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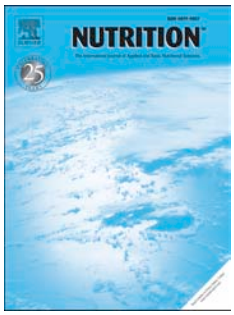

Effect of glutamine supplementation on cardiovascular risk factors in patients with type 2 diabetes

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Title page

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Running head

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Effect of glutamine supplementation on cardiovascular risk factors in patients with type 2 diabetes

Abstract

Objective: To assess clinical relevance of long term oral glutamine supplementation on lipid profile, inflammatory and metabolic factors in diabetic subjects.

Research method & procedure: Sixty-six type 2 diabetic subjects aged between 18-65 years old were randomized to receive glutamine (30g/d) or placebo three times a day, in a double-blinded, placebo-controlled trial during 6-week treatment period. Fifty-three type 2 diabetic patients completed the trial. Independent samples t-test and analysis of covariance (ANCOVA) were used.

Results: After 6- week treatment period, a significant difference was observed between the two groups in body fat mass (P=0.01) and percentage of body fat (P=0.008). Moreover, a significant reduction in waist circumference (P<0.001) and a tendency for an increases in fat free mass (P=0.03), with no change in body weight and body mass index (BMI) was found. Enhancement in body fat free mass was mainly attributed to trunk (P= 0.03). There was downward trend in systolic blood pressure (P= 0.005) but not diastolic. Fasting blood glucose (mmol/L) concentration significantly decreased after 6 weeks intervention (P=0.04). Mean HbA1c was significantly different between the groups at week 6 (P=0.04). No significant difference was detected for fasting insulin, HOMA-IR and QUICKI between groups (P>0.05). No significant difference was observed between groups in total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride. No treatment effect on C-reactive protein was found (P=0.44).

Conclusion: We demonstrated that the 6 week supplementation with 30 g/d glutamine markedly improved some of cardiovascular risk factors and body composition in patients with type 2 diabetes. Future glutamine dose-response studies are warranted in these areas.

Key words: glutamine supplementation, type 2 diabetes, body composition, Insulin, GLP-1

The present clinical trial was registered in the Iranian Registry of Clinical Trials (IRCT) and given the ID, IRCT201205279373N1 (www.irct.ir)

Abbreviations

GLP-1 Glucagon-like peptide1

HOMA Homeostasis model assessment

QUICKI Quantitative insulin sensitivity index

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia resulting from defects in insulin secretion or/ and action [1] and corresponds to 1.7% of total death in the world [2]. Type 2 diabetes is a major risk factor for cardiovascular disease development [3]. On the other hand, cardiovascular diseases is a major cause of morbidity in diabetic subjects [4] and more than 75% of all type 2 diabetes population has metabolic syndrome [5]. The rate of diabetes has risen in parallel with the increased prevalence of obesity [6]. A study by Wing et al. [7] concluded that modest losses of 5 to 10% of weight were associated with significant improvements in cardiovascular disease risk factors in overweigh and obese individual with type 2 diabetes. Type 2 diabetes is associated with decreased lean body mass and increased body fat mass compared to non-diabetics with similar BMI values [8]. Insulin resistance may accelerate development of sarcopenia [9] and type 2 diabetes is an important predictor of sarcopenia [8]. Excessive loss of muscle mass in type 2 diabetes may result in lower skeletal function, strength and quality [10].

Glutamine, a nonessential amino acid is the most abundant free amino acid in circulation [11] and pool in skeletal muscle [12]. Interestingly, the circulating glutamine concentration is reduced significantly in patients with type 2 diabetes in comparison with healthy subjects [13]. Glutamine supplementation has been shown to increase protein synthesis in catabolic stress situation [14] and decrease proteolysis in rats [15]. Studies support the hypothesis that administration of additional glutamine represents an effective dietary strategy to improve glycemic control in patients with type 2 diabetes and may be useful as an anti-obesity and anti-diabetic agent [16, 17]. However, these hypotheses are based on small trials with single doses or/ and very short study durations or in animal models. These

drawbacks mean that well conducted randomized controlled trials are required for definite results. Therefore, the aim of current study was to investigate whether 6 weeks of oral glutamine supplementation with each main meal (30 g/day) would induce and sustain improvement of cardiovascular risk factors, such as hyperlipidemia, hyperglycemia, high blood pressure, insulin sensitivity, waist circumference, body weight and body composition in type 2 diabetic patients. To the best of our knowledge, this is the first placebo controlled trial to assess clinical effects of such long term glutamine supplementation in human diabetic subjects.

Methods

Study population

Type 2 diabetic patients were recruited from diabetes clinic of Shariati hospital, Tehran. Exclusion criteria were history of drug abuse; body mass index (BMI) $\geq$35 kg/m^2; current insulin therapy; history of cancer; treatment with anti-inflammatory drugs, corticosteroid; hormonal or antibiotic agents; a restrictive diet or weight change $\geq$ 5 kg during the past 3months prior to study; systolic blood pressure >160 mmHg and/or diastolic blood pressure > 90 mmHg; fatty liver disease; liver or kidney diseases; autoimmune diseases; pregnancy and lactation; any changes in treatment with oral hypoglycemic, anti-hypertensive and anti-hyperlipidemic agents during the study and use of weight loss medications.

The present study was approved by the medical ethics committee of Tehran University of Medical Sciences and participants signed an informed consent.

Materials and Methods

Study design

Participants aged between 18-65 years old who attended Shariati hospital were randomly assigned to 2 groups that received either glutamine or placebo for 6 weeks. The patients in each group took glutamine in free form (99.99% pure) 30 g/day (10 g powder, three times /day) or resistant starch powder from corn as placebo 3 g/ day (1 g powder, three times/ day), 5-10 minutes before each main meal (breakfast, lunch and dinner) in half glass of ice-cold water [18] immediately prior to the meal for 6 weeks.

Diet and physical activity

To assess potential changes in daily food consumption and physical activity during the 6 week intervention, participants recorded 3 days food record and 1 physical activity record at baseline and the 6^{th} week of intervention. All participants were asked to maintain their usual dietary intake and habitual physical activity for the duration of trial.

Assessments

Anthropometric measurements, body composition and blood pressure

Height of patients was measured to the nearest 0.5 centimeters using a wall-mounted stadiometer. Weight and body composition were measured using Tanita BC 418 MA Segmental Body Composition Analyzer (Tanita, Japan) in light clothes and not wearing shoes [19]. BMI was calculated as body weight (kilograms) divided by the square of height (meters). Waist circumference was measured to the nearest 0.1 centimeters, midway between the lateral lower rib and the iliac crest with the patient standing upright. Systolic

and diastolic blood pressures were measured using a standard calibrated mercury sphygmomanometer on the right arm of the subjects after 5 minutes of rest.

Diet and physical activity measurements

Total energy and macronutrient content were calculated using Nutritionist IV software (The Hearst Corporation 1994). Physical activity was calculated by multiplying metabolic equivalent of task (Met) and duration (hours) per day using the international physical activity questionnaire (IPAQ) [20].

Blood sampling

After 10-12 hours fast, blood samples were taken from subjects at baseline and the 6th week of the intervention. Serum and EDTA tubes were centrifuged at 2500×g for 15 min. Serum and plasma were immediately frozen and stored at -80 $^{\circ}$C until further analysis later. Serum concentrations of glucose, triglyceride (TG), total cholesterol, HDL cholesterol, LDL cholesterol, creatinine and high sensitive C-reactive Protein (hs-CRP) were measured by a colorimetric method using Pars Azmoon kits (Pars azmoon.co, Tehran, Iran) with an auto-analyzer (Autoanalyzer Hitachi 902, Roche Diagnostics, Holliston, MA, USA). Serum insulin concentration was measured by ELISA kits (Monobind kit, Monobind Inc., Lake Forest, CA, USA). Plasma concentration of HbA1c was measured using automatic analyzer DS5 and DS5 Pink Reagent kits. The plasma concentration of glutamine was determined by HPLC.

Insulin resistance was calculated by using the homeostasis model assessment (HOMA) method [21]: fasting insulin (μU/ml) × fasting glucose (mmol/L)/ 22.5. Insulin sensitivity

was calculated using the quantitative insulin sensitivity check index (QUICKI) method [22]: 1/ (log insulin (μU/ml) + log glycemia (mg/dl)).

Statistical analysis

Statistical analyses were performed by SPSS version 18.0 for Windows (SPSS Inc., Chicago, IL). Independent samples t-test was used to compare any differences between two groups at the baseline. Whitin- group comparisons were analyzed by the paired t- test. Analysis of covariance (ANCOVA) using a general linear model, was used to test the difference between study groups at the end of study. $P<0.05$ was considered statistically significant.

Results

Sixty- six type 2 diabetic patients were randomly assigned to either glutamine or placebo group for 6 weeks. Fifty-three subjects completed the trial and were included in the statistical analyses, 27 in the glutamine group and 26 in the placebo group (Fig 1). Baseline characteristics for glutamine and placebo groups are shown in Table 1. Except for diastolic blood pressure, which was lower in the glutamine group ($P=0.03$), no significant differences were observed between the two groups at baseline (Table 1).

When the 3-day food records and physical activity questionnaires were analyzed, the glutamine and placebo groups were similar in intakes of macronutrients, energy (Table 3) and physical activity levels (Table3) at baseline and no significant differences were

observed between groups in energy, macronutrients intake (Table 3) and physical activity (Table3) over the course of the intervention.

Plasma glutamine concentration increased from baseline in glutamine group, but differences between groups were not significant (glutamine group= 4.60 ±194.74; placebo group= -50.32±159.50; P= 0.49; Figure 2).

Body weight and composition

Table1and 2 presents anthropometric and metabolic variables in week 6, respectively. After 6 weeks of supplementation, subjects in the intervention group showed a significant difference in body fat mass (glutamine group=0.05±1.34 kg; placebo group=1.05±1.36 kg; *P*=0.01) and % body fat (glutamine group=0.01±1.47%; placebo group= 1.36±1.97%; *P*=0.008) and waist circumference (glutamine group= -1.33±3.8 cm; placebo group= 2.34±3.25 cm; *P*<0.001) and fat free mass (glutamine group= 0.02±1.36 kg; placebo group= -0.76±1.22 kg; *P*=0.03) with no significant differences in body weight and BMI (*P*>0.05). Significant increase in body fat free mass in the glutamine group compared to the placebo was mainly indicated in trunk (glutamine group =0.17 ± 1.27 kg; placebo group=-0.43 ± 0.70 kg; P= 0.03) and also in apendicular (glutamine group=0.03 ±0.68 kg; placebo group=-0.32 ± 0.61 kg; P= 0.05). Trunk fat percent tended to change differently over 6 week between the treatments (glutamine group =-0.10±2.01%; placebo group=1.42±1.43%; P=0.004). Similarly, there was a significant reduction in apendicular fat percent (glutamine group = -0.12 ± 1.20%; placebo group = 1.29± 2.84%; *P*=0.02). The decrease in the mean value for waist circumference (cm) in the glutamine group over the 6 weeks was 1.33 (*P*< 0.001; Figure 3).

A significant difference (Figure 4) between the placebo and glutamine groups was detected for systolic blood pressure (glutamine group= -6.29±10.79 mmHg; placebo group= 0.76 ± 13.54 mmHg; *P*= 0.005; Figure 4 (A)). There were no significant differences for diastolic blood pressure between treatment and control (glutamine group= -1.85±9.21 mmHg; placebo group= -1.15 ± 9.93 mmHg; P= 0.20; Figure 4 (B)).

Fasting blood lipids profile

No significant differences were observed between groups in any of the general lipid profile measurements (total cholesterol, HDL-cholesterol, LDL-cholesterol and TG) (P>0.05) (Table 2). Although plasma TG concentrations decreased during the intervention period in the glutamine group (glutamine group =-10.14 mg/dl; placebo group = 3.69 mg/dl; P= 0.13), difference between groups was not statistically significant. However, after exclusion of one subject in placebo group from the analyses, the difference became significant (P=0.04).

Glycemic control:

Fasting blood glucose concentration significantly decreased after 6 week treatment in glutamine group (glutamine group = -0.79 ± 1.35 mmol/L; placebo group= -0.06 ± 1.70 mmol/L; *P*=0.04; Figure 5), and also mean HbA1c difference between the groups was significant (glutamine group=0.16±0.40%; placebo group=0.22±0.55%; *P*=0.05). However, no significant difference was detected between groups in fasting insulin, HOMA-IR and QUICKI (*P*>0.05).

Level of C- reactive protein (CRP) were similar between two groups at baseline (Table 2) and also between group differences were not significant at the end of study course

(glutamine group= 1.69±5.09 mg/dl; placebo group= 0.82 ±2.66 mg/dl; P=0.44). The effect of glutamine supplementation on cardiovascular risk factors, such as lipidemia, glycemia, blood pressure, insulin sensitivity, waist circumference and body weight and composition was assessed in population of middle aged, type 2 diabetic patients. Subjects consumed glutamine (30 g/d) or placebo three times daily. At the end of the 6-week treatment period no changes in insulin sensitivity, insulin secretion, total cholesterol, HDL-cholesterol, LDL-cholesterol, TG, CRP, weight, BMI or diastolic blood pressure were detected. However, systolic blood pressure, fasting blood glucose and waist circumference were found to be reduced and body composition improved.

Discussion

Six weeks of glutamine supplementation, in comparison with the same period of the placebo treatment, induced significant changes in total fat mass and increase in fat free mass was accompanied by a decrease in the waist circumference with no significant changes in body weight. Opara et al. [16] suggested that, glutamine attenuated body weight gain in high-fat-fed hyperglycemic mice. This means that glutamine reduces the effect of a high-fat diet on the incidence of obesity. Prada et al. [23] reported that oral glutamine supplementation reduced 50% of central fat depot of rats on a high fat diet. In the current study we found a decrease in body weight or BMI following glutamine treatment, but these decreases were not statically significant. However, the changes detected in rats could be different from those in humans [24].

Fat free mass is an important nutritional parameter, linked with immune qualification, functional status, and survival [25]. Type 2 diabetes is associated with rapid declines in skeletal muscle especially in older women [10, 26] and greater risk of body fat mass

increases [8] with no clear reasons. It has been suggested that metabolic abnormalities in type 2 diabetes may negatively affect lean body mass. Insulin resistance in type 2 diabetes may also link to reduced synthesis of whole-body proteins [10]. Besides, fat free mass declines are associated with increases in insulin resistance [27]. Additionally, type 2 diabetes and lower lean mass may increase the risk of dementia [28]. Interestingly, for the first time, our study showed the adipose changing and lean body mass increasing effect of glutamine with no significant effect in body weight and also decreases in the waist circumference in diabetic humans. It is possible, that fat mass was replaced by equal amount of lean mass. An alternative possibility is that glutamine supplementation was able to improve body compositions by increasing GLP-1(glucagon like peptide 1) levels since it is established that GLP-1 analogues induce significant reductions in body weight, fat mass and waist circumference [29]. In a recently published review article, we concluded that glutamine could be used as nutritional therapy to enhance the GLP-1 levels [30].

Obesity is a risk factor for type 2 diabetes and cardiovascular disorders [31] Therefore, obesity must be contended. However, weight loss is usually associated with significant declines in fat mass and fat free mass which could decreases basal metabolic rate (BMR) [32]. Based on the evidence, lean mass is the largest determinant of resting metabolic rate (RMR) and elevated body lean mass increases RMR [33]. Glutamine effect on RMR was not investigated in the present study, so glutamine effects on RMR remains unclear. Further investigations are required to determine the exact effect of glutamine on metabolism. Collectively, we suggest glutamine consumption in weight reduction program including hypocaloric diets. Glutamine supplementation may decrease body weight and fat mass without reductions of muscle mass or may even increase muscle mass.

A recent study found rapid decline in appendicular lean mass in patients with diabetes compared to non-diabetic subjects, especially in thigh muscle mass [10]. These data reinforce the need for effective preventative and management strategies to reduce appendicular lean mass losses risk in adults with diabetes, particularly in women. Intervention with glutamine improved appendicular and trunk lean mass, highlighting the beneficial effect of glutamine in managing loss of skeletal muscle in diabetic patients.

To the best of our knowledge, this is the first study to assess the effect of glutamine administration on arterial blood pressure. We reported that dietary supplementation with glutamine reduces systolic blood pressure but not diastolic in type 2 diabetic patients. A recent meta- analysis by Robinson et al. [34] concluded that the use of GLP-1 analogues reduces systolic blood pressure but reductions in diastolic failed to reach significant level in patients with type 2 diabetes. We hypothesize that GLP-1 may be an important factor for blood pressure-lowering effects of glutamine. The exact mechanism (s) behind the blood-pressure lowering effect of amino acids especially glutamine needs to be clarified in future studies.

The present study showed that 6 weeks of glutamine supplementation (30 g/d) significantly reduced fasting plasma glucose and also significantly changed HbA1c levels, with no significant effect on insulin concentration and blood lipid profile.

In vitro observation shows no increase in insulin secretion in pancreatic islets following use of glutamine [35] Yet, from both animal and human studies, it has become evident that glutamine functions as an insulin secretagogue [17, 23, 36]. A finding from mouse models showed that glutamine supplementation diminished the expected increase in body weight and prevented hyperglycemia and hyperinsulinemia over 5.5 month in mice fed a high fat

diet [16] Thereafter, Bakalar et al. [36] confirmed the better insulin sensitivity with glutamine administration in multiple trauma patients. A possible mechanism by which glutamine reduces glycemic was defined by Reimann et al. [37]. In their in vitro study, they concluded that glutamine stimulates the release of GLP-1 secretion more than glucose or other amino acids in the cell line GLUTag [37]. Consistent with this finding for GLP-1 secretion, two recent studies showed that oral glutamine increases circulating GLP-1conccentration in human subjects [17, 18]. One of these studies showed that increased insulin level in parallel with GLP-1, suggested that glutamine may stimulate insulin release in pancreas via GLP-1 secretion in vivo. The authors suggested that glutamine may represent a novel therapeutic strategy to stimulate insulin secretion and management of glycemia in type 2 diabetes [17]. However, the second one ruled out this effect. This study concluded that after glutamine ingestion, insulin concentration did not rise in parallel with increase in c- peptide; so it may affect insulin clearance rather than insulin secretion and also demonstrated that 30 g of glutamine or 15 g of glutamine plus sitagliptin effectively reduced postprandial glycemia in type 2 diabetic patients. These authors suggested that the glucose-lowering effect of glutamine is likely due to slow gastric emptying [18]. Similar to these findings, in the current study, glutamine effectively improved fasting glucose control in type 2 diabetes with no significant effect on insulin secretion. Our data suggest that the insulin secretion capacity of the β-cells doesn’t change in response to 30 g/day glutamine supplementation. Kjems et al. [38] indicated that GLP-1 increases insulin secretion in type 2 diabetes in a dose- dependent manner. Possibly, the result we observed in this study may be related to this phenomenon. GLP-1 is capable of suppressing glucagon secretion; a mechanism which may be able to lower fasting blood glucose [39].

Additionally, in male Wistar rats fed a standard diet or a high fat diet or high fat diet enriched with glutamine or alanine over the period of 2 months, it has been shown that the once with diet induced obesity, oral administration of glutamine increased insulin stimulated glucose uptake in skeletal muscle and insulin induced suppression of hepatic glucose output; consequently, insulin sensitivity was improved. These results were associated with enhanced adiponectin (a glucose lowering adipokine) levels [23]. Our results may be attributed to trunk fat mass [40] and enhanced adiponectin and GLP-1secretion or all of them. Although other trials have observed an increase in GLP-1 levels following glutamine consumption [17, 18], the exact responsible mechanism (s) are poorly understood.

Based on our knowledge, this trial is the first to investigate the effect of glutamine supplementation on lipid profile. We concluded that glutamine does not improve lipid profile. Although a decrease in triglyceride concentration was observed, the reduction was not statistically significant.

Previous studies reported that CRP level, the marker of inflammation, was elevated in diabetic groups. It was shown that glutamine administration reduced the expressions of inflammatory markers such as CRP, indicating that the inflammatory response was attenuated in the diabetic subjects [41]. This effect of glutamine may play a role in improvement of insulin sensitivity [42]. But in contrast with other studies, no significant changes in inflammatory index or insulin sensitivity were observed over time either in the glutamine supplemented or the placebo group.

Supplementation with glutamine at a dose of 0.5 g/ kg body weight did not seem to be accompanied by any adverse effects [43]. Because high dietary protein intake has been

shown to increase risk of developing kidney disease, serum creatinine was measured to assess potential changes in kidney function [44]. In this study no changes were observed in serum creatinine levels, so prolonged glutamine supplementation (30 g/d) does not seem to have any negative impact on kidney function. Supplements were well tolerated and there were only 2 participant complaints of gastro-intestinal symptoms which were reported in intervention group.

Adherence to the study was evaluated by the empty packages that were returned and also the plasma content of glutamine. The plasma glutamine level did not rise significantly during the treatment. These data tend to be consistent with the previous studies [45,46] and confirm that glutamine supplementation cannot rise plasma glutamine concentration in whom plasma levels are already normal before glutamine therapy is started. This lack of significant rise in plasma glutamine may be attributed to the fact that muscle glutamine utilization is increased for protein synthesis during glutamine administration [45].

Nitric oxide (NO) plays a dual role in the protection against the initiation and progression of cardiovascular disorders [47]. Since arginine is the nitrogen donor in NO generation, there has been an amplified interest in this amino acid over the past years. Data support that molecular form of glutamine affects the denovo synthesis of arginine from glutamine [48]. In agreement with this hypothesis, Boelens et al. [49] concluded that a higher citrulline production, thereby arginine production occurred when free glutamine was supplied compared with the dipeptide (L-alanyl-L-glutamine). Therefore we used glutamine in free form to benefit the clinical effect of glutamine and arginine.

The present study has limitations important to take into account. Similar to other nutrition studies, it suffers from self recall error and under reporting in overweight and obese

subjects in comparison to leans [45-47]. Despite this bias, analysis of energy and macronutrients were able to show any significant changes during study.

In conclusion, we demonstrated that the long term consumption of 30 g/d glutamine three times a day with main meals markedly reduced glycemia, blood pressure and waist circumference and improved body composition in type 2 diabetes. Glutamine may improve the complications of type 2 diabetes. Yet, the evidence of current study is not sufficient to recommend the regular use. More prolonged nutritional intervention studies are warranted in these areas to assess the proposed clinical benefits of glutamine as an effective pharmaconutrient in chronic metabolic diseases such as type 2 diabetes and also type 1 diabetes, obesity and metabolic syndrome or in muscle wasting conditions.

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Table 1. Baseline and week 6 anthropometric and body composition measurements in two groups.

Variable	Placebo group(n=26)	Glutamine group(n= 27)	*P-value*
	Mean ± SD	Mean ± SD	
Age (y)	53±5.15	50±8.01	0.09
Sex [n(%) women]	18(69.23)	18 (66.66)	0.84
Height (cm)	160.26±9.55	161.40±10.32	0.67**
Body Weight (kg) Baseline Week 6	 74.80±13.26 75.14±13.47	 77.01±13.12 76.91±13.69	 0.54** 0.18†
*P-value**	0.18	0.72	
Body Mass Index (kg/m^2) Baseline Week 6	 29.09±4.36 29.21±4.34	 29.46±3.23 29.41±3.31	 0.72** 0.22†
*P-value**	0.22	0.59	
Trunk fat free mass (kg) Baseline Week 6	 28.50±5.61 28.07±5.59	 29.02 ± 5.78 29.19±5.64	 0.74** 0.03†
*P-value**	0.005	0.48	
Trunk fat mass (kg) Baseline Week 6	 12.13±4.24 12.76±4.22	 12.94±4.00 12.98±4.27	 0.48** 0.01†
*P-value**	0.000	0.84	
Trunk fat (%) Baseline Week 6	 29.52±7.77 30.95±7.47	 30.65±6.44 30.55±6.35	 0.57** 0.004†
*P-value**	0.000	0.80	
Apendicular fat free mass (kg) Baseline Week 6	 22.75±5.40 22.42±5.59	 23.11 ±5.47 23.14±5.61	 0.81** 0.05†
*P-value**	0.01	0.82	
Apendicular fat mass (kg) Baseline Week 6	 11.48±4.97 11.90±4.72	 12.10±4.25 12.12±4.34	 0.63** 0.11†
*P-value**	0.05	0.83	

continued

Variable	Placebo group(n=26)	Glutamine group(n= 27)	*P-value*
	Mean ± SD	Mean ± SD	
Apendicular fat (%) Baseline Week 6	32.82±11.69 34.12±11.32	34.32±10.33 34.20±10.28	0.62** 0.02†
*P-value**	0.02	0.59	
Total fat free mass(kg) Baseline Week 6	51.22±10.97 50.46±11.11	52.16±11.28 52.18±11.20	0.76** 0.03†
*P-value**	0.004	0.79	
Total fat mass(kg) Baseline Week 6	23.59±8.58 24.64±8.23	25.01±7.45 25.06±7.65	0.52** 0.01†
*P-value**	0.001	0.85	
Total fat(%) Baseline Week 6	31.31±9.25 32.68±8.89	32.56±7.87 32.57±7.70	0.59** 0.008†
*P-value**	0.002	0.96	

†ANCOVA *P* value for comparison between groups at the end of study.
**Independent samples t-test *P* value for comparison between groups at the baseline.
*paired t- test *P* value for comparison whitin- group comparisons
P<0.05 was considered statistically significant.

Table 2. Baseline and week 6 glycemic control, lipid profiles, inflammatory biomarker and Creatinine in two groups.

Variable	Placebo group(n=26)	Glutamine group(n= 27)	*P-value*
	Mean ± SD	Mean ± SD	
HbA1C (%) Baseline Week 6	 6.76±1.69 7.00±1.66	 6.53±1.30 6.70±1.33	 0.59** 0.04†
*P-value**	0.05	0.20	
Fasting Insulin (µU/ml) Baseline Week 6	 8.73±5.23 10.40±8.56	 8.50±6.71 14.55±16.16	 0.90** 0.22†
*P-value**	0.30	0.06	
HOMA-IR Baseline Week 6	 3.07±1.98 3.66±2.87	 3.05±2.58 5.57±8.34	 0.97** 0.20†
*P-value**	0.29	0.08	
QUICKI Baseline Week 6	 0.33±0.03 0.32±0.03	 0.34±0.05 0.33±0.04	 0.27** 0.54†
*P-value**	0.22	0.05	
Triglycerides(mg/dL) Baseline Week 6	 159.46±91.93 163.15±93.03	 130.74±61.30 120.59±56.96	 0.18** 0.13†
*P-value**	0.74	0.18	
Cholesterol (mg/dL) Baseline Week 6	 154.61±38.84 157.5±45.88	 146.40±30.51 158.37±36.23	 0.39** 0.22†
*P-value**	0.57	0.01	
HDL-Cholesterol(mg/dL) Baseline Week 6	 45.34±9.66 45.96±10.91	 46.55±12.22 48.92±12.60	 0.69** 0.25†
*P-value**	0.56	0.05	
LDL- Cholesterol(mg/dL) Baseline Week 6	 84.11±23.44 85.23±25.26	 78.81±21.20 88.11±26.31	 0.39** 0.12†
*P-value**	0.74	0.01	

Creatinine (mg/ dL) Baseline Week 6	 0.96±0.21 0.96±0.25	 0.97±0.17 0.97±0.23	 0.81** 0.94†
*P-value**	0.84	0.87	
C-reactive protein(mg/dL) Baseline Week 6	 1.34±1.07 2.16±3.18	 1.73±1.68 3.43±5.17	 0.31** 0.44†
*P-value**	0.12	0.09	

†ANCOVA *P* value for comparison between groups at the end of study.
**Independent samples t-test *P* value for comparison between groups at the baseline.
*paired t- test *P* value for comparison whitin- group comparisons
P<0.05 was considered statistically significant.

Table 3. Baseline and week 6 energy, nutrient intake and Physical activity in two groups.

Variable	Placebo group(n=26)	Glutamine group(n= 27)	*P-value*
	Mean ± SD	Mean ± SD	
Energy (Kcal / day) Baseline Week 6	 1603±370.24 1487.80±391.37	 1731.20±608.22 1634.70±565.78	 0.46** 0.63†
*P-value**	0.07	0.35	
Carbohydrate(g/day) Baseline Week 6	 234.39±60.89 208.75±52.41	 244.29±88.18 241.23±85.31	 0.70** 0.13†
*P-value**	0.01	0.83	
Protein(g/day) Baseline Week 6	 59.80±11.76 54.88±13.17	 55.85±18.70 57±20.27	 0.47** 0.42†
*P-value**	0.15	0.79	
Total fat (g/day) Baseline Week 6	 49.47±17.88 50.92±22.82	 62.22±25.08 51.62±22.53	 0.09** 0.33†
*P-value**	0.75	0.05	
Saturated (g /day) Baseline Week 6	 13.25±4.11 12.29±4.67	 14.82±7.59 13.11±5.66	 0.46** 0.77†
*P-value**	0.30	0.43	
Monounsaturated(g/day) Baseline Week 6	 14.02±6.74 18.38±10.44	 18.75±7.92 15.22±7.90	 0.18** 0.16†
*P-value**	0.65	0.02	
Polyunsaturated (g /day) Baseline Week 6	 17.32±7.72 142.30±84.73	 21.69±10.37 16.94±9.13	 0.18** 0.16†
*P-value**	0.65	0.02	
Cholesterol (mg/ day) Baseline Week 6	 186.65±128.61 142.30±84.73	 186±101.72 176.12±111.67	0.98** 0.32†
*P-value**	0.17	0.76	
Fiber (g/day) Baseline Week 6	 13.36±5.11 15.14±5.89	 13.71±5.11 13.43±4.23	 0.84** 0.15†
*P-value**	0.16	0.75	

Physical activity (MET-h/d) Baseline Week 6	 26.92±7.76 26.38±6.25	 26.79±7.65 26.71±7.22	 0.95** 0.07†
*P-value**	0.70	0.62	

†ANCOVA *P* value for comparison between groups at the end of study.
**Independent samples t-test *P* value for comparison between groups at the baseline.
*paired t- test *P* value for comparison whitin- group comparisons
P<0.05 was considered statistically significant.

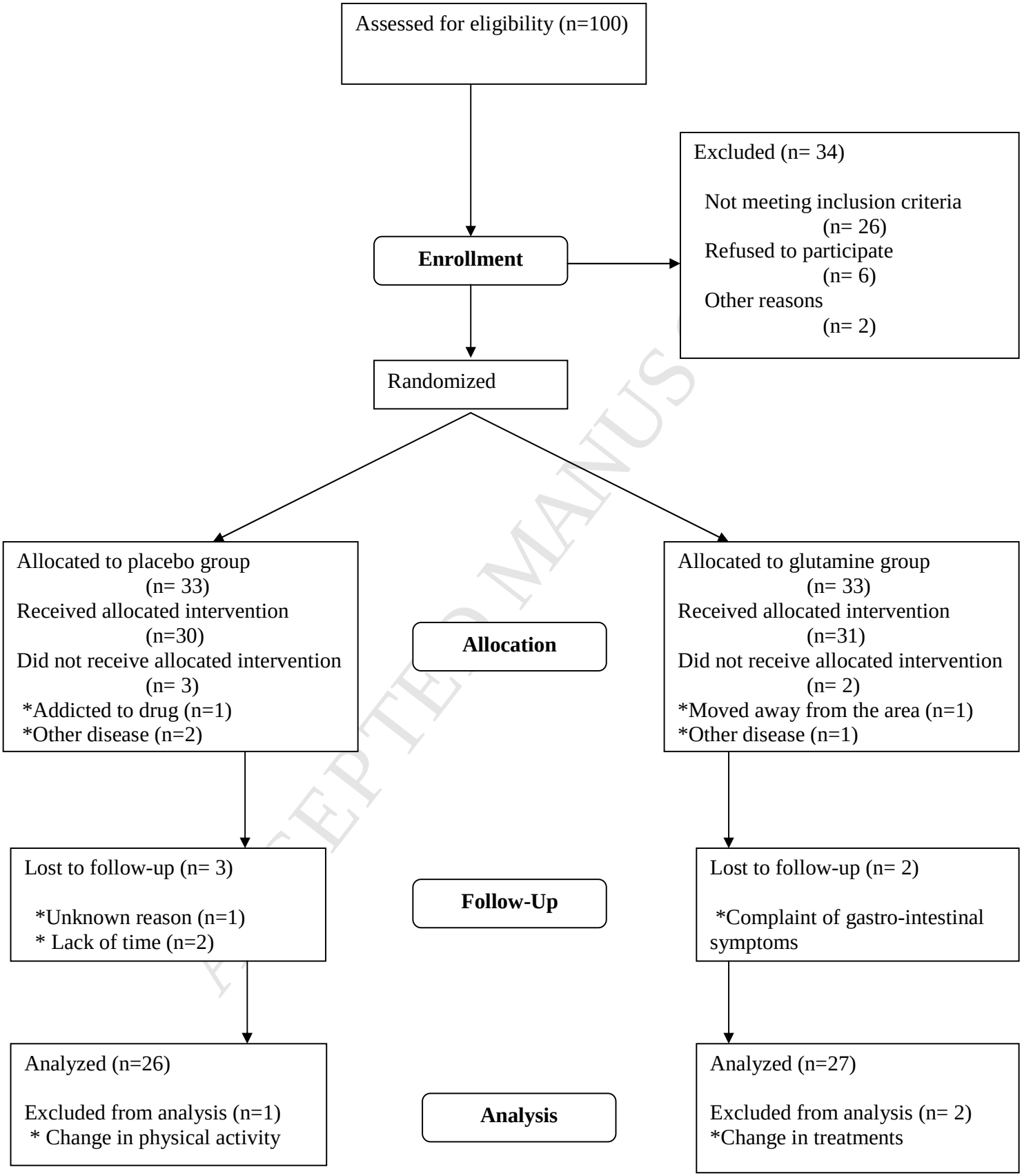

Figure1 Flowchart of participants' progress through the intervention

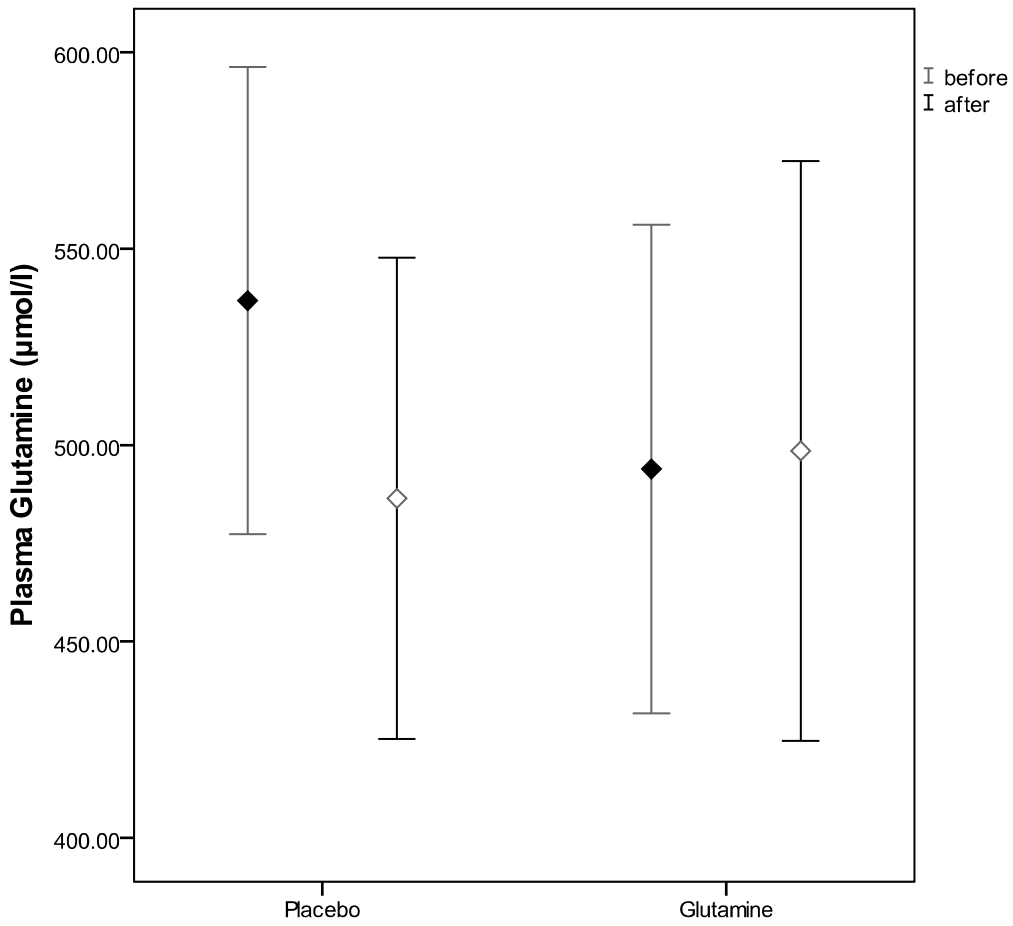

Figure 2- The effect of six- week glutamine intervention on plasma glutamine (µmol/l) in type 2 diabetes patients. There were no significant changes over time in plasma glutamine. n=26 glutamine group, n=26 placebo group. All values are means ± SD.

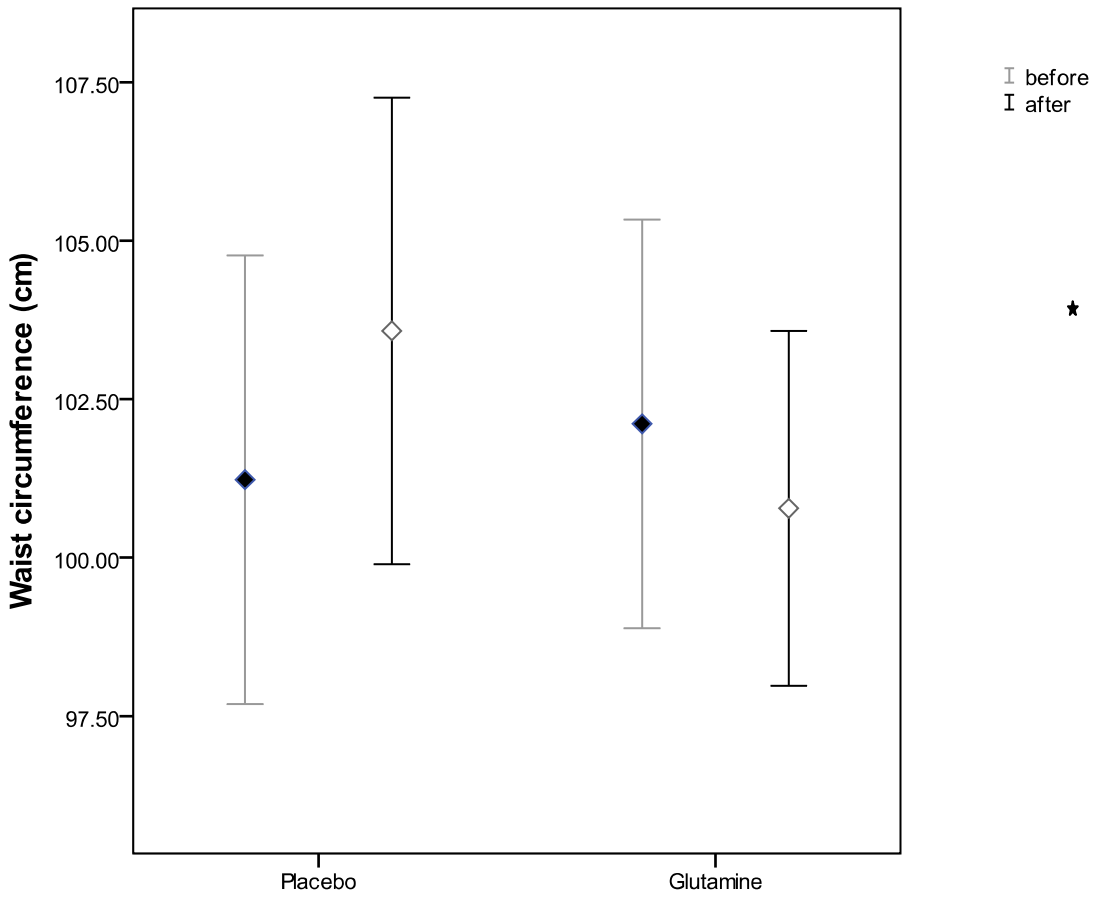

Figure 3-The effect of six- week glutamine intervention on waist circumference in type 2 diabetes patients. * P<0.001, n=27 glutamine group, n=26 placebo group. All values are means ± SD.

A

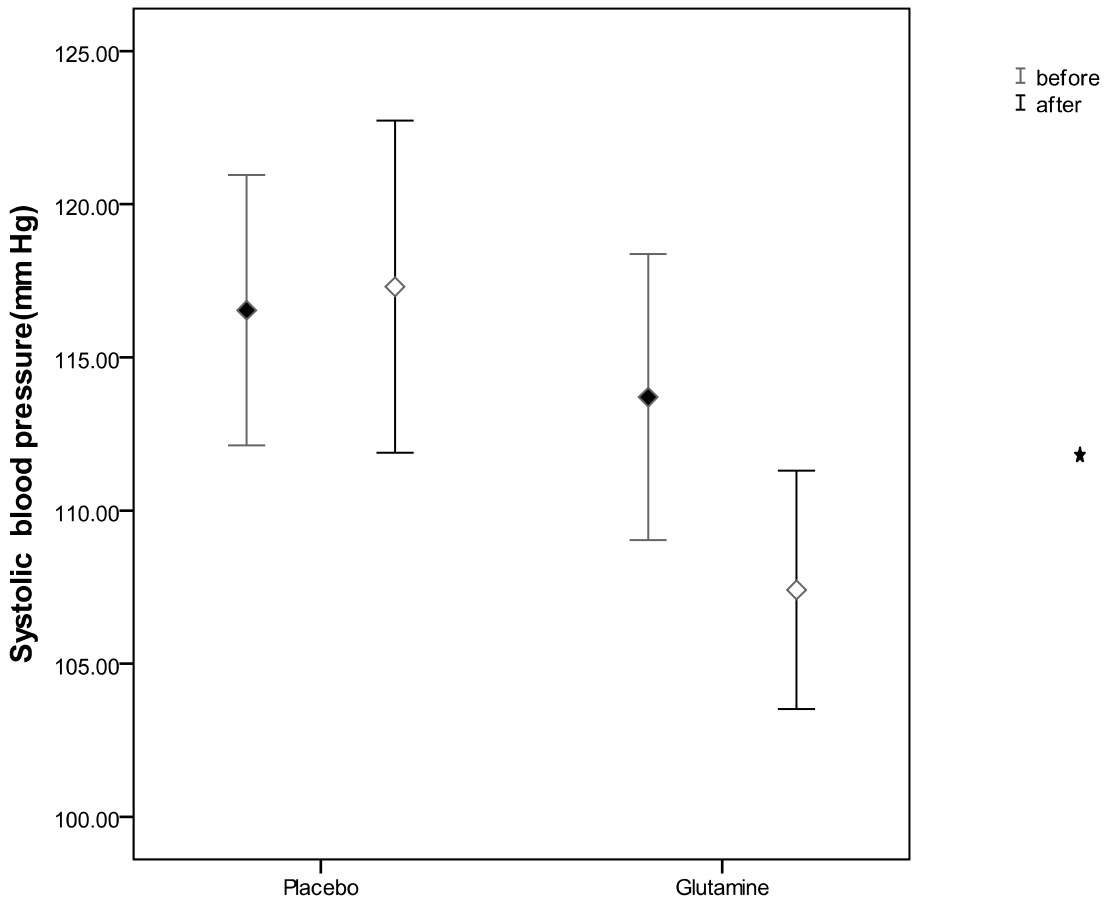

Figure 4- The effect of six- week glutamine intervention on blood pressure (mm Hg) in type 2 diabetes patients. A: Systolic blood pressure (mmHg); B: Diastolic blood pressure (mmHg).

P=0.005, There were no significant changes over time in diastolic blood pressure. n=27 glutamine group, n=26 placebo group. All values are means ± SD.

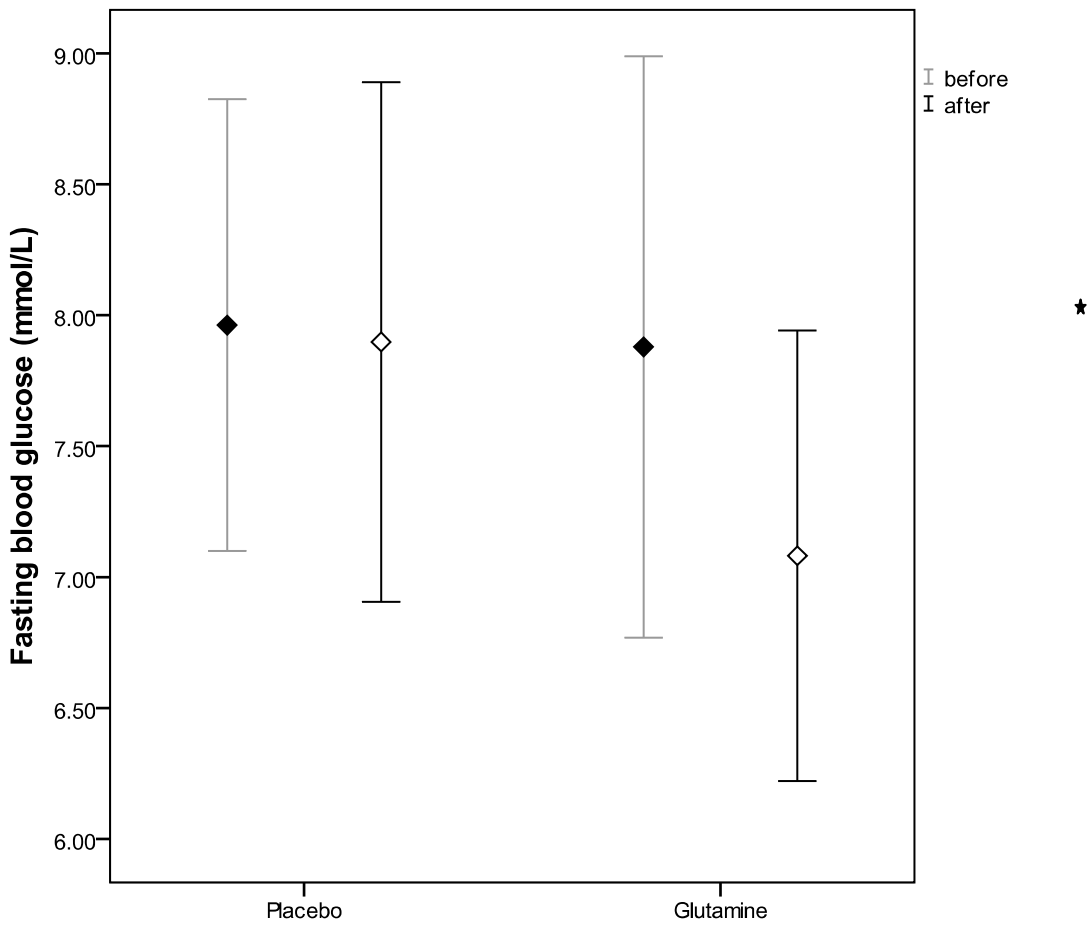

Figure5- The effect of six- week glutamine intervention on fast glucose (mmol/L) in type 2 diabetes patients. *P*=0.04. n=27 glutamine group, n=26 placebo group. All values are means ± SD.

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Highlights

- Systolic blood pressure level decreased significantly after glutamine ingestion.
- Glutamine ingestion significantly decreased fasting glucose compared with placebo.
- Waist circumference was found to be reduced in the glutamine group.
- Body composition was improved in glutamine group.