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Effects of Protein Hydrolysates on Physical Performance and Immunity in Male Players

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Article Info

Received: July 10, 2015
Accepted: August 13, 2015
Published online: Sep 1, 2015

Abstract

Aim: To investigate the effects of protein hydrolysates on Physical performance and immunity in male players aged 18-21 years.

Methods: Blood samples were drawn from 2 groups of athletes (10 subjects each). The first group ingested protein hydrolysates 0.4 g/k for 15 days, the second one ingested placebo for 15 days. All participants were subjected to Copper test as a field test for V02 max determination, according to standardized protocol of Burke (1996). The other parameters determined were, Leucocytes, Neutrophils, CD 45+ (lymphocytes) D14+ (monocytes), together with free testosterone, cortisol and insulin, using coulter counter, flow cytometer and Elisa techniques.

Results: Protein hydrolysates revealed an increased cellular parameters WBCS , CD45+ , CD14+), together with elevated free testosterone and insulin concentrations with lower cortisol. VO2 max increased following protein hydrolysates administration.

Conclusion: In response to protein hydrolysates ingestions some immunological variables are enhanced together with higher anabolic hormones and lower cortisol concentration, this might stimulate physical performance.

Key words: Protein hydrolysates, immunity, physical performance, flow cytometry.

1. Introduction:

Immunological alterations in response to a number of different exercise protocols are well documented (Pedersen, 1997 and Mackinnon 1999, 2000). Published studies have investigated changes in circulating cell populations in response to one bout of acute exercise (Gabriel & Kindermann, 1997 and Bishop et al., 1999) or repeated exercise (Ronsen et al., 2001) or different exercises (Mohamed et al., 2012). It has been suggested that decreased number of lymphocytes and/or natural killer (NK) cells during the hours after strenuous exercise may increase the risk of infections (Pedersen, 1997). In well-trained athletes, the number of circulating leucocytes and monocytes at rest are usually within normal clinical limits (Mackinnon, 2000) but NK cell numbers may be elevated in

some athletes (Nieman et al., 1995). Studies have shown that immunological variables change during different training periods and in response to variations in exercise intensity and duration (Gleeson et al., 1995 and Gabriel & Kindermann 1997). The clinical relevance of acute changes in immunological variables in blood samples after physical exercise has been debated (Mackinnon, 2000) because these changes may simply reflect a brief upset in homeostasis and a direct correlation to changed resistance to infections has not been made. However, correlation between acute changes in leucocyte pheno-types after physical exercise and physiological functions has been observed (Malm et al., 2000). Leucocyte and monocyte numbers and functions in blood samples taken at rest may be clinically relevant as an indicator of an individual's immune potential (Heshmat and Roushdy, 2010).

We have four ways to get amino acids into the bloodstream: 1) whole food proteins, 2) intact protein supplements, 3) free form amino acids, and 4) protein hydrolysates (Manninen, 2002). Protein can be hydrolyzed, producing small chains of amino acids called peptides. This process mimics our own digestive actions thus making it an ideal way to process protein.

Protein hydrolysates are produced from purified protein sources by heating with acid or preferably, addition of proteolytic enzymes, followed by purification procedures (Bucci and Unlu, 2000). Enzyme hydrolysis is greatly preferred because acid hydrolysis oxidizes cysteine and methionine, destroys some serine and threonine, and converts glutamine and asparagine to glutamate and aspartate, respectively, lowering protein quality and biological value (Bucci and Unlu, 2000).

Several studies have shown that protein hydrolysates containing mostly di- and tripeptides are absorbed more rapidly than free form amino acids and much more rapidly than intact proteins (Di Pasquale, 1997). The considerably greater absorption rate of amino acids from the dipeptide than from the amino acid mixture appears to be the result of uptake by a system that has a greater transport capacity than amino acid carrier system, thus minimizing competition among its substrates (Di Pasquale, 1997). This is a desirable trait for athletes who wish to maximize amino acid delivery to muscle.

However, whether this apparent advantage over ingestion of foodstuffs has a practical effect of faster muscle mass accretion or improved recovery from exercise has not been adequately studied in exercising individuals. Nevertheless, documented advantages (faster uptake of amino acids, higher biological value) remain attractive to consumers. In addition, there is recent evidence that protein hydrolysate ingestion has strong insulinotropic effect. Thus, this study examines some science behind protein hydrolysates applied to sports and exercise performance and immunity.

2. Methods:

Twenty healthy and injury free junior players (18-21 years old volunteered to participate in the study (mean ± SD)

(Group 1) Age = 19.3 ± 0.4 years, weight = 72,6 ± 3.4 kg, height 171,4 ± 5.4 cm.

(Group 2) Age = 19.8 ± 0.5 years, weight = 73,5 ± 4.4 kg, height 179,3 ± 6.2 cm.

2.1 VO2 max estimation:

The measurement was done using Copper test as a field test to determine the aerobic fitness of individuals test principle:

Based on the possibility of the individual to continue running for 12 min and measuring the cut distance during this period in kilometers (According to Burk, 1996).

Grade Test	Weak	Moderate	Good	Excellent
12 min Run (Kilometer)	0.2	2.4	2.8	3.2
VO2 max (ml/kg/min)	30	35-45	45-55	55-65

Immunological changes in blood were investigated by (10 ml) venous blood samples drawn from a fore arm vein before and after ingestion of protein hydrolysates or placebo for 15 days (protein hydrolysates dose 0.4 g/kg body weight after Manninen, (2004). 5 ml of blood were collected into EDTA tube for leucocyte analysis by coulter and flow cytometry for CD45+, CD14+ and 5 ml into untreated tubes for analysis of insulin, cortisol and testesterone. The blood for hormonal analysis was centrifuged and serum stored at (-20ºc). Hormonal determination by using commercial kits and Elisa technique in special lab.

2.2 Statistical Method:

Data were processed as mean ± standard deviation comparison between the mean values of the two groups were tested using t test. P value less than 0.05 was considered statistically significant.

3. Results and discussion:

Table presented in this study compared between changes from resting to post ingestions of protein hydrolysates 0.4 g/kg body weight for 15 days (experimental group), also the same happened using control group that ingest placebo for 15 days.

(Table 1, 2): VO2 max increased significantly in case of experimental group compared with control one.

(Table 3, 4): Hormonal profile (Testosterone, cortisol, insulin) before and after ingestions in experimental and control group. The results indicated an elevated values of anabolic hormones (Testosterone, insulin) in experimental group compared with control one. This was in concomitant with a drop of cortisol concentration in case of experimental group.

In case of Table (5, 6) leucocyte phenotypes significantly altered in blood from before to after protein hydrolysates ingestions compared to placebo ingestions group in the following parameters leucocytes numbers, Neutrophils, CD45+ numbers (lymphocytes) and CD14+ numbers (monocytes).

Table (1)
VO2 max (ml/kg/min) changes in control and experimental groups before and after protein hydrolysates and placebo..

Groups	Before	After	Sig.
Control Group	45.6 ± 3.5	46.2 ± 4.2	NS
Experimental Group	51.8 ± 3.8	54.7 ± 2.9	S

$P < 0.05$

Table (2)
VO2 max (ml/kg/min) changes after ingections of protein hydrolysates and placebo.

Groups	After	After	Sig.
Control Group	46.2 ± 4.2		S
Experimental Group		54.7 ± 2.9	

$P < 0.05$

Table (3)
Hormonal profile changes in control and experimental groups before and after protein hydrolysates and placebo ingestions.

Variables	Control Group		Sig.	Experimental Group		Sig.
	Before	After		Before	After	
Free testosterone n mol/l	7.2 ± 0.3	7.0 ± 0.4	NS	7.5 ± 0.4	9.6 ± 0.6	S
Insulin ng/ml	3.2 ± 0.1	3.5 ± 0.2	NS	3.4 ± 0.2	6.2 ± 0.5	S
Cortisol n mol/l	282 ± 11.6	291 ± 12.5	NS	341 ± 13.1	267 ± 14.5	S

$P < 0.05$

Table (4)
Hormonal profile changes after ingestions of protein hydrolysates and placebo.

Hydrolysate and placebo	Control (After)	Experimental (After)	Sig.
Free testosterone n mol/l	7.0 ± 0.4	9.6 ± 0.6	S
Insulin ng/ml	3.5 ± 0.2	6.2 ± 0.5	S
Cortisol n mol/l	291 ± 12.5	267 ± 14.5	S

$P < 0.05$

Table (5)
Immunological changes in control and experimental groups before and after protein hydrolysates and placebo.

Variables	Control Group		Sig.	Experimental Group		Sig.
	Before	After		Before	After	
Leucocytes cells x 103 ml	6.9 ± 0.4	7.1 ± 0.5	NS	7.2 ± 0.5	8.9 ± 0.6	S
Neutrophils cells x 103 ml	3.6 ± 0.2	3.5 ± 0.3	NS	3.8 ± 0.4	4.7 ± 0.5	S
CD45+ (cell □l)	651 ± 14.3	655 ± 16.2	NS	639 ± 12.1	684 ± 11.7	S
CD14+ (cell □l)	531 ± 16.6	522 ± 17.8	NS	542 ± 13.4	581 ± 12.5	S

$P < 0.05$

Table (6)
Immunological changes after ingestions of protein hydrolysates and placebo.

Variables	Control (After)	Experimental (After)	Sig.
Leucocytes cells x 103 ml	7.1 ± 0.5	8.9 ± 0.6	S
Neutrophils cells x 103 ml	3.5 ± 0.3	4.7 ± 0.5	S
CD45+ (cell □l)	655 ± 16.2	684 ± 11.7	S
CD14+ (cell □l)	522 ± 17.8	581 ± 12.5	S

$P < 0.05$

Discussion:

The immunological response to exercise described in most studies (increased neutrophil and decreased lymphocyte number) has partially been attributed to increased cortisol after exercise (Pedersen, 1997 and Mackinnon, 1999). As indicated by findings in the present studies, the action of cortisol on various leucocyte subpopulations is rather complex.

The presence of cortisol responders and non responders among subjects, the subjects training status and resting cortisol concentration are factors which may affect the influence of cortisol on leucocytes and monocytes during rest and after exercise (Weicker and Werle, 1991).

In the present study (Table 3, 4) resting cortisol was lower in case of experimental group compared to control. This result agreed with information presented by Mougios (2006) which added that measuring cortisol at rest may aid in estimating physical or mental stress, while measuring cortisol after exercise may show how the organism receives a particular load. Because cortisol causes proteolysis in muscle, high concentration of it are undesirable.

The data presented in our study revealed a higher free testosterone concentration at rest after the ingestion of protein hydrolysates (Table 3, 4). This was in accordance with the studies of Manninen (2004) and Mougios (2006).

They also added that testosterone have anabolic effects on skeleton and muscles as it promote protein synthesis and curb proteolysis and increase erythropoietin synthesis and the responsiveness of bone marrow cells to the hormone thus promoting erythropoiesis. Also due to these actions high concentrations of it are desirable. Malm et al., (2000) reported that resting testosterone correlated to the immunological response to physical stress and that CD14 and CD45 on monocytes were influenced by testosterone concentration at rest. Weicker and Werle, (1991) stated that the athlete (including hormone concentration and VO2 max) in part determines the immunological response to exercise.

The data presented in table (3, 4) indicated a higher insulin concentration after protein hydrolysates ingestions for 15 days compared to placebo ingestions. This result agrees with the study of Manninen (2004).

Principal actions of insulin. Ganong (2001).

Rapid (seconds)

Increased transport of glucose, amino acids, and K+ into insulin-sensitive cells

Intermediate (minutes)

Stimulation of protein synthesis

Inhibition of protein degradation

Activation of glycolytic enzymes and glycogen synthase

Delayed (hours)

Increase in mRNAs for lipogenic and other enzymes.

From our understanding of insulin physiology we can see different ways in which insulin might be a performance-enhancing agent:

1. Through facilitating glucose entry into cells in amounts greater than needed for cellular respiration it will stimulate glycogen formation. Thus, insulin will both increase muscle glycogen concentrations prior to exercise and in the recovery phase after exercise.
2. Insulin is also being used in more haphazard way, particularly to increase muscle mass in bodybuilders. It has been long known that insulin-treated patients with diabetes have an increase in lean body mass when compared with matched controls (Sonksen, 2001)

Formerly, it was believed that insulin secretion was controlled almost entirely by the blood glucose concentration. However, as more has been learned about the metabolic functions of insulin for protein and lipid metabolism, it has become apparent that blood amino acids and other factors also play important roles in controlling insulin secretion.

Protein meals, infusion of physiological amino acid mixtures, or certain individual amino acids cause insulin release in humans even under conditions where the blood sugar changes little from its basal level (Newgard and Matschinksy 2001). However, changes of blood sugar levels markedly influence the responsiveness of beta cells to individual amino acids.

VO2 max increased significantly after protein hydrolysatesd ingestion compared to control group (Table 1, 2). This indicated that protein hydrolysates ingestion has strong

anabolic effect and increase anabolic hormones such as insulin and testosterone and might help fitness and physical performance. This was in accordance with the study of Manninen (2004) who added that protein meals, infusion of physiological amino mixtures or certain individual amino acids cause anabolic hormones release namely insulin even under conditions where the blood sugar changes little from its basal level (Newgard and Matchinksy, 2001). Fernando et al., (2000) demonstrated that VO2 max levels reflect the performance criteria in player physical exercise in characterized by an increase in oxygen consumption by the whole body and particularly by the muscle. The higher level of VO2 max of experimental group may be attributed to their higher physical performance.

Ganong, (2010) demonstrated the major histocompatibility complex genes (MHC) on the surface of the antigen presenting cells bind to appropriate T cells. Therefore, receptors on the T cells must recognize a wide variety of complexes. Most of the receptors on circulating T cells are made of two polypeptide units designated α and β. They form heterodimers that recognize the MHC proteins and the antigens fragments with which they are combined. These cells are called αBT cells. CD8 occurs on the surface of cytotoxic T cells that bind MCH-1 proteins and CD4 occurs on the surface of helper T cells that binds MCH11 proteins. The CD8 and CD4 proteins facilitate the binding of the MHC proteins to the T cell receptors, and they also foster lymphocyte development. CD45+ proteins facilitate the binding of the MHC proteins to lymphocyte receptors and CD14+ facilitate the binding of the MHC proteins to the monocytes receptors and they foster lymphocytes and monocytes development.

The data presented on Table (5, 6) revealed a significant increased CD45+ numbers and CD14+ numbers in case of experimental group compared to control one, denoting an increased development and function of lymphocytes and monocytes. These results were in accordance with that of Malm et al., (2004). Also monocytes and lymphocytes higher cell surface density of CD45+, CD14+ in endurance athletes compared with untrained subjects have been noted (Gabriel et al., 1997). Thus, it can be suggested that both adhesion and signaling properties of cells in circulation can be altered with exercise.

Nevertheless, increased receptor density on circulating leucocytes and monocytes suggests an increased capacity for adhesion and signaling in trained vs untrained subjects (Gabriel et al., 1997).

4. Conclusion:

In response to protein hydrolysates ingestions some immunological variables are enhanced together with elevated anabolic hormones studied (Insulin and free testosterone) and lower cortisol concentration (stress), all with higher VO2 max might stimulate physical performance.

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