles and an increase in intestinal aminoacid absorption (21). These findings might explain that fact, confirmed by our own investigations, that Pollisport™ given in conjunction with hydrolyzed protein was more effective than given on its own. This problem requires more extensive investigation.

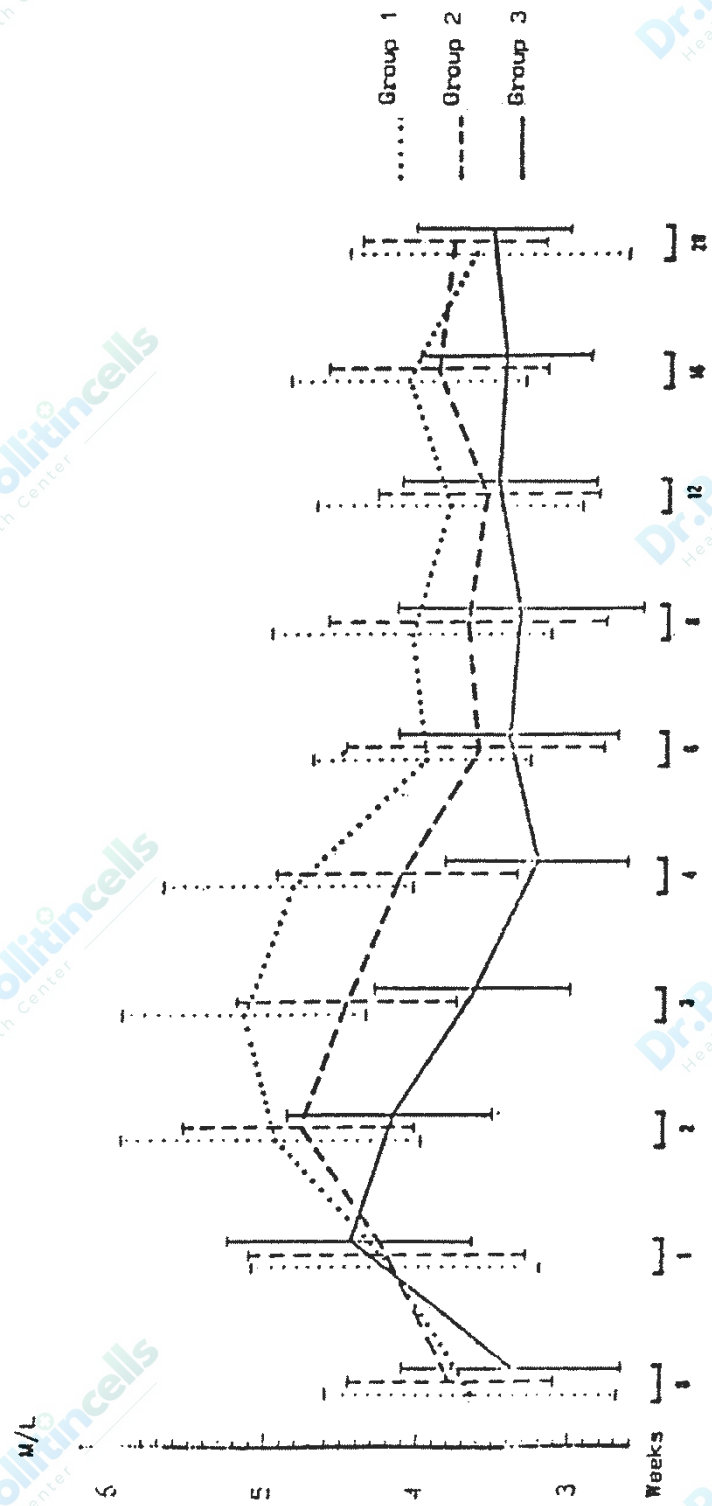


Fig. 4 Changes in lactate level before and during the stay in the Near East.

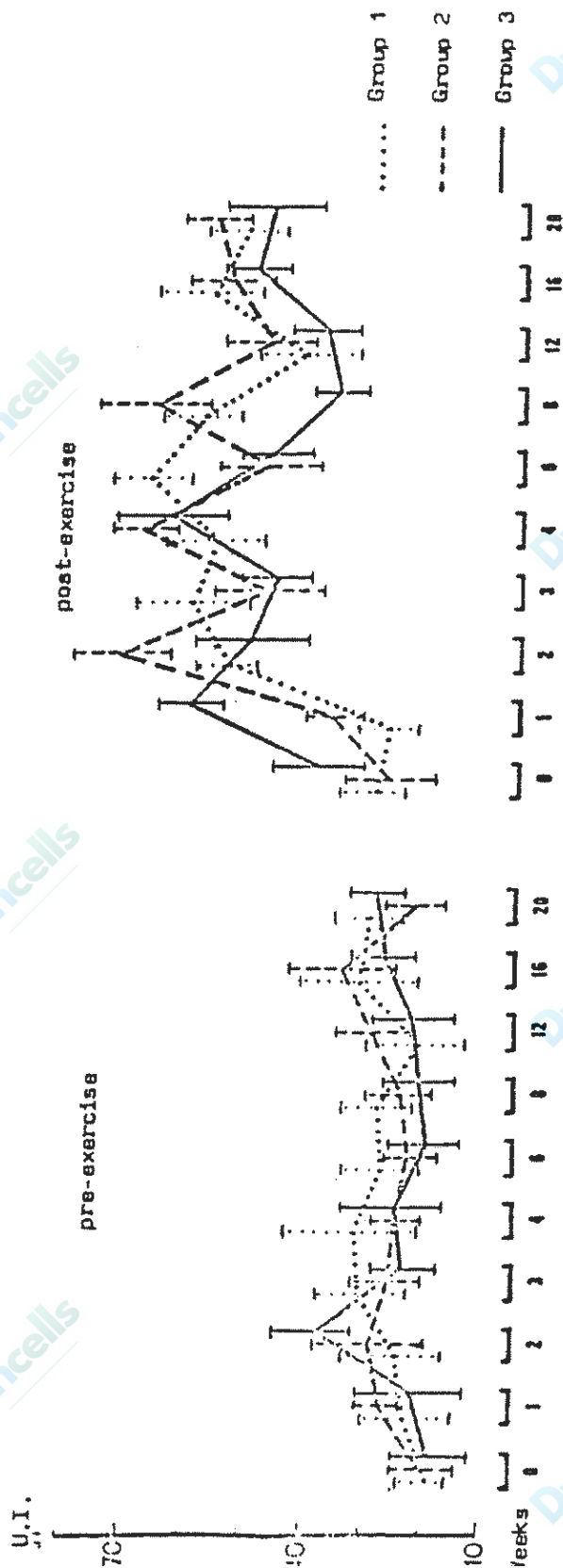


Fig. 5 Pre- and post-exercise changes in aspartate aminotransferase activity before and during the stay in the Near East.



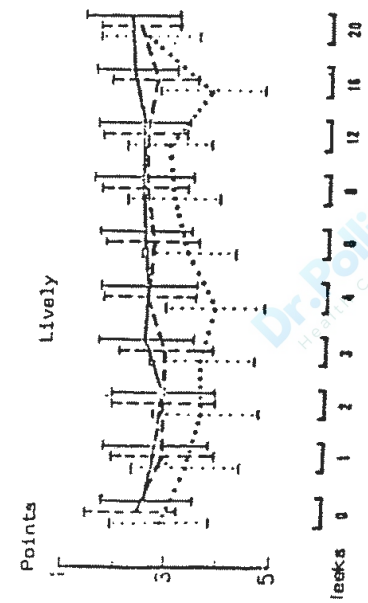
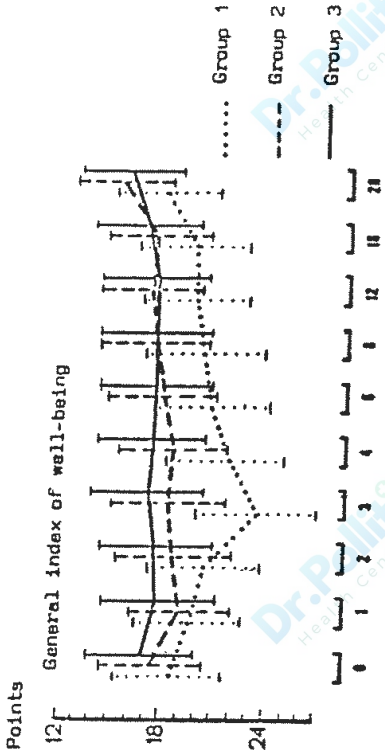
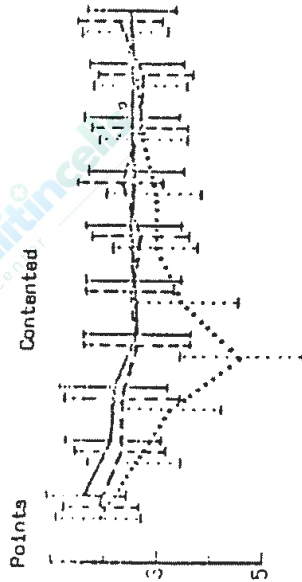
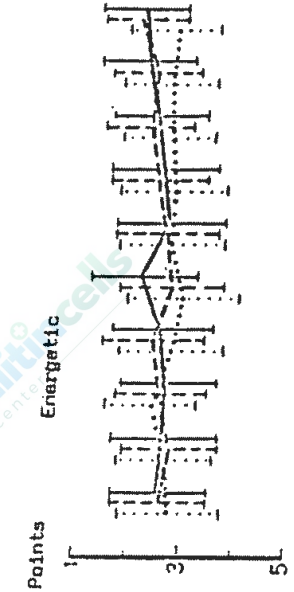


Fig. 6 Changes in selected parameters of subjective sense of well-being before and during the stay in the Near East.

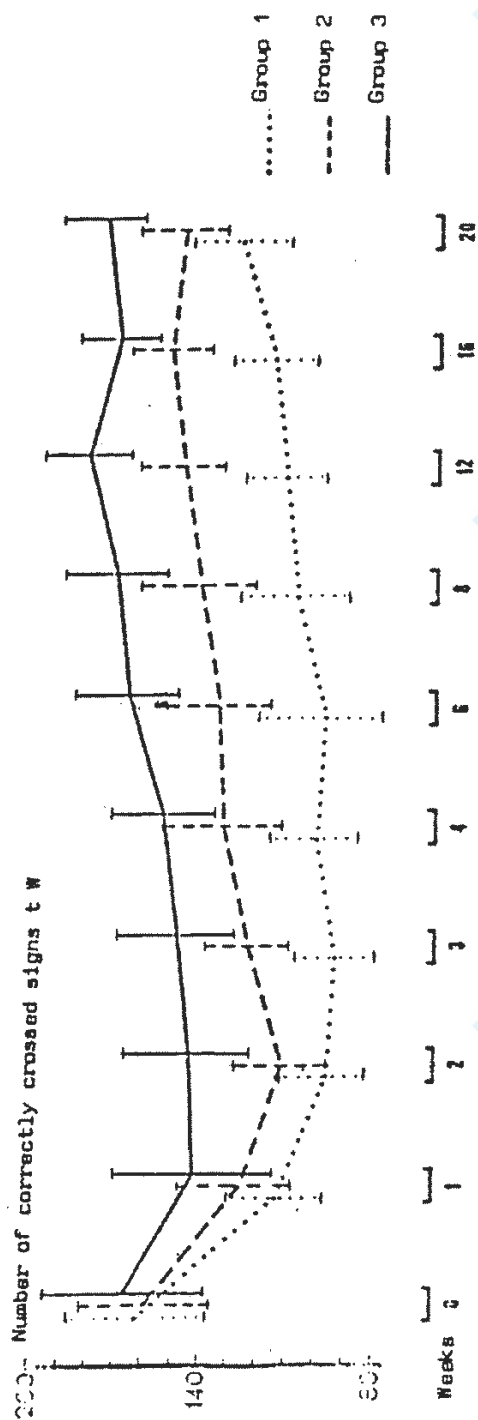


Fig. 7 Changes in ability to concentrate before and during the stay in the Near East.

## CONCLUSIONS

1. Rapid transport (by air) of Polish Military Special Unit drivers into sub-tropical conditions resulted in reduced physical fitness, physical capacity, ability of observation, and adversely affected their subjective sense of well-being. The last two factors are essential in the work of professional drivers and their impairment can lead to the occurrence of road accidents.
2. The period of acclimatization to subtropical conditions was much longer in the servicemen studied than had been assumed on the basis of preliminary tests. The average length of this period was 4-6 weeks, depending on the experimental group.
3. The most significant and longest-lasting changes were found in the group, which received neither Cernitin™ nor hydrolyzed protein.
4. Administration of Cernitin™ and hydrolyzed protein significantly shortened the period of acclimatization as measured by the rate of normalization of physical fitness factor; it also had a beneficial effect on psychological function and on the subjective sense of well-being. It was particularly noticeable in the group receiving both of these substances.
5. The beneficial effect of Cernitin™ and hydrolyzed protein in physical fitness and capacity is probably related to the effect of these substances on oxygen debt tolerance and to an increase in anaerobic capacity.

## REFERENCES

1. Brouha L.: Effect d l' environnement sur les reactions physiologiques an cours d' un travail repete. "Le travail humain" 1965, No. 28, p. 5.
2. Brouha L., Smith P., De Lanne R., friaxfield M.: Physiological reactions of men and women during muscular activity and recovery in various environments. "Journal Appl. Physiol." 1961, Vol. 16, p.133.
3. Clarke H.: Application of measurements in physical education and hygiene (Warsaw 1967).
4. Coronelli P.: The experimental testing of semiprofessional, and amateur footballers. Symposium for Sportsmen. Eelsinborg 1972. "Pollisport™" on professional, Report of Pollisport™. AB Cernelle Engeihoim.
5. Denisiuk L., Milicer H.: The development of motor ability in young chidren and chidren of school age. PZWS Warsaw 1969.
6. Fijalkowski A., Lemieszek G., Luczak.Szczurek A., Roguski K., Stepien B., Zateska E., Zarzecki K.Report on investigation of the effect of preparation "Pollisport™" and "Pollen Stark™ Protein" on the performance of weight lifters. PKOI 1973.
7. Galubinska K., Pecion T., Lechowska-Pastek M., Kacprzak W. Effect of Near East Environment on the psychological state of Polish servicemen in Polish Military Special Unit. Lekarz Wojskowy (Army Doctor 1979, 1-2, 22).
8. Glomme J.: Studies on the effect of Cernitin™ (pollen extract) in the diet, using animal test material. Y. Keshygeinisk Institute Dept. of Social Hygene, Oslo 1972.
9. Glomme J.: The effect of Cernitin™ on upper respiratory tracts infections. University Heart Service, University of Oslo. International Symposium for Sportsmen, Sportwomen and Coaches, London 1973.
10. Holmer I.: Oxygen uptake during swimming in man. "Journal Appl. Physiol." 1972, 701.33, p.502.
11. Jethon Z.: Der Emfluss von Stark Protein auf die physische und psychische Arbeitskapazität in subtropischen Klima. Symposium: Aminosäuren und Sportliche Hochleistung. Hamburg 1978.
12. Jethon Z.:Effeti dilla somministrzione additive di minerali e vitamine sulle capacita fisiche di atleti. Conference: coadiuvarti delle resetenze orgeniche nella nutrizone Firenze 1978.
13. Jethon Z., Luczak-Szczurek A., Put A.: Effect of additional intake of mineral salts and vitamins in the effort capacity of certain sports. 5 International Symposium on Nutrition in Sportsmen, Warsaw 1975.
14. Catalogue of tests and norms used in physical education. Ministry of Defence, Warsaw 1975.
15. Mellerowicz H.: Ergometrie. Munchen-Berlin-Wien 1975.
16. Noyes C.: The use of Cernitin™, an extract of organic pollen, to increase body weight and to increase resistance toward infections. Report of Pollisport™. Pymposium for Sportsmen. Helsinborg 1972.
17. Phammacological Studies of Cernilton® Cernitin™ BGX™ T60™. Tobishi Pharmaceutical Co. Ltd., Tokyo 1968.
18. Pilicz S. Methods of assessment of physical fitness in students. Wychowanie Fizyezni I Sport (Physical Education and Sport) 1963, No. 4 p.447.
19. Pugh L.: Rectal temperatures, weight losses and sweat rates in marathon running. "Journal Appl. Physiol." 1967, vol. 25 p.347.
20. Soulairac A.: "The effect of "CP" powder on the mortality, changes in body weight, food conversion ratio, speed of cicatrization in male and female rats. Unpublished report AB Cernelle.
21. Szymanski A. Effect of Cernitin™ on the absorption of amsnoacids in the intestine (Unpublished).
22. Wenzel H.: Indoor climatic conditions: Physiological aspects evaluation and optimum levels. Ergonomics and Physical Environmental factors. Ilo, Geneva 1970, p.287.
23. Wyndham C., William von Rahden: A physiological basis of the "optimum" level and energy capacities. 'Nature' 1962, no.195, p.1210.



### Effect of Nutritional Substances\* on Work Capacity during Stay in a Subtropical Climate

**Ignacy Dabrowski, M.D.**

**Academy of Physical Education, Cracow, Poland**

Through a recently performed experiment involving 90 Polish soldiers – divided into three groups during a stay in a subtropical climate – it was possible to demonstrate deterioration in physical and mental capacity during the period of adaptation. Administration of preparations containing pollen extracts and amino acids significantly improved physical performance, for example, in long-distance running, long jumping, the formation of lactate after exercise on a bicycle ergonometre, as well as concentration and subjective well-being.

As yet, no explanation can be offered for the effects observed.

In 1978, some Polish soldiers participated in the peacekeeping forces stationed by the United Nations at the Suez Canal following the Egyptian-Israeli conflict.

The region has a subtropical climate, with an average temperature approaching 30°C during the six months of summer. Past experience has shown that such temperatures normally affect people from more temperate climates. The result is that work capacity and physical performance are impaired over a fairly long period of adaptation. In addition, concentration and subjective well-being are adversely affected.

In order firstly to survey the extent of such changes and secondly to establish whether the changes could be affected by the administration of pollen extract and amino acids, 90 soldiers were subjected to a thorough examination before and during a 5-month stay in this region. The chosen method of therapy was partly based on an experiment by Jethon and others on weightlifters, in which these preparations were shown to affect significantly the weightlifters' physical performance.

#### Test Subjects:

90 Polish soldiers, with special training as drivers, were assigned at random to 1 of 3 companies to serve at the Suez Canal. Ages varied between 20 and 33 years, average weight was 66 kg and average height was 171 cm. All were in good health, and were mentally and physically fit.

#### Procedure:

Soldiers in two of the three platoons were given nutritional preparations, while the third served as a control group. One of the first two groups was given only pollen extract (Pollitab Sport – 4 tablets – daily, at mealtimes). The second group also received extra amino acids ("Stark-protein", 1/4 g daily), also at mealtimes. All groups had identical diets, duties and training programs.

The soldiers' physical performance was analyzed before their departure from Poland, then after 1, 2, 3, 4,

\*Pollen extracts and amino acids. isks in the subtropical climate.

A number of measures of performance were used to evaluate physical performance, including running for various periods of time, press-ups, long-jumping and increases in the blood's level of lactic acid following

standard exercise on a bicycle ergonometre. Physical performance was measured at the same times of day, including by the Bourdon test of speed and mental efficiency, the Wiersma test of concentration, and subjective assessments of well-being on an analogue 7-point scale.

The results obtained were dealt with using normal statistical procedures and the Student t-test.

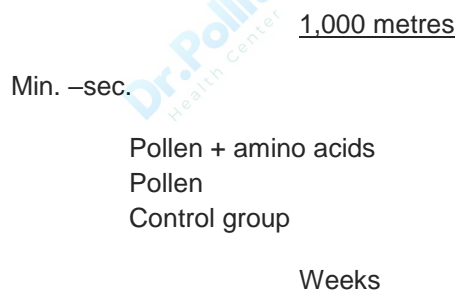
### Results:

Full results from the experiment were published in a doctoral dissertation (Dabrowski, 1980). The main positive findings are summarized below.

In practically all indications obtained of physical and mental performance, there was a significant, distinct deterioration during the first two to three weeks in the specific climate. This corresponds fully to findings from previous experiments (Falkiewics, 1966, 1971 and Galubinski, 1979). A gradual recovery was then observed during the 5-month long stay, but the degree of recovery differed considerably from individual to individual. In the majority of individuals and variable values, the recovery never matched the original values.

The interesting result of this experiment was the consistent and typical difference obtained for most variables throughout the three groups. Recovery in the control group was slowest and the least complete, while recovery in the groups given the nutritive was clearly fuller and more rapid. The recovery in performance was greatest in the group given the combination of pollen extract and amino acids. A comparison between the latter group and the control group revealed a significant difference ( $p < 0.01$ ) for 1,000 metres running, long jumping, lactate increase after exercise and tests of concentration and subjective well-being. (Figures 1-5).

### Figure 1



### Figure 2



Pollen + amino acids  
Pollen  
Control groups  
Weeks

Figure 3

mMol/l

Lactate  
Control  
Pollen  
Pollen + amino acids  
Weeks

Figure 4

Points

Concentration  
Pollen + amino acids  
Pollen  
Control group  
Weeks

Figure 5

Points

Well-Being  
Pollen + amino acids  
Pollen  
Control group  
Weeks

Discussion:

This experiment clearly confirms previous findings – for example, by Jethon and others –that physical performance is improved by the administration of certain nutritional substances. It may also be said that the changes observed are remarkably substantial and it would be desirable for the results to be explained, for example, by metabolic change ( lactate formation during exercise, enzyme effect, etc.). However, such

an explanation cannot easily be offered without further research. Although amino acids make a valuable contribution to physical build-up and enzyme synthesis, the quantity of protein administered is too little, in comparison to a normal daily protein intake (70 g), to have any significant impact on the nitrogen balance. It is possible that administering a balanced intake of all the amino acids in this way could be beneficial, in comparison with intake via a normal diet. However, since both milk and meat formed part of the diet, this hypothesis does not provide an adequate explanation either. Similarly, it is hard to account for the effect of the pollen extracts. In previous experiments, it has been observed that these extracts had a “performance-raising” and roborant effect (Dubrisay, 1978). However, the problem here is that an extract knowledge of all the active ingredients in pollen extracts is not available. A number of different growth steroids with growth-stimulating effects in plants could be the active agents.

Nevertheless, considerably more research is needed in this area if these strong, but difficult to explain, correlations between nutritional preparation and performance are to be accounted for.

#### Summary:

Through a recently performed experiment involving 90 Polish soldiers – divided into three groups during a stay in a subtropical climate – it was possible to demonstrate a deterioration in physical and mental capacity during the period of adaptation. Administration of preparations containing pollen extracts and amino acids significantly improved physical performance, for example, in long-distance running, long jumping, the formation of lactate after exercise on a bicycle ergometer, as well as concentration and subjective well-being.

As yet, no explanation can be offered for the effects.

#### Bibliography

Dabrowski, Ignacy: Effect of Cernitin and hydrolysed protein on adaptation to physical effort in subtropical conditions. Thesis, Warsaw, 1980.



Dubrisay: Une nouvelle thérapeutique naturelle des états de dénutrition protéique résultants d'une étude Clinique menée en double-aveugle. Gazette Medicale 79, No. 40, pages 7674-7683. 1982.

Falkiewicz, B.: Biogeographical aspects of human adaptation. Roczniki Naukowe WSWF (Scientific Annals of WSWF), Posan. 1981, 19, 281.

Falkiewicz, B.: Effect of seasons on thermal adaptation. Roczniki Naukowe WSWF (Scientific Annals of WSWF), Posnan. 1966, 12, 373.

Galubinska, K., Plecion, T., Lechowsla-Postek, M., Kacprzak, W.: Effect of the Near East environment on the psychological state of Polish servicemen in Polish Special Military Units. Lekarz Wojskowy (Army Doctor), 1979, 1-2, 22.

Jethon, Z.: Physical Capability. Roczniki Naukowe WSWF (Scientific Annals of WSWF), Warsaw. 1979, 24, 91.

Jethon, Z. Luczak-Szczuewk, A. & Put, A.: Physical performance for weightlifters after administration of nutritional substances. Biol. Med. 1/82.



### Effects of Pollen Extract upon Adolescent Swimmers

**R.J. Maughan, BSc, PhD and S.P. Evans, BSc**

Department of Sports Science, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF

#### Abstract

Many competitive sportsmen in this country regularly use pollen extract as a dietary supplement in the belief that it can lead to an improvement in performance. We have investigated the effects of a six-week course of pollen extract administration on a variety of physiological parameters in a group (n=20) of adolescent swimmers. At the time of the study, all subjects were training on a daily basis. During the course of the study, maximum oxygen uptake increased in both the treatment group and the placebo group, no differences between the response of the two groups being observed. Vital capacity showed a significant increase in the treatment group, but not in the placebo group. The results indicate that no positive benefit was obtained from the use of pollen supplementation. However, the number of training days missed due to upper respiratory tract infections was much less in the pollen treatment group (4 days) than in the placebo group (27 days). In a study of longer duration, this difference could lead to an improved performance by the pollen treatment group due to fewer interruptions to training.

**Keywords:** Performance, Swimming, Pollen, Diet.

#### Introduction

The sporting situation is an intensely competitive one in which athletes will search for any improvement in performance, however small it may be. Accordingly, a wide range of ergogenic aids in the form of dietary supplements is extensively employed in this field. One of the more recent innovations is the use of a pollen extract which has been claimed to produce improvements in athletic performance. This product has been marketed by A. B. Cernelle as "Pollitabs," containing pollen extract as well as vitamins B, C and E. The two main beneficial effects of this product have been claimed to be an increased resistance to respiratory tract infection (Lindahl, 1978; Mark-Vendel, 1978) and an effect on protein synthesis (Dubrisav, 1972). A large number of the studies carried out in this area have been rather poorly controlled, and it was the aim of the present study to determine whether or not administration of a pollen extract could influence performance.

#### Methods

Twenty young competitive swimmers were used as subjects, comprising 16 males and 4 females. Mean age of the subjects was 15.7 years (range 11.5 to 20 years). All subjects were training on a daily basis, and all had been training for some months prior to the beginning of the test period. Subjects were divided into two groups, each containing 8 males and 2 females, the selection being random otherwise. A series of tests as detailed below, was then performed on each subject. These tests were repeated after a six-week period during which subjects ingested either Pollitabs or placebo (cod-liver oil capsules). Treatment administration was performed on a double-blind basis in order to minimize any subjective bias. Differences between groups were assessed by the Student's t-test, and differences within each group as a result of treatment were assessed by a paired t-test.

Height and weight were recorded, in addition to percentage body fat according to the method of

Durnin and Rahaman (1967). Right and left hand grip strength measurements were made using a grip dynamometer (Taketiki Vogyo, Japan). Quadriceps isometric strength (MVC) and endurance time at 50% of MVC were measured using an isometric chair constructed after the manner of Thorstensson (1976).

Maximum oxygen uptake ( $VO_2$  max) was assessed by using stepwise increases in workload on a friction braked Monark bicycle ergometer. The attainment of  $VO_2$  max was established according to the levelling off criterion of Astrand and Saltin (1961). Respiratory variables were assessed on a P.K. Morgan automatic gas analysis system comprising a Fleisch pneumotachograph, paramagnetic  $O_2$  analyzer and infra-red  $CO_2$  analyzer.

Vital capacity (VC) and forced expiratory volume ( $FEV_1$ ) was obtained using a Vitalograph spirometer.

Haemoglobin (Hb) concentration was estimated by conversion to cyanmethemoglobin using Drabkin's reagent (BDH); haematocrit was obtained using a Hawksley micro-haematocrit system.

## Results

Results are presented in Table 1.

Body weight and height increased in both groups during the test periods ( $p < 0.01$ ). There were no differences between groups either before or after the test period. There were no differences in body fat content between the groups, and no change took place in either group.

### Strength and Endurance Tests

No significant changes took place in right hand grip strength, isometric leg strength and isometric endurance time, with no differences between the groups. However left hand grip strength showed significant improvement in both groups ( $p < 0.05$  in both groups). No differences, however, were found to exist between the groups.

### Blood Measurements

A significant decrease ( $p < 0.05$ ) in blood haemoglobin concentration took place during the trial period with mean ( $\pm$  SD) reductions of  $1.05 \pm 0.91$  and  $0.61 \pm 0.67$  g/100 mls for placebo group and Pollitabs group respectively.

The haematocrit (percentage) was found to decrease in the Pollitabs group significantly ( $p < 0.05$ ); however there were no differences between the two groups.

### Aerobic Capacity

TABLE I

Comparison of Pollitabs and placebo groups. Values are means  $\pm$  SEM. The right hand column shows the differences between the changes observed in the two groups. A full explanation of the tests employed is given in the text.

	Test Group			Control Group			
	Pre	Post	Difference	Pre	Post	Difference	Test v Control
Height (cm)	167.1 $\pm$ 2.2	167.8 $\pm$ 2.2	+0.7	165.2 $\pm$ 3.7	165.8 $\pm$ 3.7	+0.6	+0.1
Weight (kg)	56.2 $\pm$ 3.3	58.3 $\pm$ 3.3	+2.1	58.2 $\pm$ 3.1	60.0 $\pm$ 2.8	+1.8	+0.3
Body fat (%)	16.0 $\pm$ 1.2	15.4 $\pm$ 1.1	+0.4	18.0 $\pm$ 2.0	18.5 $\pm$ 2.0	+0.5	-0.1
Grip strength (R, kg)	38.8 $\pm$ 4.0	39.6 $\pm$ 4.0	+0.8	37.1 $\pm$ 2.4	36.7 $\pm$ 2.1	-0.4	+1.2
Grip strength (L, kg)	35.3 $\pm$ 3.6	36.6 $\pm$ 3.6	+1.3	34.1 $\pm$ 2.1	35.8 $\pm$ 2.2	+1.7	-0.4
Leg strength (kg)	40.7 $\pm$ 3.7	44.8 $\pm$ 3.4	+4.1	42.8 $\pm$ 2.3	47.0 $\pm$ 4.0	+4.2	-0.1
Endurance (sec)	81 $\pm$ 6	72 $\pm$ 8	-9	73 $\pm$ 7	72 $\pm$ 8	-1	-8
VC (l)	4.03 $\pm$ 0.33	4.99 $\pm$ 0.30	+0.16	4.88 $\pm$ 0.38	4.96 $\pm$ 0.38	+0.08	+0.06
FEV <sub>1</sub> (%)	86.8 $\pm$ 2.0	85.9 $\pm$ 1.6	-0.9	81.0 $\pm$ 1.0	82.0 $\pm$ 1.1	+1.0	-1.9
$VO_2$ max (l/min)	3.05 $\pm$ 1.8	3.30 $\pm$ 2.0	+0.25	3.05 $\pm$ 0.25	3.32 $\pm$ 0.21	+0.27	-0.02
$VO_2$ max (mls/kg/min)	54.4 $\pm$ 2.1	56.6 $\pm$ 1.4	+2.1	52.6 $\pm$ 2.2	55.1 $\pm$ 1.8	+2.5	-0.4
Hb (g%)	16.8 $\pm$ 0.3	16.0 $\pm$ 0.3	-0.8	16.7 $\pm$ 0.4	15.6 $\pm$ 0.5	-1.1	+0.3
Hct (%)	43.5 $\pm$ 0.9	42.3 $\pm$ 0.9	-1.2	42.0 $\pm$ 1.4	41.1 $\pm$ 1.3	-0.9	-0.3

### Anthropometric Measurements

The  $VO_2$  max, expressed in l/min, showed a significant improvement in both the Pollitabs and

placebo group ( $p < .05$ ), with mean ( $\pm$ SD) increases of  $0.27 \pm 0.09$  and  $0.24 \pm 0.09$  l/min respectively. If allowance is made for the increase in body weight which occurred during the test period, this increase in  $VO_2$  max, expressed in ml/kg/min does not assume statistical significance.

#### *Respiratory Parameters*

Vital capacity increased considerably in the group taking Pollitabs ( $p < 0.05$ ); a small increase was also observed in the control group, but this did not attain statistical significance. No significant difference was found between the groups.

Force expiratory volume in 1 second was not found to change in either group nor was any significant difference found between the groups.

#### **Discussion**

The subjects used for the present study were healthy adolescents differing from the normal population only in that they were all engaged on a strenuous programme of physical training. They may thus be considered to represent the group at which the beneficial effects of pollen supplementation are aimed.

If the body weight and height of these subjects are compared with non-athletic children of comparable age, almost all were heavier and taller than average (Bayer and Bayler, 1976); this is in agreement with results obtained by Eriksson et al (1977) for a comparable population of swimmers. The results did not indicate the administration of Pollitabs had any effect on body weight, height or body fat content. It would not, however, be expected that any such effects would become apparent within the time scale of this experiment.

The normal training programme undertaken by the subjects included two weight training sessions weekly, with the aim of increasing muscular strength. In spite of this, there were no significant increases in either group recorded for right-hand grip strength or for quadriceps strength. In contrast to this finding the left-hand

grip strength showed comparable increases in both groups. This may be explained by the fact that the left-hand strength is generally weaker than the right; any bilateral training carried out would thus represent a greater stimulus to the left side and consequently produce a greater improvement in performance.

The significance of the changes in vital capacity is not immediately clear. The results show an increase in VC in the Pollitabs group but not in the placebo group. This change, however, is not sufficiently large to cause a significant difference to exist between the two groups. The values obtained for all subjects in the present study are higher than those of normal adolescents (Engstrom et al, 1962). This finding is in agreement with other results obtained from swimmers (Andrews et al, 1972; Eriksson et al, 1977) and is also in agreement with the suggestion that a large VC is required for success in competitive swimming (Astrand et al, 1963).

A higher correlation has also been shown to exist between  $VO_2$  max recorded during work on a bicycle ergometer and swimming performance (Astrand et al, 1963). Although both groups recorded a higher value for  $VO_2$  max following the test period there was no difference between the two groups, and the difference can therefore be ascribed to the effects of the training regimen.

The changes in hematological variables (Hb and Hct) which were recorded would appear to be of little consequence and probably reflect the haemodilution which normally accompanies a period of physical training.

The results would appear to indicate that there is no beneficial effect to be obtained by administration of pollen extract to swimmers. Before this conclusion can be stated with any certainty however, two points must be born in mind. The first of these is that the present test lasted only six weeks; by comparison with the time scale which most training programs are conducted, this is an extremely short space of time and may not be of sufficient duration to produce a measurable effect. Secondly, it was noted that the placebo group, during the 6-week

experimental period, missed a total of 27 days of training through illness while the Pollitabs group missed 4 days in total. All days missed in both groups were the results of upper respiratory tract infections. Because of the small numbers involved, these data are not readily amenable to statistical evaluation. They do, however, suggest that the swimmers taking Pollitabs might expect to miss only 1 day in 105 due to upper respiratory tract infection; this compares extremely favorably with the placebo group who might expect to miss 1 day in 16. Such a difference might be expected to have important consequences for the athlete whose performance is dependent on the ability to engage in consistent physical training.

1. Andrews, G.M., Becklace, M.R., Galero, J. S. and Bates, D. V., 1972 "Heart and lung functions in swimmers and non-athletes during growth". *J.Appl.Physiol.* 32: 245-251.
2. Astrand, P. O. and Saltin, B., 1961. "Oxygen uptake during the first minutes of heavy muscular exercise". *J.Appl.Physiol.* 16: 971-976.
3. Astrand P.O., Engstrom, L., Eriksson, B., Korlberg, P., Nylander, I., Saltin, B. and Thoren, C., 1963 "Girl swimmers". *Acta Paediatr. Scand. Suppl.* 147.
4. Bayer, L. and Bayler, N., 1976. *Growth Diagnosis.* University of Chicago Press, Chicago.
5. Dubrisav, J., 1974 "A new approach to the natural treatment of protein-administration". *Gazette Medicale de France* 40: 7674-7683.
6. Durnin, J. V. G. A. and Rahaman, M. M., 1967. "The assessment of the amount of fat in the human body from measurements of skinfold thickness". *Brit.J.Nutr.* 21: 681-689.
7. Engstrom, L., Karlberg, P. and Savarty, C., 1962 "Relation between mechanical properties of the lungs, lung volumes and ventilator capacity in healthy children 7-15 years of age". *Acta Paediatr. Scand.* 51: 68-80.
8. Eriksson, B. O., Berg, K. And Taranger, J., 1977 "Physiological analysis of young boys starting intensive training in swimming". In: *Swimming Medicine IV.* Ed. B. E. Eriksson and B. Furberg. University Park Press, Baltimore.
9. Lindahl, O., 1978 "Mechanical effects of the pollen-based preparations". In: *Symposium on coadjuvants of organic resistance in nutrition.* Pharm.Ass.Florence, Florence pp. 44-49.
10. Mark-Vendel, S., 1978 "Twenty-year experience of a specialist in paediatrics in the therapeutical use of pollen-based preparations". In: *Symposium on coadjuvants of organic resistance in nutrition.* Pharm.Ass.Florence, Florence pp. 49-52.
11. Thorsetensson, A., 1976 "Muscle strength, fiber types and enzyme activities in man". *Acta physiol.Scand. Suppl.* 443: 1-72.

## References

