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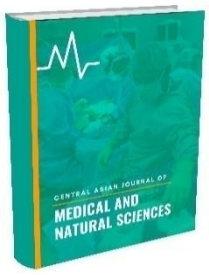

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Determination of Some Biochemical Parameters in Healthy Younger Man who Taking Amino Acid Supplement

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Abstract: Weightlifting, often referred to as Olympic-style lifting, is one of the most accepted methods to enhance power output among athletes. Because the exercises involve rapid acceleration against resistance throughout the movement, power outputs are quite high (1).

1. Introduction

1.1 Weight lifting

Weightlifting, often referred to as Olympic-style lifting, is one of the most accepted methods to enhance power output among athletes. Because the exercises involve rapid acceleration against resistance throughout the movement, power outputs are quite high (1).

Indeed, the snatch and clean and jerk afford the highest power outputs recorded in sport (2).

Given the intent to move the load as quickly as possible, weightlifting exercises stimulate greater motor unit synchronization and therefore improve the ability to generate power (3).

The high levels of force development as well as improved muscle action speed associated with weightlifting can enhance performance in sports that require explosive dynamic movements (4).

Weightlifters are arguably the most powerful athletes. As such, the training methods and modalities used in weightlifting are often looked at for the training of other athletes in sports in which strength, speed, and power contribute to elite athletic performance. In addition to the musculoskeletal and mechanical adaptations, cardiorespiratory, motor behavior, and psychological adaptations also result from weightlifting training (5).

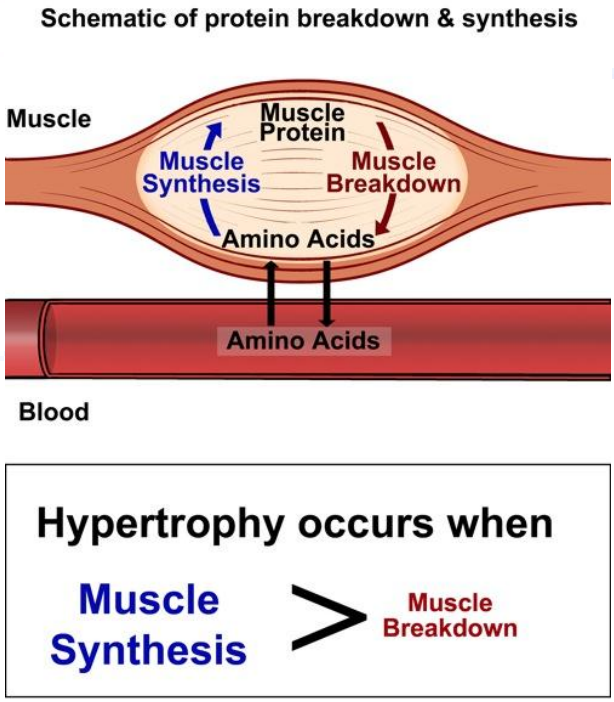
1.1.1 Nomenclature

Weightlifting is defined as the sport in which athletes attempt to lift the most weight in the snatch and the clean and jerk. Strength and conditioning professionals should be clear to differentiate between weightlifting and weight or resistance training, which is the broad category of exercise against

resistance . The term Olympic lifting, although commonly used, is inappropriate for most athletes, as this should be reserved for the elite individuals who compete in weightlifting at the Olympics games . Similarly, the term weightlifter refers distinctly to individuals training and competing in weightlifting(6).

1.2 Protein and amino acids for athletes

The main determinants of an athlete's protein needs are their training regime and habitual nutrient intake. Most athletes ingest sufficient protein in their habitual diet. Additional protein will confer only a minimal, albeit arguably important, additional advantage. Given sufficient energy intake, lean body mass can be maintained within a wide range of protein intakes. Since there is limited evidence for harmful effects of a high protein intake and there is a metabolic rationale for the efficacy of an increase in protein, if muscle hypertrophy is the goal, a higher protein intake within the context of an athlete's overall dietary requirements may be beneficial. However, there are few convincing outcome data to indicate that the ingestion of a high amount of protein ($2–3\ g \cdot kg^{-1}\ BW \cdot day^{-1}$, where BW=body weight) is necessary. Current literature suggests that it may be too simplistic to rely on recommendations of a particular amount of protein per day. Acute studies suggest that for any given amount of protein, the metabolic response is dependent on other factors, including the timing of ingestion in relation to exercise and/or other nutrients, the composition of ingested amino acids and the type of protein(7)



1.3 Protein intake and health

Nutritional assessment of diets and populations is most often concerned with dietary adequacy and the potential adverse effects of low or inadequate nutrient intakes. However, for a proportion of the population in the developed countries, many nutrients are not only abundant in the usual diet, but also may be taken as dietary supplements, so that overall intakes may be far in excess of requirements and recommended intakes. This is especially true for protein and amino acids. Average protein intakes of populations consuming the mixed diets of developed countries will usually be considerably in excess

of recommended intakes, especially for meat-eaters. In addition to this, protein and amino acid supplements are readily available to the general public through pharmacies, grocery stores, and Internet vendors of nutritional products. Protein supplements are the most widely consumed ergogenic aid, whereas single amino acids are consumed for a wide variety of reasons, most of which have little or no secure scientific foundation. There are several issues which arise from the potential for protein intakes to be in excess of the recommended intake. One issue is the potential for harm. The possibility of toxicity resulting from consuming very large amounts of individual amino acids is outside the scope of this report, but has been examined in various publications (8,9). While the previous report did not review the issue of protein intakes in excess of requirements, concern has been expressed in several national reports. In the United Kingdom, in the context of guidance on high intakes, several potential adverse effects were identified and it was concluded that it was prudent for adults to avoid protein intakes of more than twice the reference dietary amount (i.e. 1.5 g protein/kg) (10). It has since been pointed out that such intakes are easily exceeded by physically active individuals on normal diets, and that much higher levels of protein are consumed through the protein enriched diets typical of men involved in body-building (11).

Protein intakes in excess of recommended intakes may confer health benefits, i.e. it may be that optimum protein intakes are greater than a recommended intake derived. Several studies examines the relationship between protein intakes and long term health in relation to a number of specific disease states, and also whether it is possible to identify a maximum level of protein that can be consumed without adverse effects.

1.3.1 Renal function

There is clear evidence that high intakes of protein by patients with renal disease contribute to the deterioration of kidney function (12). However, the suggestion that the decline of glomerular filtration rate that occurs with advancing age in healthy subjects (13) can be attenuated by reducing the protein in the diet appears to have no foundation. However, it seems unlikely that this mechanism would operate in humans, in whom the decline in kidney function occurs through a fall in filtration by non-sclerotic nephrons (14). In a group of subjects covering a wide range of dietary protein intakes, the glomerular filtration rate was related to the protein intake, but albumin excretion, an indication of renal disease, was not (15). Moreover, protein restriction lowers glomerular filtration rate, suggesting that the decline of glomerular filtration rate with age is a natural consequence of the decline in protein intake as age progresses, and is unrelated to deterioration of renal function (16). As concluded by Walser (14), protein restriction on the grounds of renal function is justifiable and prudent only in subjects who are likely to develop kidney failure owing to diabetes, hypertension, or polycystic kidney disease.

1.3.2 Bone health

The relationship between protein intake and bone health appears to be more complex than was previously believed. Thus the potential negative effect of protein on calcium balance is a function of the overall dietary acid–base balance. In addition, protein seems to have direct anabolic effects on the bone matrix. It is well documented that diets containing high protein can result in an increase in urinary calcium excretion (17), amounting to a 50% increase in urinary calcium for a doubling of protein intake (18). Bone mineral balance is very sensitive to acid–base balance, and calcium can be mobilized from bone in response to the need to buffer the acid load produced by oxidation of the sulfur-containing amino acids, methionine and cysteine (19). Accordingly, increased resorption of bone has been shown to occur as a consequence of increased protein intake (20).

1.3.3 Kidney stones

High-protein diets had an increased occurrence of renal stones. Renal stones occur very commonly, and have been estimated to affect 12% of the United States population at some time (21). The urine contains high concentrations of calcium and oxalate, which can accumulate in the kidney as calcium oxalate stones, the most common form of renal stone. Initial studies showed that an increase in dietary animal protein resulted in an elevation of urinary calcium and oxalate, which was estimated to increase the risk of forming stones by 250% (22). Moreover, prospective studies of the effect of dietary calcium and other nutrients on the risk of kidney stones showed that a higher intake of calcium decreased, and a higher intake of animal protein increased, the risk of stones (23).

1.3.4 Cardiovascular disease

There is a complex relationship between protein intake and cardiovascular disease which has yet to be fully resolved. There is a body of experimental studies in rodents pointing to animal protein intakes being more hyper cholesterolaemic and atherogenic compared with intakes of vegetable protein, especially when fed as part of cholesterol-free, purified diets (24). However, this effect is not observed in other species, such as pigs and humans (25), and as yet no convincing mechanisms have been identified. Moreover, evidence has accumulated from human studies that diets with a higher proportion of protein are beneficial for the heart (25).

1.3.5 Cancer

As the incidence of cancer is clearly influenced by environment, the role of diet in the development and growth of malignant tumors has received much attention, although the unequivocal identification of dietary influences has proved most difficult. Furthermore, whereas there have been many large scale studies to investigate the roles of specific foods or food sources, as well as energy substrates and micronutrients, on specific cancers, few have examined dietary protein specifically. Thus potential influences of protein have to be surmised from studies examining the major protein-containing food groups such as meat, dairy foods, eggs and fish. Recent large studies have shown that high intake of red and processed meat is associated with greater incidence of colorectal cancer (26), that meat and dairy consumption do not influence the incidence of gastric cancer (27), and that vegetable and fruit consumption reduces the risk of breast cancer (28).

1.4 Protein requirements of adults

The protein requirements of adult men and women of various body weights are shown in Table (1.1) For adults, the protein requirement per kg body weight is considered to be the same for both sexes, at all ages, and for all body weights within the acceptable range. The value accepted for the safe level of intake is 0.83 g/kg per day, for proteins with a protein digestibility-corrected amino acid score value of 1.0. No safe upper limit has been identified, and it is unlikely that intakes of twice the safe level are associated with any risk.

However, caution is advised to those contemplating the very high intakes of 3–4 times the safe intake, since such intakes approach the tolerable upper limit and cannot be assumed to be risk-free(29).

Table(1.1): Safe level of protein intake for adult men and women.

Body weight (kg)	Safe level of protein intake (g/kg per day)[b]
40	33
45	37
50	42
55	46
60	50
65	54
70	58
75	62
80	66

[a] All ages >18 years.
[b] 0.83 g/kg per day of protein with a protein digestibility-corrected amino acid score value of 1.0.

1.5 Liver and amino acid supplements

Excessive carbohydrate intake is causatively linked to obesity/diabetes. Low-carbohydrate, high-fat/high protein diets like Atkins diet are often recommended to the obesity patients to promote weight loss (31). More importantly, typical western meat-rich foods contain both high protein and high fat (32). Although the hazardous effect of high fat upon hepatic structure/function is well-recognized, the impact of concomitant high protein intake upon HF-induced liver injury remains unclear (33). Branched chain amino acids (BCAA, including leucine, isoleucine, and valine) are a group of essential amino acids. Relatively abundant in food, they account for 20% of total protein intake (34). Part of the high-protein diet often recommended for obese patients, BCAA intake reduces body weight (35). However, recent studies demonstrate elevated circulating BCAA are strongly associated with NAFLD-related metabolic disorders, such as obesity, metabolic syndrome, and type 2 diabetes mellitus (36).

Materials and Methods

Chemical instrument and sample

2.1 Instruments

Table(2.1): The instrument used in the study

Company	Instrument
EBA 20	Centrifuge with maximum speed 5000 r. p. m
PD-303	Spectrophotometer
Memert	Incubator

2.2 Patients

The study group included of (30) healthy man aged range (17-40) classified into two groups, group1 consisted of 15 healthy man taking amino acid supplement aged range (20-40), and group 2 consisted of 15 healthy man don't take any amino acid supplement aged range (17-38)

2.3 Samples and Blood

Blood samples(3ml) were obtained from patients vein puncture. Blood samples allowed to clot at room temperature then centrifuged at 1500*g for 5 minutes. The serum specimens were stored at-16 to one month and at -20 to two months until used.

2.4 Methods

2.4.1 Determination of Total Protein

2.4.1.1 Principle:

In the biuret reaction, a chelate is formed between the Cu^{+2} ion and the peptide bonds of the protein in alkaline solutions to form a violet colored complex whose absorbance is measured photometrically . The intensity of the color produced is proportional to the concentration of protein in the sample.

$$Cu^{+2} + \text{Serum protein} \xrightarrow[(25\text{-}37)\ C]{pH>12} \text{Copper-protein complex}$$

2.4.1.2 Reagent composition

R1: Biuret reagent. Cupric sulfate 6mmol/L, sodium potassium tartrate 21mmol/L, potassium iodide 6mmol/L and sodium hydroxide 0.75 mole/L C R:34

CAL: Protein standard. Bovine serum albumin 7g/dL (70 g/ L) concentration value is traceable to standard reference material 927

2.4.1.3 Materials required

1-Spectrophotometer : to measure absorbance at 540 +-20.5.1.3

2-Micropipettes : to measure reagent and samples.

2.4.1.4 Procedure

1- pipette into labeled tubes

Tubes	Blank	Sample	CAL. Standard
R1.Biuret	1ml	1ml	1ml
Sample	------	20µl	-------
CAL. Standard	------	------	20µl

Mix and incubate the tubes 5minutes at 37C

2-Read the absorbance(A) of the samples and the standard at 540 nm 3-

against the reagent blank.

The color is stable for at least 1hour.

2.4.1.5 Calculation

(A sample/ A standard) *C standard = g/dL total protein

2.4.1.6 Reference values

Adults: 6.6-8.7g/dL

2.4.2 Determination of Albumin

2.4.2.1 Principles

The method is based on specific binding of bromocresol green (BCG), and anionic dye, and the protein at acidic pH with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed is proportional to the concentration of albumin in the sample.

BCG + Albumin ⟶ pH 4.3 BCG-albumin complex

2.4.2.2 Reagent composition

Vial R2:bromocresol green

Materials	Concentration
Succinic acid	83 mmol/L
Bromocresol green	167 mmol/L
Sodium hydroxide	50 mmol/L
Polyoxyethylene monolauryl ether preservation	1.00 g/L

Vial R2:Standard

Bovine albumin 4.0 g/dL

2.4.2.3 Materials required

1-Spectrophotometer : to measuer absorbance at 630nm +-20.

2-Micropipettes : to measuer reagent and samples.

2.4.2.4 Procedure

1-Bring reagents and samples to room temperatue.

2-Pipette into labelled tubes:

Tubes	Blank	Standard	Assay
R2.reagent	1ml	1ml	1ml
Demineralised water	10µl	------	-----
Specimen	------		10µl
Standard	------	10µl	-----

3-Mix and let the tubed stand 1 minute at room temperatuer.

4-Read the absorbance (A) of the samples and the standared at 630 nm against the blank.

2.4.2.5 Calculations

(A sample/ A standard) *C standard = g/dL Albumin

2.5.2.6 Table (Expected values)

Albumin	g/dL	[µmol/L]
14-18) year(	3.2-4.5	[482-677]
18-60) year(	3.4-4.8	[512-722]

2.5 Determination of AST activity

2.5.1 Principle

Aspartate Aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenyl-hydrazine

α-oxoglutarate+L-asprarate ⟶ L-glutamate + Oxaloacetate

Reagents composition 2.5.2

2.5.3 Procedure

Wavelength Hg 546nm (530-550nm)

Cuvette 1cm light path

Incubation Temperature 37 ^{0}C

Measurement against Reagent Blank

Pipette into test tubes

	Reagent	Blank
Sample	---	0.1 mL
Buffer(R1)	0.5 mL	0.5 mL
Distilled water	0.1 mL	---
Mix incubate for exactly for 30 Min at 37 ^{0}C		
2,4-dinitrophenylhydrazine(R2)	0.5 mL	0.5 mL
Mix allow to stand for exactly 20Mix at 20 to 25 ^{o}C		
Sodium Hydroxide(R3)	0.5 mL	0.5 mL

Mix read the absorbance of sample (A sample) against the reagent blank after 5 Min .

2.5.4 Calculations

Obtain the activity of AST in the serum from the table

Absorbance	U/L	Absorbance	U/L
0.020	7	0.100	36
0.030	10	0.110	41
0.040	13	0.120	47
0.050	16	0.130	52
0.060	19	0.140	59

Absorbance	U/l	Absorbance	U/l
0.070		23	
0.150			67
0.080		27	
0.160			76
0.090		31	
0.170			89

2.6 Determination of ALT activity

2.6.1 Principle

Alanine Aminotransferase measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenyl – hydrazine

L-glutamate + ⟶ **- oxoglutrate +L-alanine α pyruvte**

Alanine Aminotransferase measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenyl – hydrazine.

2.6.1 Reagents composition

Contents Initial concentration of Solution

R1.Buffer

Phosphate buffer 100mmol/L PH7.4

L-aspartate 100mmol/L

α-oxoglutarate 2mmol/L

R2. 2,4-dinitrophenylhydrazine 2.0mmol/l

R3. Sodium Hydroxide

CAL. Pyruvate Standard

2.6.2 Procedure

Wavelength Hg 546nm (530-550nm)

Cuvette 1cm light path

Incubation Temperature 37 ^{o}C

1.Measurement against Reagent Blank

Pipette into test tubes

	Reagent Blank	Sample
Sample	- - -	0.1 mL
Buffer(R1)		0.5mL
	0.5 mL	
Distilled water		0.1 mL
	- - -	
Mix incubate for exactly for 30 Min at 37 °C		
2,4-dinitrophenylhydrazine(R2)	0.5 mL	
0.5mL		
Mix allow to stand for exactly 20Mix at 20 to 25 °C		
Sodium Hydroxide(R3)		5.0mL

0.5mL

Mix read the absorbance of sample (A sample) against the reagent blank after 5 Min .

2.6.3 Calculations

Obtain the activity of ALT in the serum from the table

Absorbance	U/L	Absorbance	U/L
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	65
0.375	21	0.125	67
0.150	25	0.40	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88

2.7 Statistical analyses

The data throughout this study reported in the form of (mean value ± the standard deviation).The data were compared by SPSS version 20, where difference is considered as highly significant when ($p<0.001$), significant when ($p<0.05$), and non-significant when ($p>0.05$).

3. Results and discussion

Liver has to perform different kinds of biochemical, synthetic and excretory functions, so no single biochemical test can detect the global functions of liver. All laboratories usually employ a battery of tests for initial detection and management of liver diseases and these tests are frequently termed "Liver function tests", although they are of little value in assessing the liver function per se. In spite of receiving a lot of criticism for this terminology, the phrase 'Liver function tests' is firmly entrenched in the medical lexicon. It might be argued that 'Liver injury tests' would be a more appropriate terminology. Moreover, the clinical history and physical examination play important role to interpret the functions. The role of specific disease markers, radiological imaging and liver biopsy can not be underestimated (37,38).

Classification of liver function tests

A. Tests of the liver's capacity to transport organic anions and to metabolize drugs- Serum bilirubin, urine bilirubin, urobilinogen etc

B. Tests that detect injury to hepatocytes (serum enzyme tests) – Aminotransferases, alkaline phosphatase, ã glutamyl transpeptidase, nucleotidase, leucine aminopeptidase etc

C. Tests of the Liver's biosynthetic capacity- Serum proteins, albumin, prealbumin, serum ceruloplasmin, procollagen III peptide, a 1 antitrypsin, a feto protein, prothrombin time etc (40).

In our study we determined some parameters to find if there is a difference in these parameters between men who taking amino acid supplement from those who don't taking any supplement to assess the effect of these supplement on liver function.

The results obtained from our study are list in table (3.1) ,we find that there isn't any significant differences in the all parameters we determined between the study groups.

Table (3.1): Levels of total protein, albumin, globulin, albumin/globulin ,GOT,GOT specific activity, GPT and GPT specific activity in our study groups .

Groups	Group 1 Taking amino acid supplement	Group2 Don't taking amino acid supplement	P value
No.	15	15	----
Total protein (g/dl)	9.69± 1.49	9.55± 1.72	0.808
Albumin (g/dl)	2.66± 0.56	2.80± 0.63	0.534
Globuline (g/dl)	7.03± 1.48	6.75 ±1.841	0.643
Albumin/Globulin	0.39± 0.13	0.46± 0.23	0.354
GOT(U/L)	22.26 ±10.29	20.60±9.98	0.656
GOT specific activity(U/g)	2.37±1.16	2.28±1.34	0.836
GPT(U/L)	5.46±2.03	6.80±4.24	0.282
GPT specific activity(U/g)	1.02±1.66	0.73±0.56	0.534

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