Evaluation of the effect of oral taurine supplementation on levels of fibroblast growth factors, β-Klotho co-receptor, some biochemical indices and body composition in obese women on a weight-loss diet: a study protocol for a double-blind randomized controlled trial

Fatemeh Haidari
Ahvaz Jondishapour University of Medical Sciences
Maryam Asadi (maryamasadi136@gmail.com)
Ahvaz Jondishapour University of Medical Sciences Faculty of Paramedical Sciences
https://orcid.org/0000-0002-2510-3722
Javad Mohammadi-asl
Ahvaz Jondishapour University of Medical Sciences Faculty of Medicine
Kambiz Ahmadi-angali
Ahvaz Jondishapour University of Medical Sciences

Study protocol

Keywords: taurine supplementation, weight loss diet, fibroblast growth factors, obesity

Posted Date: March 22nd, 2019

DOI: https://doi.org/10.21203/rs.2.219/v2

Version of Record: A version of this preprint was published on May 31st, 2019. See the published version at https://doi.org/10.1186/s13063-019-3421-5.

Abstract

Background: according to studies, the function of FGFs are disturbed in obesity. So that, serum level of FGFs are correlated with insulin resistance and obesity. Besides the effects of eicosapentaenoic acid on serum FGF21 concentrations, the effect of other nutrients on FGFs is not clear. Since obesity as an important health problem is rising in the world and on the other side, taurine (Tau) biosynthesis is reduced by adipose tissue-derived factors in obesity, the effects of Tau and a weight loss diet on obesity need to be investigated further. Methods: We will conduct a 2 month's double-blind, parallel-group, randomized controlled clinical trial to investigate the effect of Tau supplementation on serum levels of fibroblast growth factors, β-Klotho co-receptor, some biochemical indices and body composition in obese women on a weight-loss diet. Discussion: we will determine the other advantages of a weight loss diet on metabolic risk factors. Moreover, for the first time, the effects of a weight loss diet along with Tau supplementation on these variables will be assessed.

Background

Obesity is rising around the world. Since excess body weight is associated with a higher incidence of cardiovascular disease, hyperlipidemia, hypertension, type 2 diabetes mellitus and some cancers; obesity is the fifth cause of mortality all over the world (1, 2). According to the new studies, some novel obesity-related hormones may have an important role in obesity treatment as metabolic regulators (3).

Fibroblast growth factors (FGFs) 19 and 21 as members of FGFs are different from canonical FGFs. FGF19 and FGF21 could circulate in vessels as hormones. They increase total energy expenditure. Furthermore, they may decrease blood glucose, insulin, triglycerides, fat mass and body weight (4). FGF19 and FGF21 link to a unique dual receptor complex consisting of β-klotho and activate tyrosine kinase FGF receptors (FGFR1-4) via a low-affinity interaction with heparan sulphate glycosaminoglycans (HSGAGs). β-klotho links to those and facilitates FGFR activation. β-klotho mainly expresses in metabolic organs including liver, adipose tissue and pancreas (5, 6).

Some evidence showed that FGF19 (released by intestine) and FGF21(released by the liver and adipose tissue) play an important role in glucose and lipid metabolism. Some evidence has shown an impaired FGF19 and 21 biosynthesis in obesity (7). According to the result of a cross-sectional study, serum level of FGF21 and FGF19 was high and low in the obese subjects, respectively. Furthermore, β-klotho gene expression was decreased. Adipose-derived Pro-inflammatory factors could decrease gene expression of β-klotho in obesity. Since the function of FGF19 and FGF21 is dependent to β-klotho as a co-receptor; decreased expression of β-klotho causes to metabolic disorders (8). Thus, β-klotho co-receptor has been considered as a new marker in metabolic diseases(9, 10). Since β-klotho expression is afected by Adipose-derived Pro-inflammatory factors, weight loss could be effective in reduction of resistance to FGF21 and β-Klotho co-receptor up-regulation.

Adiponectin has an important role in glycemic and lipid homeostasis. Recent evidence has showed that FGF21 increases adiponectin expression (7). Generally, the serum level of FGF21 is directly associated with insulin resistance and increases the liver stress markers in obese individuals. In addition, serum level of FGF19 is reversely associated with insulin sensitivity and improvement in lipid metabolism. It seems that these two FGFs overlap in the body metabolism function (8). Although a study showed the increasing effect of eicosapentaenoic acid (EPA) on serum FGF21 (11), the effect of other nutrients on FGFs is not clear.

Taurine (2-aminoethanesulfonic acid, Tau) is a sulfur amino acid that biocynthesizes endogenously from systeine (Cys) or methionine (Met) . Additionally, this amino acid could provide by diet specially sea foods. Diet-derived Tau carries in blood circulation in a low amount to other tissues (12). There is some evidence that shows the serum level of taurine is reduced in obesity (13, 14). Tau is involved in many biochemical functions such as regulation of glucose and lipid metabolism, enhancement of energy expenditure, anti-inflammatory effects and appetite control. The most important effect of Tau in obesity is its direct effect on adipose tissue (12). Tau increases genes expression that are related to energy expenditure including peroxisome proliferator–activated receptor (PPAR) α, PPAR γ and PPAR γ co-activator protein (PGC) -1α. Thus, this amino acid increases energy expenditure in the white adipose tissue. In the other side, Tau induces PGC-1α gene expression in the brown adipose tissue (15). Also, in adipose tissue, it decreases the number of M1 macrophages (secreting pro-inflammatory cytokines and reactive oxygen species (ROS)) and increases the number of M2 macrophages (involved in the clearance of free fatty acid (FFA) and inhibition of lipotoxicity). Hence, Tau could reduce inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP) (12, 15). Also, this amino acid is anti-inflammation and anti-oxidant through its sulfonic acid group and non-participation in protein structure (in free form) (16). Although result of studies are controversy, there is some evidence that shows the beneficial effect of Tau on energy expenditure, weight and Body composition in obesity and diabetes in both animals and humans (15). Tau has a putative role in increasing energy expenditure, fatty acid β-oxidation and adipose tissue hypertrophy reduction (15). Since serum FGFs concentration is associated with visceral obesity (7) and the effect of Tau supplementation on serum FGFs concentration is not clear, we decided to conduct a randomized controlled clinical trial (RCT) investigating the effect of Tau supplementation on fasting serum levels of fibroblast growth factors (FGF19, FGF21), β-Klotho co-receptor, some of the metabolic risk factors and body composition in obese women on a weight-loss diet.

Study aims

The primary aims of the present RCT is the assessment of Tau supplementation on fasting serum levels of biochemical parameters and body composition. Furthermore, the secondary aims will evaluate the associations between changes in concentrations of fibroblast growth factors (FGF19, FGF21) and β-Klotho co-receptor with other variables.

Methods

Design and setting

We will be performed a double-blind, parallel-group, clinical RCT. The proposed RCT will be conducted at the Private Nutrition Therapy Clinics in Ahvaz for 8 weeks to evaluate the effect of daily 3 gr Tau supplementation in obese individuals. Fig 1.

Participants

Participants will be 50 non-menopause obese women. Inclusion criteria will be included: women 18 to 49 years old; body mass index (BMI) range between 30 to 40 kg/m2; Exclusion criteria will be included: menopause, pregnancy and lactation; having history of food allergy, cancer, acute or chronic renal failure, acute or chronic hepatic failure, thyroid disorders, gastrointestinal diseases, taking Multivitamin/mineral supplements, taking herbal supplements or weight-loss drugs, surgery for weight loss and any weight loss over the past six months and taking Tau supplement less than 90% of total prescribed capsules at the end of the study.

Ethics and trial registration

The eligible participants will be notified about the study protocol. The protocol is approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences that is in accordance with the Declaration of Helsinki (approval number: IR.AJUMS.REC.1397.590). Each participant will sign an informed contest form. All collected data will be held confidential. This study registered on Iranian Registry of Clinical Trials (registration number: IRCT20131125015542N2).

Sample size

The sample size was calculated based on the effect of Tau supplementation on changes in hs-CRP in obese people that was conducted by Rosa et al.(12). It was computed by considering 95% confidence interval and 80% power ($\alpha = 0.05$ and $\beta = 0.2$). In addition, the mean and SD of hs-CRP levels in the mentioned study was as follow: $\mu1=14.30$; $\mu2= 10.50$; $SD1= 2.90$; $SD2= 2.40$. we considered 20% attrition rate. Finally, 25 subjects considered for each group. All individuals will be included in the RCT if they will be met the inclusion criteria and willing to participate in the study to achieve the estimated sample size.

Randomization and blinding

Eligible subjects will be divided and stratified based on age (within ten years intervals) randomly into two groups including control (standard weight loss group + taking placebo, n=25) and intervention (standard weight loss group + Tau supplementation, n= 25). Randomization will be performed using the computer-generated random numbers by a third party to reduce the probable bias. The third party will generate a random block design in blocks of ten.The naming of Tau or placebo bottles will be done based on random numbers and odd or even numbers will be allocated randomly to the A or B group. To preserve the blindness in case of any side effects, the third party will use unique codes instead of A or B.To achieve blinding, the bottles will be sealed and we will be assured from the similarity of appearance and

their weight. The researcher and participants will be blinded to the treatment allocation. Randomization codes of RCT will be unlocked only after all individuals complete the study protocol.

Intervention

All subjects will follow a hypocaloric diet, which energy needs will calculate by Mifflin Jeor St equation. Then, 30% of estimated energy requirements will deduct. The intervention group will receive 1 gr Tau capsule three times a day after breakfast, lunch and dinner. The Tau supplementation dose determined according to the previous study (12).Tau supplement will provide by Nutricost Company (USA). In addition, Placebo capsules will provide by the Pharmacy Faculty of Ahvaz Jundishapur University of Medical Sciences in the same form and size of Tau capsules. All capsules will be given to participants in the similar packing every 15 days. The macronutrients of a hypocaloric diet will be 50% carbohydrate, 30% fat and 20% protein. Considering the general principles of diet, a trained dietitian will give a dietary exchange list and an individualized diet according to the subjects' dietary habits. The same dietitian will follow subjects to check compliance through phone calls or SMS every three days. Figure 2 shows schedule for enrollment, intervention and assessment based on SPIRIT.

Measurements

An individual information questionnaire including demographic situations, history of diseases, supplementations and medications will be filled at the baseline. Dietary intake will be evaluated by 3 days 24-hour recall quistionaires (2 weekdays and 1 weekend day) at the beginning, middle and the end of study. Total calorie and macroniutrients intake will be calculated using Nut IV (the Hearst Corporation, San Bruno, CA). The participants will be asked not to change their physical activity level (PAL). Physical activity levels will be assessed by the International Physical Activity Questionnaire (IPAQ) at the beginning and end of the study. The physical and anthropometric measurements will be given with minimal clothing and without shoes. Body weight will be measured using a 100 g accuracy scale (Seca). Height will be measured using a 0.5 cm accuracy Seca stadiometer. BMI will be computed by dividing body weight (kg) to the square of height (m). Waist circumference will be measured using tape meter at the midpoint between the lowest rib and iliac crest and at the end of normal expiration to the nearest 0.5 cm. TANITA BC-418 body analyzer will be applied to estimate total body fat, fat percent, fat-free mass and fat free mass percent. 5 mL of venous blood sample (in regular tube) will be taken after 10-12 h overnight fasting at the baseline and end of study. Blood samples will be centrifuged at 1500 g for 15-20 min to separate serum. Serum will be stored at -80 °C and will be used to measure biochemical analysis such as FGF19 (μg/mL) , FGF21 (μg/mL), β-klotho co-receptor (μg/mL), leptin (μg/mL), adiponectin (μg/mL), hs-CRP (μg/mL), insulin (μU/mL), fasting blood sugar (FBS) (mg/dL), total cholesterol (TC), high-density lipoprotein (HDL-C), triglyceride (TG), alanine transferase (ALT), aspartate transaminase (AST) and gamma-glutamyl transferase (GGT) in serum. Enzyme-linked immunosorbent assay (ELISA) kits will be applied to measure serum FGFs, β-klotho co-receptor, leptin, adiponectin, hs-CRP and insulin. Lipid profile and serum glucose concentration. Also, serum hepatic enzymes will be measured using enzymatic method by pars-azmoon kits (Tehran, Iran). Low-density lipoprotein (LDL-C) concentrations

will be computed by the Friedewald equation. Homeostasis model assessment – insulin resistance (HOMA-IR) will be calculated as follow: FBS (mg/dL) × fasting serum insulin (μU/mL)/405.

Statistical analysis

We will be used intention to treat (ITT) and pre-protocol (PP) populations in the analysis. The ITT population consists all individuals who will be randomized, whereas the PP population consists all participants who complete the 8-week intervention. The data will be revised randomly to check accuracy and completeness. All data will be reported as mean ± SD. The changes percent for each variable will be computed by the following formula: [(E – B) / B × 100], where E and B are the end value and the baseline value of variable, respectively. The data normality will be analysed using Kolmogorov-Smirnov test. To compare parametric continuous data between and within the groups, independent sample t-test and the paired sample t-test will be used, respectively. In addition, to compare the differences in asymmetric variables between and within the groups, the Mann-Whitney test and Wilcoxon test will be applied, respectively. The analysis of covariance (ANCOVA) test will be used to control confounding variables. To evaluate the association between changes in fibroblast growth factors (FGF19, FGF21), β-Klotho co-receptor concentrations and other variables, linear regression models will be used. SPSS version 21 (IBM, Armonk, NY, USA) will be used to data analysis. The p value < 0.05 will be considered statistically significant.

Safety, adverse effects and monitoring data

There are no known side effects for 3 gr/day Tau supplementation (12). However, this RCT will supervise by a Data Monitoring Committee (DMC). In addition, any possible side effects will be reported to the Ethics Committee of the Ahvaz University of Medical Sciences.

Discussion

The discovery of the FGFs (FGF19 and FGF21) and their influences on the body energy balance as hormones demonstrate a significant progress in obesity and type 2 diabetes studies. It seems FGF21, FGF19 and β-klotho concentrations are correlated with risk factors of metabolic diseases especially in subjects with abdominal obesity. Weight loss may diminish obesity risk via regulation of adipose tissue-derived factors, finally modulating concentrations of FGFs (FGF19, FGF21) and β-klotho co-receptor. In addition, According to the studies, Tau may regulate adipose tissue-derived factors. Therefore, a weight loss diet can promote the useful effects of Tau supplementation. This study will determine another beneficial effect of Tau supplementation through regulation of FGFs and β-klotho co-receptor along with a standard weight loss diet.

Abbreviations

ALT: alanine transferase; AST: aspartate transaminase ; ANCOVA: analysis of covariance; BMI: body mass index; Cys: systeine; EPA: eicosapentaenoic acid; FBS: fasting blood sugar; FGFs :fibroblast growth

factors; FGFR: FGF receptors; FFA: free fatty acid ;GGT: gamma-glutamyl transferase; HDL-C:high-density lipoprotein; HOMA-IR: Homeostasis model assessment – insulin resistance ; hs-CRP: high-sensitivity C-reactive protein; ITT: intention to treat; IPAQ: International Physical Activity Questionnaire; LDL-C: Low-density lipoprotein ; Met: methionine; PAL: physical activity level; PPAR: peroxisome proliferator–activated receptor; PGC: PPAR γ co-activator protein; ROS: reactive oxygen species; RCT: randomized controlled clinical trial; Tau: taurine;; TC: total cholesterol; TG: triglyceride.

Declarations

Acknowledgements

Not applicable.

Funding

This research is funded by Ahvaz Jundishapur University of Medical Sciences.

Availability of data and materials

The results will not be available before publishing.

Authors' contributions

FH and MA: contributed to design and data extraction; FH, MA, JM and KAA: prepared the manuscript; FH, MA, JM and KAA: performed the critical review. The manuscript has been revised and approved by all authors.

Consent for publication

Not applicable.

Competing interests

None of the authors declares a competing of interest.

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Figures

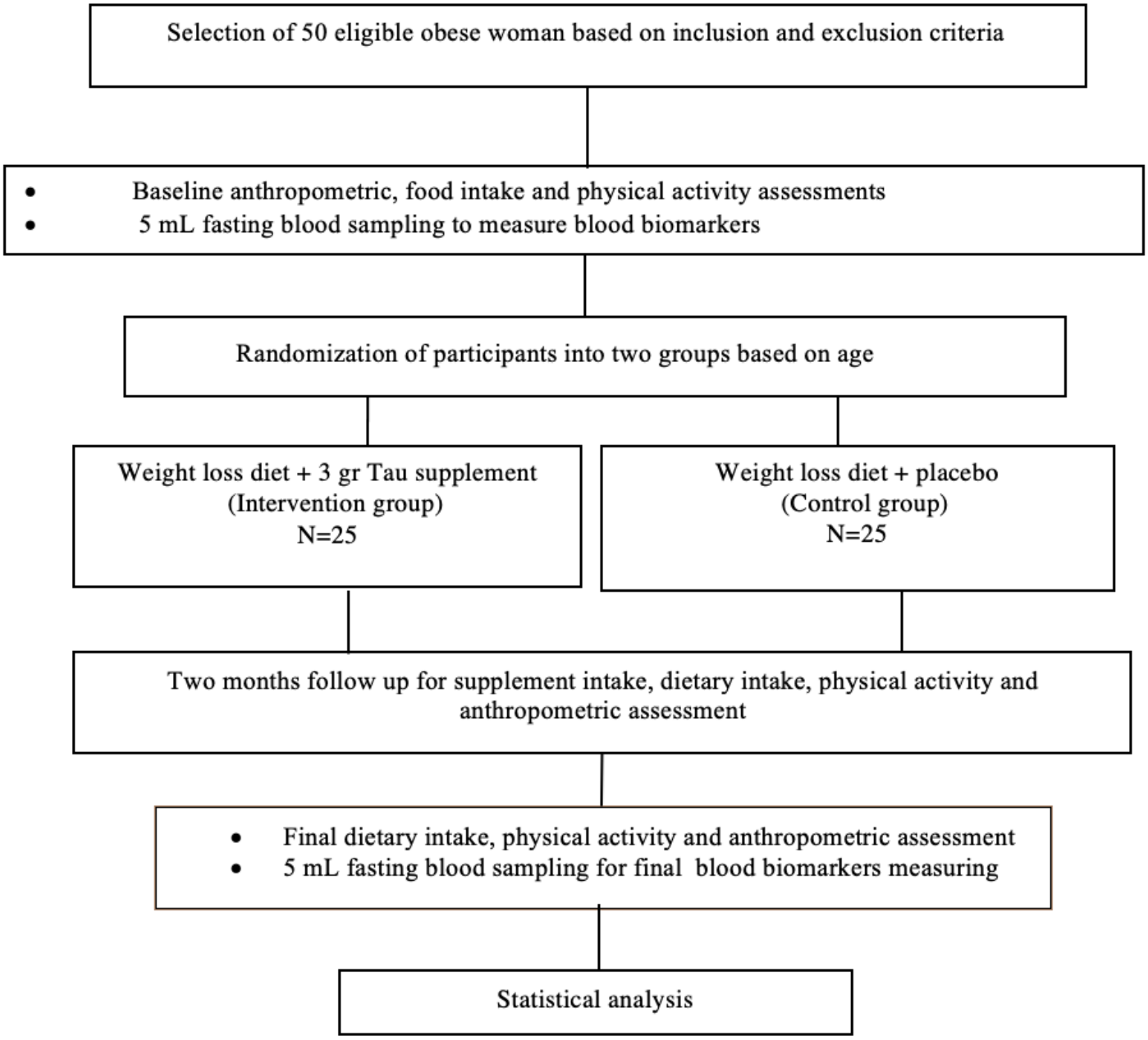

Figure 1

Protocol flow diagram; we will perform a 2 months double-blind, parallel-group, randomized controlled trial to assess the effect of Tau supplementation on serum levels of FGFs, β-klotho co-receptors, glycemic status, lipid profile, adipocytokines, hs-CRP and body composition in 50 premenopausal obese women on a weight-loss diet.

	STUDY PERIOD							
	Enrolment	Allocation	Treatment phase		Follow up			
TIMEPOINT**	-1 week	Baseline (week 0)	Baseline (week 0)	Weeks 8	8 weeks after baseline			
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Allocation		X						
INTERVENTIONS:								
[Hypocaloric diet +Tau]			◆—	—◆				
[Hypocaloric diet + placebo]			◆—	—◆				
ASSESSMENTS:								
[Blood tests, anthropometric measurements, 24 hr. recall and etc.]		X	X					
[Blood tests, anthropometric measurements, 24 hr. recall and etc.]					X			

Figure 2

Schedule of enrolment, interventions, and assessments.*

Supplementary Files

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- supplement1.doc