

A soy-based supplement alters energy metabolism but not the exercise-induced stress response

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ABSTRACT

Objective

To determine the changes in endurance capacity as well as in metabolic, hormonal and inflammatory markers induced by endurance training combined with a soy protein based supplement.

Design

Randomized controlled study consisting of moderate endurance training without (G0) or with (G1) a soy protein based supplement.

Subjects

Two groups of 15 subjects (10 males and 5 females in each group): healthy sports students aged 23.6 ± 1.9 years.

Measurements

Body composition (body mass (BM), body density (BD) by air displacement) and physical fitness (determined by treadmill ergometry) were measured at baseline and after 6 weeks of the intervention; changes in circulating metabolic and hormonal parameters (glucose, lactate, urea, uric acid, ammonia, cortisol, insulin, IGF-1), and exercise-induced stress and inflammatory markers (CK, LDH, myoglobin, hs-CRP, IL-6, IL-10, blood cell counts) were determined after the intervention period in a field test (11.5 km running on hilly ground).

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Results

30 participants completed the 6-week study; 28 students were able to perform the field test. No significant changes in BM and BD were noted after intervention with only slight increases in running performance and maximum aerobic capacity in the total group (2%, $p=0.016$). Subjects in the G1 group showed significant improvements in running velocity and lower lactate values following the intervention (-12%, $p=0.003$). In addition, the G1 group showed significantly lower differences in the exercise-induced increase of metabolic parameters (triglycerides, uric acid) and insulin in the post-exercise recovery period.

Conclusions

Our data suggest that moderate endurance training in combination with a soy-based protein supplement improves aerobic energy supply and metabolic function in healthy sports students, even without changes in body composition and without changes in the exercise-induced stress and inflammatory reaction.

INTRODUCTION

The ratio of the different dietary macronutrients has a distinct influence on body composition and metabolic functions. However, the exact amount and the underlying mechanisms are still under discussion for both physically inactive and overweight as well as active and normal-weight subjects (22). Recently, it has been shown that protein rich diets may not only increase thermogenesis, but improve glycaemic control, induce weight loss, help to stabilize weight following weight reduction and limit the loss of muscle mass (11, 12). These facts may be of special interest for overweight and insulin resistant subjects (10, 20), but also normal weight and physically active subjects may benefit from an improved carbohydrate metabolism and insulin sensitivity known to be of high relevance for fuel selection and in particular for the utilization of fatty acids (19, 41). Therefore, a protein-rich diet could help to improve aerobic capacity and metabolic flexibility in combination with an endurance-training programme.

So far, ingestion of protein or essential amino acids has been predominantly linked to an increased muscular protein synthesis. Although results are equivocal, studies in humans suggest that protein ingestion can enhance skeletal muscle hypertrophy in response to chronic resistance training. The evidence linking protein intake to an enhanced endurance capacity is still scarce (34). Recently, some authors suggested that protein intake during or after exercise may induce an ergogenic effect on endurance performance (7, 14, 30). Nevertheless, there is no consensus whether the ergogenic effect of protein is only an effect of adding calories, or may actually be a unique and specific benefit of the protein intake itself (8, 28). Therefore, we performed an intervention study in which the combination of endurance training and a soy-based protein supplement in healthy sports students was tested. The food supplement was a commercially available soy-yoghurt-honey product (Almased®) which has been recently tested for its significant benefits in body composition and metabolism control (11, 12, 20).

The aim of the study was to answer the following questions:

1. Is there an association between the intake of a protein-rich supplement over 6 weeks and changes in body composition?
2. Does the intake of the supplement influence endurance performance and energy supply during exercise?
3. Does the intake of the supplement induce changes in circulating hormone levels?
4. Does the intake of the supplement influence the exercise-induced muscular or systemic stress reactions?

METHODS

The study was designed as a RCT (Randomized Controlled Trial) according to GCP (Good Clinical Practice). 30 clinically healthy sports students of both genders were randomized to a verum (G1; N=15, 10 males, 5 females) and control (G0; N=15, 10 males, 5 females) group. Both groups were instructed to perform a moderate endurance training (60 minutes per day, 5 times a week) at the aerobic threshold over a period of 6 weeks. In addition, the subjects of the verum group (G1) were instructed to take two 50 g servings of the supplement (Almased® containing 53.3 g protein, 30.5 g carbohydrates, 2.0 g fat, 354 kcal per 100 g) solubilized in 200 ml water every day for 6 weeks, until the day of the field test. During the study period one participant dropped out due to a viral infection in each group resulting in 2 x 14 completers according to the study protocol.

Three examinations were carried out: U-1 as pre-intervention examination including health control, anthropometric measurements, blood drawing (blood specimen “a”) and performance diagnostics (5); U-2 as post-intervention examination after the 6-week training period (both groups) and intake of the protein-rich supplement (verum group) with the same conditions as in U-1; and finally U-3 as a post-intervention running stress test (cross-country race on hilly ground with a distance of 11.5 km) starting at 11:00 a.m., three hours after breakfast. At U-3 blood samples were drawn at 8:00 a.m. at rest in a fasting state (“b”) as well as one (“c”) and four hours (“d”) after performing the field test; an additional blood sample was drawn at 8:00 a.m. at rest in a fasting state the morning after the field test (“e”).

The following parameters concerning muscular energy supply and exercise-induced stress reaction were measured: blood glucose, blood lactate, serum urea, uric acid, ammonia; creatine kinase (CK), lactate dehydrogenase (LDH), myoglobin, cortisol, insulin, IGF-1, hs-CRP, blood cell count, IL-6, IL-10; all variables were measured with standardized methods of clinical chemistry.

In addition, body composition was analyzed by air displacement plethysmography with the Bod-Pod technology (13) before and after intervention (U-1, U-2). Individual data regarding health status, training and dietary behavior were collected by weekly protocols.

For statistical evaluation the SPSS 18.0.2 program was used. For intra- and inter-individual comparisons the groups were examined with the Friedman test (non-parametric equivalent of ANOVA), intra-individually over all time points as well as inter-individually with the principle of the repeated measurements. To reject the null hypotheses, a significance level of $p < 0.05$ was used.

The study protocol was approved by the Ethical Commission of the University of Freiburg. All participants were educated by an informational conversation supported by written information and gave their written consent to participate in the study.

RESULTS

Both groups were comparable in gender, age, body composition and physical fitness at the beginning of the study (Table 1). After the 6-week intervention, in both groups, no changes in body mass or body density were detectable (Table 2). An increase in body mass by the additionally supplied calories could not be established in the verum group. In addition, there were no differences in health status, training or dietary behaviour between the groups.

Tab. 1: Personal and anthropometric data of the subjects (N=30) examined

	G0 (N=15)	G1 (N=15)
Sex [male/female]	10/5	10/5
Age [yrs]	24.0 ± 2.1	23.3 ± 1.6
Weight [kg]	67.5 ± 10.6	68.3 ± 12.3
Height [m]	1.76 ± 0.10	1.73 ± 0.11
Body mass [kg/m²]	21.8 ± 2.21	22.5 ± 2.15
VO₂max [ml/kg/min]	50.8 ± 6.43	51.1 ± 5.66

The performance capacity of the total group of students, described as maximum running velocity (km/h) in the treadmill test, showed a minor increase of +2% after the training programme of 6 weeks; however, these changes were statistically significant ($p=0.022$ and $p=0.016$, respectively) (Table 2). In the verum group, running velocity at the defined lactate thresholds (at 2 mmol/l and 4 mmol/l blood lactate) increased significantly by about 15% ($p=0.011$, Table 2).

Only in the verum group blood lactate values in the stress test after intervention were significantly reduced (Table 2): at the aerobic threshold -20% ($p=0.009$), at the anaerobic threshold -11% ($p=0.009$), and for total lactate production (calculated as sum of lactate of each stress step) -12% ($p=0.003$). In contrast to the changed lactate kinetics of the stress test, there were no differences with respect to the results of the field test (G0: 60.2 ± 8.8 min versus G1: 61.4 ± 9.9 min) or

Tab. 2: Anthropometric and performance data before and after intervention in both groups; intra-group significance (before/after intervention) given as index * ($p < 0.05$) and ** ($p < 0.01$)

	G0 (before)	G0 (after)	G1 (before)	G1 (after)
Weight [kg]	67.5 ± 10,6	68.7 ± 11,2	68.3 ± 12.3	68.2 ± 12.1
Density [kg/l]	1.067 ± 0.019	1.066 ± 0.017	1.065 ± 0.016	1.064 ± 0.018
Running velocity max [km/h]	16.1 ± 2.07	16.4 ± 1.65	16.2 ± 1.80	16.4 ± 1.83
Running velocity 2mmol L [km/h]	10.1 ± 2.04	10.4 ± 2.22	9.28 ± 2.75	10.6 ± 2.45 **
Running velocity 4mmol L [km/h]	13.2 ± 1.49	13.5 ± 1.58	12.8 ± 2.00	13.3 ± 2.16 **
Lactate [mmol/l] resting state	1.38 ± 0.22	1.48 ± 0.25	1.44 ± 0.29	1.26 ± 0.23 *
Lactate [mmol/l] aer threshold	1.52 ± 0.45	1.55 ± 0.48	1.81 ± 0.60	1.44 ± 0.50 **
Lactate [mmol/l] anaer threshold	3.06 ± 0.49	3.06 ± 0.48	3.31 ± 0.60	2.94 ± 0.49 **
Lactate [mmol/l] sum over all	22.9 ± 8.26	21.4 ± 8.77	25.9 ± 6.12	22.7 ± 6.17 **

the training amount (G0: 250 ± 54.5 min/week versus G1: 231 ± 58.2 min/week) between both groups.

Blood glucose metabolism was not influenced by the intervention; as expected, exercise induced a significant fall in blood glucose concentration of about approximately -15 mg/dl 1 hour after strain ($p=0.001$) in both groups (Table 3). The production of urea during and after the field test was significantly influenced by the intervention. In both groups there was a significant increase of urea production after exercise (Table 3); however, the increase in urea during endurance exercise was more pronounced in the verum group ($p=0.005$). Within one day after the field test, urea values comparable to pre-stress levels were reached in both groups again (Table 3). In contrast, there was a more pronounced increase in uric acid in the control group (Table 3) as an end metabolite of the purine nucleotide cycle (PNC); particularly in the morning after the field test ($p=0.028$) (Table 3). In both groups a stress-induced increase in blood ammonia was seen ($p=0.001$); this increase was also more pronounced in the control group ($p=0.06$ for inter-individual comparison) (Table 3).

The regulation of the triglycerides in the recovery phase showed a correlation to the time as well as the assigned group (Table 3). In intra-individual comparison,

the triglycerides showed a stronger increase ($p=0.016$) in the control group compared with the verum group; this led to significantly different triglyceride values 4 hours after the field test between the groups ($p=0.018$) (Table 3 and Figure 1b).

Markers of muscular (Table 4), systemic (Table 4), and immunological stress (Table 5) showed significant changes after exercise in both groups (all $p=0.001$ for CK, LDH and myoglobin, for the blood cell counts as well as for IL-6 and IL-10); hs-CRP was significantly elevated in both groups the day after the field test ($p=0.013$, Table 5). However, regarding these parameters there was no evidence for significant effects of the intervention.

Tab. 3: Metabolic substrates before and after intervention in the course of the field test; n.e. (not estimated); intra-group significance (before/after field test) given as index * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$)

Glucose [mg/dl]	G0	G1
a	80.5 ± 8.58	81.6 ± 9.24
b	90.6 ± 5.51	90.33 ± 5.17
c	75.1 ± 16.6 ***	74.3 ± 11.2 ***
d	90.7 ± 14.7	90.4 ± 10.9
e	86.73 ± 9.20	86.6 ± 3.70

Urea [mg/dl]	G0	G1
a	34.0 ± 6.94	33.0 ± 7.71
b	30.7 ± 5.69	34.8 ± 7.14
c	34.8 ± 6.69 ***	43.4 ± 7.23 ***
d	36.3 ± 6.66 ***	48.4 ± 9.25 ***
e	33.6 ± 8.11 ***	35.8 ± 5.62 *

Uric acid [mg/dl]	G0	G1
a	4.86 ± 0.99	4.89 ± 0.94
b	4.49 ± 0.65	4.66 ± 0.85
c	5.53 ± 0.79 ***	5.53 ± 0.85 ***
d	5.37 ± 0.85 ***	5.31 ± 0.89 ***
e	5.11 ± 0.82 ***	4.88 ± 0.87

Ammonia [μmol/l]	G0	G1
a	n.e.	n.e.
b	29.9 ± 6.70	27.1 ± 4.22
c	42.0 ± 13.7 ***	35.5 ± 6.94 ***
d	37.2 ± 9.12 ***	31.9 ± 7.55 ***
e	n.e.	n.e.

Triglycerides [mg/dl]	G0	G1
a	77.3 ± 38.1	88.9 ± 45.2
b	90.9 ± 33.8	88.0 ± 36.3
c	126 ± 71.3 *	90.1 ± 39.4
d	150 ± 85.0 *	87.2 ± 39.6
e	87.1 ± 57.5	77.5 ± 45.4

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Tab. 4: Muscular stress indicators (CK, LDH activity, myoglobin concentration) and blood cell counts (Tsd, thousands of cells) before and after intervention in the course of the field test, intra-group significance (before/after field test) given as index * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$)

CK [U/l]	G0	G1
a	199 ± 141	243 ± 258
b	152 ± 104	146 ± 151
c	253 ± 145 ***	258 ± 236 ***
d	460 ± 475 ***	409 ± 329 ***
e	749 ± 426 **	441 ± 395 ***

LDH [U/l]	G0	G1
a	181 ± 23.8	188 ± 52.2
b	173 ± 29.1	179 ± 42.7
c	230 ± 39.2 ***	225 ± 47.1 ***
d	216 ± 41.2 ***	233 ± 50.6 ***
e	200 ± 54.3 ***	200 ± 39.3 ***

Myoglobin [µg/l]	G0	G1
a	71.4 ± 15.6	72.3 ± 22.7
b	64.6 ± 9.07	64.5 ± 5.79
c	255 ± 147 ***	297 ± 161 ***
d	199 ± 123 ***	243 ± 140 ***
e	82.1 ± 24.6 ***	80.4 ± 45.0 ***

Leucocytes [Tsd/µl]	G0	G1
a	6.63 ± 1.12	6.25 ± 0.94
b	6.29 ± 1.27	6.68 ± 1.56
c	11.7 ± 2.46 ***	12.3 ± 2.71 ***
d	12.0 ± 2.62 ***	12.4 ± 1.57 ***
e	6.56 ± 1.17	6.39 ± 1.32

Lymphocytes [Tsd/µl]	G0	G1
a	2.55 ± 0.34	2.66 ± 0.31
b	2.50 ± 0.47	2.53 ± 0.64
c	1.61 ± 0.33 ***	1.46 ± 0.47 ***
d	1.99 ± 0.55 *	2.03 ± 0.46 *
e	2.41 ± 0.39	2.22 ± 0.48

Neutrophils [Tsd/µl]	G0	G1
a	3.28 ± 1.15	2.88 ± 0.56
b	3.03 ± 1.01	3.31 ± 1.34
c	9.01 ± 2.15 ***	9.37 ± 2.51 ***
d	9.07 ± 2.39 ***	9.10 ± 1.25 ***
e	3.39 ± 1.01	3.33 ± 1.16

Tab. 5: Systemic and immunological stress indicators (serum concentration of hs-CRP, IL-6, and IL-10) before and after intervention in the course of the field test; intra-group significance (before/after field test) given as index * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$)

hs-CRP [mg/dl]	G0	G1
a	0.76 ± 0.63	0.80 ± 0.72
b	0.75 ± 0.59	0.69 ± 0.63
c	0.97 ± 0.69	1.10 ± 1.59
d	1.01 ± 0.69	1.40 ± 2.25
e	1.74 ± 1.10 *	2.49 ± 3.66 *

IL6 [pg/ml]	G0	G1
a	0.86 ± 0,80	0.51 ± 0.37
b	0.81 ± 0,62	0.97 ± 1.27
c	2.33 ± 1,44 ***	2.96 ± 1.13 ***
d	1.20 ± 1,39	1.35 ± 0.96
e	0.82 ± 0,71	1.51 ± 1.83

IL10 [pg/ml]	G0	G1
a	0.45 ± 0.48	0.42 ± 0.30
b	0.51 ± 0.57	0.59 ± 0.56
c	16.2 ± 12.6 ***	13.8 ± 11.0 ***
d	1.52 ± 1.59 *	6.16 ± 11.5 *
e	3.13 ± 6.78 *	2.23 ± 3.45 *

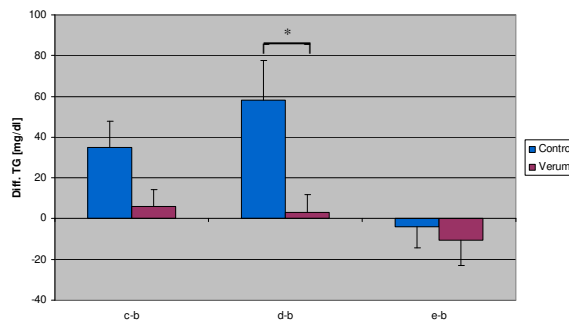
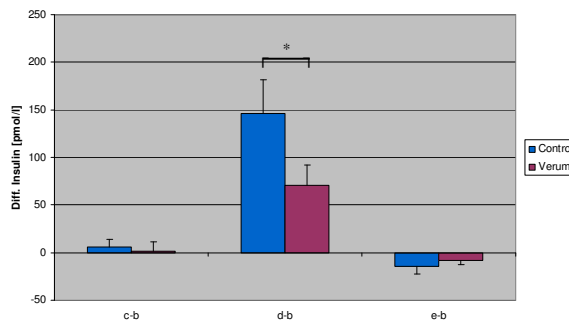


Fig. 1a and 1b: Insulin (1a) and triglyceride (1b) response after the endurance stress test (11.5 km on hilly ground) in the control and verum group (* $p < 0.05$)

Tab. 6: Blood hormones (serum concentration of insulin, cortisol, IGF-1 and HGH) before and after intervention in the course of the field test; intra-group significance (before/after field test) given as index * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$)

Insulin [pmol/l]	G0	G1
a	46.5 ± 23.6	40.5 ± 19.8
b	57.0 ± 33.2	51.6 ± 12.3
c	63.4 ± 36.5	53.1 ± 34.4
d	203 ± 154 ***	122 ± 84.3 **
e	42.6 ± 21.5	43 ± 16.9

Cortisol [nmol/l]	G0	G1
a	621 ± 209	683 ± 205
b	709 ± 170	746 ± 194
c	736 ± 233	723 ± 278
d	426 ± 199 ***	404 ± 225 ***
e	631 ± 161 *	696 ± 189 **

IGF-1 [ng/ml]	G0	G1
a	220 ± 77.0	231 ± 63.1
b	253 ± 63.8	293 ± 58.4
c	265 ± 66.7	288 ± 54.4
d	261 ± 68.8	296 ± 55.1
e	259 ± 78.3	304 ± 69.8

HGH [ng/ml]	G0	G1
a	2.56 ± 3.58	3.00 ± 5.94
b	3.15 ± 4.55	2.27 ± 3.29
c	2.84 ± 2.72	2.66 ± 2.18
d	1.24 ± 2.21 *	1.14 ± 1.78 *
e	4.45 ± 6.95	1.58 ± 2.31

In both groups a significant increase in serum insulin after the field test was detectable in the recovery phase (4 hours after field test: $p = 0.001$) (Table 6). Being specified for the group factor, this increase was more pronounced in the control group ($p = 0.09$; Figure 1a). The IGF-1 levels of both groups showed neither stress-induced nor intervention related changes (Table 6). The cortisol levels showed a stress-induced decrease in the recovery phase in both groups (4 hours after field test: $p = 0.001$); however, group-specific differences were not detectable (Table 6).

DISCUSSION

The main result of the present study was that a 6-week soy-based protein supplementation promotes an increased aerobic energy provision during endurance exercise of moderate intensity. It was shown that the concentration of lactic acid at both aerobic and anaerobic threshold and also the total lactate production during the treadmill test to exhaustion was lower following the soy protein supplementation.

With respect to anaerobic energy supply, a reduced ammonia and uric acid production was found following the 11.5 km running test. Increased concentrations of ammonia and uric acid indicate adenine nucleotide breakdown and the utilization of the anaerobic purine nucleotide cycle (3, 5). It has been shown previously that endurance exercise leads to an increased utilization of some free amino acids for energy provision (15). As the exercise-induced changes in urea were more pronounced in the verum group, it can be assumed that there was an additional energy supply from protein in this group. Finally, the significant differences in the post-exercise regulation of triglyceride and insulin metabolism suggest an improved use of fatty acids for energy metabolism (1, 18, 32) induced by the regular intake of the soy-based dietary supplement. This effect could only be shown in the verum group, whereas in the control group an increased hepatic synthesis of triglycerides by the unutilized free fatty acids has to be assumed (19). This observation is in accordance with the results of studies investigating molecular effects of soy protein in rats (27)(24).

The results are also consistent with experimental data of a recently published trial in which the specific effects of this soy protein supplement on postprandial fuel selection and appetite regulation were investigated (21). The glycaemic and insulinaemic responses were considerably higher after a standardized breakfast with a high GI than following the soy protein supplement. In addition, the postprandial decrease in fat oxidation was significantly less pronounced after intake of the supplement; this effect was also detectable after lunch as a "second-meal" effect. It has been demonstrated by several groups that a lower GI of a pre-exercise meal was associated with a higher fat oxidation both before and during exercise (9, 35, 36, 38, 42). This effect can mainly be attributed to lower pre-exercise insulin concentrations which will lead to enhanced peripheral lipolysis, increased plasma FFA and increased β -oxidation in skeletal muscles. On the one hand, this effect could be used to improve training gain in endurance type sports, on the other hand, it has a glycogen sparing effect, thereby minimizing the ergolytic effects of carbohydrate depletion. Glycogen sparing means that an increased fat oxidation during intense, prolonged endurance exercise reduces the relative proportion of carbohydrate oxidation. The lower rate of carbohydrate oxidation will preserve the intramuscular and intrahepatic glycogen stores. These stores can be used in the later stages of exercise and prevent premature fatigue (35, 42).

Considerable research has been done during the last decades to elucidate the effect of macronutrient composition on physical performance, particularly endurance performance (25). For endurance athletes, a high-carbohydrate diet (6, 16) has been recommended by sports nutritionists. The diet should contain 6 to 8 grams of carbohydrates per kilogram of body mass (31). However, it should be mentioned that this amount of carbohydrates can easily reach up to 2,400 kilocalories of carbohydrate per day and may eventually interfere with an adequate intake of protein (4, 17, 23, 37). In addition, dietary carbohydrates, particularly with a high glycaemic load, generate high blood glucose and insulin levels which could impair fat metabolism (2). A higher intake of protein may be a feasible way to burn more fat (10, 21). Furthermore, there is experimental evidence that soy protein influences cellular energy metabolism by molecular mechanisms. Soy

protein improves insulin resistance and lipid levels by activating peroxisome-proliferator activated receptors (PPARs) (39). PPARs are known as nuclear receptors which control metabolic processes, particularly affecting energy metabolism, by regulating the expression of genes involved in glucose homeostasis, lipid metabolism, and fatty acid oxidation (26, 27, 33, 43). It has been shown (26) that consumption of isoflavone-rich soy protein improves glucose tolerance, insulin resistance and hepatic triglyceride concentrations in rats. In addition, these investigators showed that isoflavone-rich soy extracts increased the gene expression of PPARs in cell culture studies, suggesting that the beneficial effects of soy protein on glucose and lipid metabolism may be mediated by PPAR activation.

More recently, it was also demonstrated (27) that soy protein feeding in rats increased the activity and mRNA levels of several skeletal muscle enzymes involved in fatty acid oxidation, including carnitine palmitoyltransferase (CPT1) activity and beta-hydroxyacyl-CoA dehydrogenase (HAD), acyl-CoA oxidase, and medium-chain acyl-CoA dehydrogenase. Moreover, PPAR gamma coactivator-1 (PGC1)-alpha and PPAR-alpha mRNA levels were also found to be elevated, suggesting that soy protein intake stimulates skeletal muscle fatty acid oxidation by activating PPAR pathways leading to a reduced accumulation of body fat (24). It may be assumed that soy protein works in the same or a similar manner in human organisms, however, comparable results from experimental studies in humans are still lacking. Therefore, further research is needed to confirm this assumption.

In the present study, it was further investigated if the intake of the supplement could also prevent muscle soreness and exercise-induced inflammatory stress. It has been assumed that an improvement in these parameters may enhance regeneration and help to achieve a stable fitness level (29)(40). However, there is no consensus whether an additional protein intake could prevent or reduce post-exercise muscular or systemic stress (4, 28, 34).

Apart from an increased post-exercise glycogen resynthesis, many other mechanisms have been discussed which could improve the immune response during and following exercise (28). These include an increased central drive, a blunting of exercise-induced muscle damage (8), and a modification in the pattern of exercise-related cytokine production. However, in our study we found no indices to assume such mechanisms. It could be possible that the study protocol (moderately endurance-trained subjects, 6 weeks of supplementation, duration of stress test 60 minutes) was not suitable to induce respective changes. Either the supplementation period was too short or the stress test was not appropriate. In addition, it has to be critically remarked that many studies so far have failed to demonstrate improved stress tolerance or altered immune function by measuring stress markers in the blood.

The intake of the protein-rich food supplement was not associated with changes in body composition within the 6-week period. The combined effects of protein supplementation and physical training on body composition and particularly muscle mass in healthy and trained subjects have been equivocal. It can be assumed

that in the present design, the length of supplementation and training volume (frequency x intensity x duration) were not sufficient to induce alterations in body composition.

In conclusion, the results support the hypothesis that the soy-based food supplement promotes aerobic energy supply during moderate endurance training. In addition, in these healthy and normal-weight sports students, the intervention led to an increased endurance performance. It can be assumed that the supplement significantly influences the supply of fat as a source of energy during exercise. The group-specific behaviour in the post-exercise triglyceride and insulin kinetics, which were evident in the regeneration phase, suggests an altered mitochondrial metabolism of the muscle cells and an improved use of fatty acids for energy metabolism following additional soy protein intake.

Research related to this article was funded by the Almased GmbH, Bienenbüttel, Germany

REFERENCES

1. Achten J and Jeukendrup AE. Optimizing fat oxidation through exercise and diet. *Nutrition* 20: 716-727, 2004.
2. Achten J and Jeukendrup AE. Relation between plasma lactate concentration and fat oxidation rates over a wide range of exercise intensities. *Int J Sports Med* 25: 32-37, 2004.
3. Banister EW and Cameron BJ. Exercise-induced hyperammonemia: peripheral and central effects. *Int J Sports Med* 11 Suppl 2: S129-S142, 1990.
4. Beelen M, Burke LM, Gibala MJ and van Loon LJ. Nutritional strategies to promote postexercise recovery. *Int J Sport Nutr Exerc Metab* 20: 515-532, 2010.
5. Berg A, Jakob E, Lehmann M, Dickhuth HH, Huber G and Keul J. [Current aspects of modern ergometry]. *Pneumologie* 44: 2-13, 1990.
6. Bergstrom J, Hermansen L, Hultman E and Saltin B. Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71: 140-150, 1967.
7. Betts JA, Toone RJ, Stokes KA and Thompson D. Systemic indices of skeletal muscle damage and recovery of muscle function after exercise: effect of combined carbohydrate-protein ingestion. *Appl Physiol Nutr Metab* 34: 773-784, 2009.
8. Betts JA and Williams C. Short-term recovery from prolonged exercise: exploring the potential for protein ingestion to accentuate the benefits of carbohydrate supplements. *Sports Med* 40: 941-959, 2010.
9. Brand-Miller JC, Holt SH, Pawlak DB and McMillan J. Glycemic index and obesity. *Am J Clin Nutr* 76: 281S-285S, 2002.
10. Clifton PM, Bastiaans K and Keogh JB. High protein diets decrease total and abdominal fat and improve CVD risk profile in overweight and obese men and women with elevated triacylglycerol. *Nutr Metab Cardiovasc Dis* 19: 548-554, 2009.
11. Deibert P, Konig D, Schmidt T, Zaenker KS, Frey I, Landmann U and Berg A. Weight loss without losing muscle mass in pre-obese and obese subjects induced by a high-soy-protein diet. *Int J Obes Relat Metab Disord* 28: 1349-1352, 2004.

12. Deibert P, Solleder F, Konig D, Vitolins MZ, Dickhuth HH, Gollhofer A and Berg A. Soy protein based supplementation supports metabolic effects of resistance training in previously untrained middle aged males. *Aging Male* 14: 273-279, 2011.
13. Dempster P and Aitkens S. A new air displacement method for the determination of human body composition. *Med Sci Sports Exerc* 27: 1692-1697, 1995.
14. Flakoll PJ, Judy T, Flinn K, Carr C and Flinn S. Postexercise protein supplementation improves health and muscle soreness during basic military training in Marine recruits. *J Appl Physiol* 96: 951-956, 2004.
15. Haralambie G and Berg A. Serum urea and amino nitrogen changes with exercise duration. *Eur J Appl Physiol Occup Physiol* 36: 39-48, 1976.
16. Hermansen L, Hultman E and Saltin B. Muscle glycogen during prolonged severe exercise. *Acta Physiol Scand* 71: 129-139, 1967.
17. Howatson G and van Someren KA. The prevention and treatment of exercise-induced muscle damage. *Sports Med* 38: 483-503, 2008.
18. Hurley BF, Nemeth PM, Martin WH, III, Hagberg JM, Dalsky GP and Holloszy JO. Muscle triglyceride utilization during exercise: effect of training. *J Appl Physiol* 60: 562-567, 1986.
19. Koivisto V, Hendler R, Nadel E and Felig P. Influence of physical training on the fuel-hormone response to prolonged low intensity exercise. *Metabolism* 31: 192-197, 1982.
20. König D, Deibert P, Frey I, Landmann U and Berg A. Effect of meal replacement on metabolic risk factors in overweight and obese subjects. *Ann Nutr Metab* 52: 74-78, 2008.
21. Konig D, Muser K, Berg A and Deibert P. Fuel selection and appetite-regulating hormones after intake of a soy protein-based meal replacement. *Nutrition* 28: 35-39, 2012.
22. Larsen TM, Dalskov SM, van Baak M, Jebb SA, Papadaki A, Pfeiffer AF, Martinez JA, Handjieva-Darlenska T, Kunesova M, Pihlsgard M, Stender S, Holst C, Saris WH and Astrup A. Diets with high or low protein content and glycemic index for weight-loss maintenance. *N Engl J Med* 363: 2102-2113, 2010.
23. Lemon PW, Yarasheski KE and Dolny DG. The importance of protein for athletes. *Sports Med* 1: 474-484, 1984.
24. Manzoni MS, Rossi EA, Carlos IZ, Vendramini RC, Duarte AC and Damaso AR. Fermented soy product supplemented with isoflavones affected fat depots in juvenile rats. *Nutrition* 21: 1018-1024, 2005.
25. Maughan RJ, Greenhaff PL, Leiper JB, Ball D, Lambert CP and Gleeson M. Diet composition and the performance of high-intensity exercise. *J Sports Sci* 15: 265-275, 1997.
26. Mezei O, Banz WJ, Steger RW, Peluso MR, Winters TA and Shay N. Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW 264.7 cells. *J Nutr* 133: 1238-1243, 2003.
27. Morifuji M, Sanbongi C and Sugiura K. Dietary soya protein intake and exercise training have an additive effect on skeletal muscle fatty acid oxidation enzyme activities and mRNA levels in rats. *Br J Nutr* 96: 469-475, 2006.
28. Negro M, Giardina S, Marzani B and Marzatico F. Branched-chain amino acid supplementation does not enhance athletic performance but affects muscle recovery and the immune system. *J Sports Med Phys Fitness* 48: 347-351, 2008.

29. Northoff H, Berg A and Weinstock C. Similarities and differences of the immune response to exercise and trauma: the IFN-gamma concept. *Can J Physiol Pharmacol* 76: 497-504, 1998.
30. Nosaka K, Sacco P and Mawatari K. Effects of amino acid supplementation on muscle soreness and damage. *Int J Sport Nutr Exerc Metab* 16: 620-635, 2006.
31. Rodriguez NR, DiMarco NM and Langley S. Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. *J Am Diet Assoc* 109: 509-527, 2009.
32. Romijn JA, Coyle EF, Sidossis LS, Rosenblatt J and Wolfe RR. Substrate metabolism during different exercise intensities in endurance-trained women. *J Appl Physiol* 88: 1707-1714, 2000.
33. Ronis MJ, Chen Y, Badeaux J and Badger TM. Dietary soy protein isolate attenuates metabolic syndrome in rats via effects on PPAR, LXR, and SREBP signaling. *J Nutr* 139: 1431-1438, 2009.
34. Stearns RL, Emmanuel H, Volek JS and Casa DJ. Effects of ingesting protein in combination with carbohydrate during exercise on endurance performance: a systematic review with meta-analysis. *J Strength Cond Res* 24: 2192-2202, 2010.
35. Stevenson E, Williams C, McComb G and Oram C. Improved recovery from prolonged exercise following the consumption of low glycemic index carbohydrate meals. *Int J Sport Nutr Exerc Metab* 15: 333-349, 2005.
36. Stevenson EJ, Williams C, Mash LE, Phillips B and Nute ML. Influence of high-carbohydrate mixed meals with different glycemic indexes on substrate utilization during subsequent exercise in women. *Am J Clin Nutr* 84: 354-360, 2006.
37. Tarnopolsky M. Protein requirements for endurance athletes. *Nutrition* 20: 662-668, 2004.
38. Thomas DE, Brotherhood JR and Brand JC. Carbohydrate feeding before exercise: effect of glycemic index. *Int J Sports Med* 12: 180-186, 1991.
39. Velasquez MT and Bhathena SJ. Role of dietary soy protein in obesity. *Int J Med Sci* 4: 72-82, 2007.
40. Weinstock C, König D, Harnischmacher R, Keul J, Berg A and Northoff H. Effect of exhaustive exercise stress on the cytokine response. *Med Sci Sports Exerc* 29: 345-354, 1997.
41. Wolfe RR. Fat metabolism in exercise. *Adv Exp Med Biol* 441: 147-156, 1998.
42. Wu CL and Williams C. A low glycemic index meal before exercise improves endurance running capacity in men. *Int J Sport Nutr Exerc Metab* 16: 510-527, 2006.
43. Xiao CW, Mei J and Wood CM. Effect of soy proteins and isoflavones on lipid metabolism and involved gene expression. *Front Biosci* 13: 2660-2673, 2008.