Adipokines: The possible association between low carbohydrate diet score and depression

Leila Setayesh
https://orcid.org/0000-0002-5617-7310
Sara Pooyan
Reyhane Ebrahimi
Habib Yarizadeh
Elaheh Rashidbeygi
Negin Badrooj
Hossein Imani (dr.imani20@gmail.com)
Khadijeh Mirzaei (mirzaei_kh@tums.ac.ir)

Research

Keywords: Depression, Adipokines, Low Carbohydrate Diet, Galactin1, TGF-β, PAI, Obesity

Posted Date: February 25th, 2020

DOI: https://doi.org/10.21203/rs.2.24419/v1

Abstract

Background Due to lack of enough date to confirm the association of obesity and depression in middle east, We aimed to explore the possible mediatory role of adipokines such as galactin1, transforming growth factor beta (TGF-beta), and endothelial plasminogen activator inhibitor (PAI-1) in the association between obesity and depression.

Methods A total of 256 women ranged between 18-48 years old were grouped based on their low carbohydrate diet (LCD) score. Body composition and dietary intake were assessed. Enzyme-linked immunosorbent assay was used to measure serum adipokines levels.

Results There was a negative association between LCD score as a covariant and depression as an independent variable (P= 0.02) (beta ±SE= -0.143±0.031) (CI= -0.129 to -0.08). A regression model linear analysis using galactin1, TGF-β, and PAI 1 as a covariant indicated the mediatory effects of these adipokines (P > 0.05). The higher adherence to LCD was associated with a decrease of anthropometric components (p < 0.05).

Conclusion We indicated that a higher adherence to LCD would probably ameliorate depression through the mediatory role of the study adipokines. The higher adherence of LCD in depressive obese individuals due to possible improvement effects on mental health.

1. Background

Obesity is defined as an excessive accumulation of fat in white adipose tissue (WAT) with negative effects on health. It is a chronic disease resulting from a positive energy balance in conditions of energy excess or an imbalance between energy intake and expenditure [1–3]. Obesity is growing as a public health problem with a high prevalence in both developed and developing countries [1]. In 2014, World Health Organization (WHO) estimates that almost 40% of adults are overweight (body mass index (BMI) > 25 kg/h2) and nearly a third of them are obese (BMI ≥ 30)[4].

A mental disorder clinically refers to a dysfunction of individual's cognition and emotional or behavioral regulation [5]. It is usually associated with significant distresses in scotia, profession, work and other important activities. Recent studies reported that psychiatric diseases such as major depressive disorder (MDD), dysthymia, manic, and hypomanic episodes have raised more in obese patients compared to normal weight people. One of the most common psychiatric diseases is MDD which is clustered based on its symptoms and other clinical features [3].

Supporting evidences on obesity and associated comorbidities have led to understand the role of adipose tissue as a highly active endocrine organ in controlling the related physiological and pathological processes [6]. Adipose tissue plays an important role in mediating the biological effects on metabolism and inflammation. Moreover, it maintains the energy homoeostasis and secretes adipokines which are powerful peripheral signals from adipose tissue to brain in the long term control of appetite and energy

balance [7]. Adipokines have been suggested as critical mediatory factors in association between obesity and psychopathology [6]. Since the macronutrient composition of diet including the balance of carbohydrates and proteins is a key factor in the regulation of overall body weight and metabolism, and also it regulates the secretion of adipokines such as leptin, then, a high carbohydrate and glycemic index in diet may change the amount of these adipokines as reported before [2]. Several studies have shown the beneficial effects of low carbohydrate diet (LCD) on short and long term weight loss programs, body composition, cardiovascular and metabolic features i.e. abdominal fat, high density lipoprotein (HDL), fasting glucose, circulating insulin, and blood pressure and also psychological outcomes in contrast to the diet with macronutrient composition. In addition, individuals with a higher LCD score have lost weight faster than ones using other conventional diets like low fat diet or Mediterranean diet. Hence, LCD is a more effective way to reach a quick treatment for obesity [7].

A possible association between LCD and depression through following different adipokines have been investigated in obese people with mood disorders in previous studies. However, the precise mechanisms of adipokines galactin1, transforming growth factor beta (TGF-beta), and endothelial plasminogen activator inhibitor (PAI-1) are not well known, and their functions in the control of mood and obesity are even less understood. In the current study, we investigated the secretion of biological substances playing role in improving obesity and MDD and also their association with the amount of major components in the diet. Moreover, the mediatory effects of LCD on MDD though adipokines like galactin1, TGF-beta, and PAI-1 are examined too.

2. Methodes

2.1. Study population

We performed a cross-sectional study on 256 randomly selected healthy women ranged between 17 to 69 years who referred to health centers in Tehran. Participants had a BMI with a range of 25–40 kg/m2. A self-administered questionnaire was provided from all participants for their health status and the exclusion criteria of study were followed as: an absence of any acute or chronic inflammatory disease or a regular use of medicine like birth control pills, history of hypertension, cardiovascular disease, diabetes mellitus, impaired renal and liver function, intake of alcohol or drug abuse, smoking, thyroid disease, malignancy, pregnancy and lactation period. Furthermore, we excluded those participants with chronic diseases affecting their diet as well as those who have followed an arbitrary special dietary regimen or with any body weight fluctuations over the past year.

Blood samples were collected following an overnight fasting and the serum was centrifuged, liquated and stored at a temperature of − 80 °C until the analysis was performed. All samples were analyzed by using a single assay according to manufacturer’s protocol. All measurements were taken at the Endocrinology & Metabolism Research Institute (EMRI) Bionanotechnology laboratory of Tehran University of Medical Science. Triglyceride (TG) and fasting blood glucose (FBG) were measured by Randox Laboratories Kit (Hitachi 902). Serum concentrations of galactin 1 (Human Galactin-3*96 T, ELIZA kit Crystal Company),

TGF-beta (HUMAN TGF-BETA 1 Quantikine ELIZA kit R&D System- usa), and PAI (Human PAI-1*96 T ELIZA kit Crystal Company) were measured in triplicate.

2.2. Dietary Assessment

Over the past year Dietary intake and nutritional status were evaluated using a semi-quantitative food frequency questionnaire (FFQ). This procedure was designed according to the Willett study including a list of 147 food items along with a standard serving size for each nutrient. [8]. The reliability and validity of this FFQ have been approved in 2010 in Iran [9]. FFQ data were analyzed by Nutritionist-4 software.

2.3. Anthropometry measurements (Body Composition Analysis)

An impedance fat analyzer (InBody 720, Korea) was utilized to acquire weight, BMI, total body water (TBW), Body Fat Mass (BFM), fat mass (FM), fat-free mass (FFM), body cell mass (BCM), body fat percentage (%), waist to hip ratio (WHR), and visceral fat area (VFA) of the subjects following a standardized procedure. In details, a low-level electrical current is sent through the body into the electrodes of hands and feet. Then, the impedance of currents is measured with the aim of evaluating the body composition.

2.4. Calculation of the LCD score

Data was collected from completed FFQ forms by participants in Tehran Health center. These data which were based on the percent of energy intakes such as carbohydrate, protein and fat, were used to calculate the score of LCD. A higher score defines a higher amount of protein and fat intake with a lower intake of carbohydrate.

We separated the study participants in eleven classifications regarding their carbohydrate, vegetable, refined grain, protein, monounsaturated fatty acid (MUFA), and n3/n6 poly unsaturated fatty acid (PUFA), consuming as well as fiber and glycemic load (GL) as a percentage of energy intake. Dietary GL was estimated as total available carbohydrate per 100 and total glycemic index in which is expressed as gr/d. Woman with the highest intake of vegetable, protein, MUFA, PUFA, and fiber in their macronutrient, received 10 points and thus, woman with the lowest received 0 points. In contrast, those with lowest level of refined grain, GL, and carbohydrate intake got 10 points and those with highest intake of these macronutrients got 0 points. We considered the percent of consumed energy instead of absolute intake to decrease the bias resulting from under-reporting of food and representing the food components. We divided the study participants into tertiles of protein, fat, and carbohydrate as a percentage of energy intakes. Those with the lowest tertile of carbohydrate intake were regarded as 3 scores and those with the highest tertile of carbohydrate were regarded as 0 scores. Moreover, fat and protein were scored as the same too but the order was considered reverse. (The lowest tertile of fat and protein intakes were regarded as 0 scores while the highest were regarded as 3 scores). For estimating the LCD score, we summed these macronutrient scores in which 0 showed the highest carbohydrate intake and the lowest protein and fat intakes and 12 showed the lowest carbohydrate intake and the highest protein and fat intakes [10]. To create the final diet score, we summed the points of each items which arranged from 0

(the highest carbohydrate intake and the lowest fat and protein intake) to 30 (the lowest carbohydrate intake and the highest fat and protein intake). Therefore, the highest score was referred to those who followed the pattern of LCD termed as the “LCD score”

2.5. Depression assessment:

The DASS (Depression anxiety stress scales) is a forty-two-item self-report questionnaire to evaluate the three negative emotional states of stress, depression, and anxiety. To determine the total scores for each subscale, a respondent indicates a four-point scale with the degree of each item applied over the last week and z-scores can be used for values comparison. A normal range is defined as a z-score of 0.5, a mild state is a z-score of 0.5 to 1, a moderate state is a z-score of 1 to 2, a severe state is a z-score of 3, and an extremely severe state is often defined as a z-score more than 3 [11].

2.6. IPAQ assessment:

The International Physical Activity Questionnaires (IPAQ) are designed to provide an information of walking, moderate, and vigorous physical activity for a comparable evaluation especially for research purposes. These questionnaires can be used by young and middle-aged adults (18–65 years). Here, we recorded the needed information on physical activity of our study participants according to the standard methods for adapting these data to our country’s study centers. For weekly physical activity assessment, a short form (9 items) was applied using MET (metabolic equivalent) scores for each type of activity. According to the IPAQ scoring protocol, MET scores were demonstrated as 3 and 6 METs for moderate activities and as 6 METs for vigorous activities to show the overall physical activity. For reporting the total physical activity from all activity categories, MET scores across all these sub-components were summed and MET-minutes per week (MET-min/wk) were estimated [12].

2.7. Statistical Analysis:

Continuous variable with normal distribution was provided with mean ± standard error of mean (SEM) and skewed distributed variables was provided with median (IQR). ANOVA with Scheffe post hoc was used for normal distributed data, and Kruskal wails and Bonferroni correction post hoc were performed for non-normal distributed data. Then, ANCOVA analysis was used to remove effect of potential confounder. The linear regression model (LRM) analysis was used for finding the modulatory role of adipokines levels on LCD score and depression relationship. The stepwise method for finding the modulatory role of galactin1, TGF-beta, and PIA in the LRM was also used. Multiple stepwise linear regression was performed to identify significant predictor of these adipokines with different anthropometric and laboratory variables. Multinomial logistic regression was performed to identify the risk of MDD with regarding to adipokines levels. The significance level was set at a probability of ≤ 0.05 for all tests. Statistical analysis was performed using SPSS version 22.0 (SPSS, Chicago, IL, USA).

3. Results:

3.1. Study population characteristics

The minimum, maximum, mean age, height, weight, and BMI of the study participants were 24.20, 49.6, 36.49 years (SD8.38), 161.38 cm (SD 5.90), 31.4 kg (SD 4.31), and 34.09 kg/m2 (SD 2.72), respectively (Table 1). In order to examine the association of serum low carbohydrate score with variables and depression, the participants were grouped based on LCD score, group 1 (n = 85), group 2 (n = 85) and group 3(n=84) which group 3 showed the most adherence to LCD score in comparison with group 1. (Table 2). The differences between low- and high-LCD score groups were analyzed by independent ANOVA for fat percentage, FFM, FFM per weight, RMR per weight, VFA, BMI, and lipid profile. The results showed that there were statistically significant differences in obesity degree percentage (p = 0.02), DBP (p = 0.01), FMI (p = 0.05), TG (p = 0.01), GPT (p = 0.03), depression (p = 0.04), weight (p= 0.03), height (p= 0.02), fat free mass (p=0.00), and skeletal muscle mass (p=0.00) after adjustment for age. However, there were no significant differences in terms of fat percentage, FFM, height, SBP, BFM, BMI, visceral fat level, glucose, cholesterol, HDL-C, LDL-C, GOT, GPT, hs.CRP, galactin 1, PAI, TGF-beta, HOMA-IR, quantitative insulin sensitivity checks index (QUICKI), total antioxidant capacity (TAC), and cross laps between the two groups (p > 0.05) (Table 2). This study revealed that there is a negative association between LCD score adherence and depression.

We diagnosed 46.45% of our participant had depression by DASS self-administered questionnaire. In our study, we analyzed the association of normal participants (n=136) and patients with mild and moderate depression (n=72) and patients with severe and major (n=46) depression with all variables and LCD score by ANOVA test. The results showed that there were statistically significant differences between depression and PAI (P= 0.04), TG (0.01), FMI (P=0.05), obesity degree percentage (p=0.04), and DBP (p=0.01). Moreover, there was no significant change due to the three states of depression in age, weight, height, SBP, BMI, BFM, FFM, skeletal muscle mass, fat percentage, VFA, glucose, cholesterol, HDL-C, LDL-C, GOT, hs.CRP, LCD score, insulin. PAI, TGF-beta, and galactin1 (p>0.05). (Table 3)

3.2. Association of depression and LCD score

We performed ANOVA test to define the relationship between depression and LCD score, then we used LCD score as a covariant and depression as an independent variable which revealed a significant association (P= 0.02) (confidence interval=5.491 to 9.448) (beta ±SE = -0.134±0.027). After adjustment for age, BMI, energy, and IPAQ, the association between LCD score and depression remained significant (P= 0.02) (confidence interval=-0.129 to -0.08) (beta ±SE =-0.143±0.031) (table 4).

3.3. Regulatory effect of adipokines on LCD score and depression

Recent studies showed that mental disorders may be decreased by LCD and also it revealed that some adipokines may play important roles in this manner. The adipokines we used in our study are galactin1, TGF-beta, and PAI. We used these adipokines as covariates according to correlation method. According to our measurements, LCD with mediatory effects on galactin1, TGF-beta, and PAI may alter the probability of depression. In another words, the regression model linear analysis revealed that after adjustment for

age, IPAQ, BMI, and energy, these mediatory effects of galactin1, TGF-beta, and PAI did not remain significant as provided in table 5 (p = 0.69, p= 0.4, and p=0.39 respectively). Thus, results demonstrated that LCD may improve the depression through the regulatory effects of galactin1, TGF-beta, and PAI.

4. Discussion

This cross-sectional study strengthens this concept that LCD with mediatory role on galactin1, TGF-beta, and PAI levels may alter the probability of depression. Remarkably, the regression model linear analysis revealed that these adipokines have mediatory role in the relation of obesity and depression.

Obesity as a gateway disease is related to higher rates of morbidity and mortality driven by its comorbidities such as nonalcoholic fatty liver disease (NAFLD), [13] diabetes [14], cancer [15], immune-mediated disorders, [16] and cardiovascular disease (CVD). Many studies indicated that obesity can be linked to depression through different pathways such as chronic inflammation. On the other, some shared disorders like CVD may be an outcome of the negative mood states or stresses in which enhance the possibility of premature mortality. Furthermore, one of the most important risk factors of myocardial infraction is determined as MDD.

Proteins secreted by adipose tissue termed adipokines have several effects on many physiological processes such as controlling the food intake, energy homeostasis, insulin sensitivity, angiogenesis, regulation of blood pressure, and blood coagulation. Visceral fat release a great amount of adipokines, including leptin, resistin, adiponectin, PAI, galactin1, and TGF-beta and is also connected to inflammation which in this manner, it is more notable than subcutaneous fat [17]. Since the association of obesity and adipokines together with metabolic disturbances are reported before, and also depression and obesity are both linked to inflammation, the importance of visceral fat as an active endocrine organ secreting different biological active components may be considered as an important issue in monitoring the inflammatory and depressive disorders [4]. Moreover, any change in adipokines levels may be a conceivable prospective regulation of obesity-depression association. However, it is not completely discovered

In the context of mood states, adipokines are involved in sending signals from adipose tissue to the brain. They straightly contribute to weight regain or predict body weight dynamics [18]. Metabolic and neuroendocrine systems and also autonomic responses are important factors related to adipokines which keep the balance of energy storage in body or cause to a constant weight loss [19]. In addition to the role of adipokines in metabolic regulation, they also work as a bidirectional communicator between adipose system and hypothalamic pituitary adrenal (HPA) axis. Moreover, their levels are reduced by the stimulatory effects of chronic or high intensity stress and also are affected by glucocorticoids and inflammatory factors. However, this conceivable association remains unknown and have not been well-defined yet.

In this manner, Taylor et al. studied the role of adipokines as anti-obesity hormones and observed that cortisol may be suggested as a pathophysiological mediator in the excess weight gain of their study

population [20]. Evidence showed that weight gain and cortisol levels have mutual influence on each other. Cortisol is one of the suggested pathophysiological regulators of weight gain and has been supported by a recent study emphasizing obesity followed by the early increase of intracellular cortisol. Moreover, recent studies stated that an excessive intracellular cortisol known as pseudohypercortisolism may also happen in obesity. This could be resulted from an increase in the activation of 11β-hydroxysteroid dehydrogenase- 1 (11β-HSD-1), the enzyme which reduces cortisone to cortisol and is capable of elevating the intracellular cortisol levels in adipose tissue [21, 22]. Moreover, it was reported that the most usual biological change in obesity and MDD is dysregulation of HPA axis [23]. Indeed, the antidepressants used in the treatment of depression can affect the function of HPA axis with reducing the free cortisol levels and exacerbating the glycemic control [22].

It should be noted that adipokines can be dysregulated in mental disorders linking hypercortisolemia with signs of metabolic syndrome such as insulin resistance or visceral obesity. For instance, the association of obesity and depression through regulating adipokines has been investigated in a recent study indicating their positive correlation particularly in women. Additionally, a higher activity of HPA axis was reported in MDD patients [24] which was associated with the secretion of adipokines. Thus, previous studies showed that adipokines are affiliated with free cortisol levels in depressed patients and also are related to risk factors of insulin resistance and obesity, including BMI and QUICKI [25].

Previous studies showed the role of several adipokines such as adiponectin, leptin, and resistin in the pathophysiology of mental disorders. For instance, a study demonstrated that adiponectin levels were significantly lower in MDD patients compared to controls, while leptin levels are significantly higher [26]. In addition, the leptin insufficiency and/or leptin resistance as a predisposing agent for mental disorders like depression was also stated before. In details, a study on rats indicated that those who were exposed to chronic stress showed decreased levels of leptin followed by a depressive like behavior [27]. Anyway, despite the fact that many researchers examined the levels of adipokines in depressed people, it is an unknown matter because of the existed conflicts. Indeed, there are still some adipokines which their role in this manner is in its infancy. Hence, the present study may help us to find new discoveries about obesity and depression as one of the most important physical and psychological reason of disability in the world. Although variables such as weight, cardiovascular risk factors, medical comorbidities, and pharmacotherapies may have influences on the role of these adipokines in depression. Consequently, here, we examined the possible regulatory role of circulating PAI, galactin1, and TGF-beta as mediatory adipokines in the obesity-depression association. All the mentioned adipokines in these study regulate HPA axis in some way, therefore all of them can affect the circulating levels of cortisol.

It should be noted that weight loss due to diet and mood are related to each other with an improvement in the mood after losing weight. Food containing high proteins will produce more sense of fullness and less tiredness or sleepiness compared to food containing high carbohydrates [33, 34]. In this manner, dietary management like low calorie and fiber consumption may be effective in the regulation of adipokines levels. Remarkably, mood and mental states are related to the proportionate levels of brain-derived neurotropic factors, and are reduced by high fat diets which are likely as a consequence of corticosterone

levels variations. LCD is a growing diet with high proteins and fats among people. In this diet, after emptying of glycogen stores due to the lack of dietary carbohydrate, fat oxidation is started to convert them into ketone bodies (KBs). KBs have lower efficacy but act as a fuel for body especially brain [35]. Then, they are used medically to control epilepsy and seizures. Moreover, LCD has long term effects on cognitive function in contrast to diets with enough carbohydrates. [36–38]. Therefore, in the present study, we aimed to identify the role of galactin1, TGF-beta, and PAI in depression through LCD score.

To explain our findings, randomized control trials performed by Samaha F.F. et al. and Foster G.D. et al. in 2003 indicated that LCD has a remarkable result in losing weight over a 6 to 12 months period [39, 40]. Additionally, a clinical study represented by Brinkworth G.D. et al. introduced LCD as an effective alternative dietary pattern for weight loss. The effects of LCD compared to other restricted calorie diets on fast improvement of mood are same in short terms (8 weeks) but in long terms (12 months) LCD group achieved a better outcome. Furthermore, in short terms, LCD could reduce weight more rapidly [41]. There are some suggested mechanisms for the inhibitory effect of LCD on appetite. One of them is the intake of only one class of food instead of variety of classes. Then, fewer intension and more fullness would happen after meals [42–44]. Although, more loss weight is acquired in LCD compared to low fat diet, but the mechanism for mood improvement due to LCD is still unknown. However, in a study on animal model, the antidepressant effects of LCD was observed. Then, LCD may be used as a mood stabilizer in depressive disorders [45]. In addition, glucocorticoids have some extra effects in the intake of food. An elevated tendency for consuming delicious and high calorie foods is a consequence of increased glucocorticoids levels due to chronic stress. Hence, the outcomes are an accumulation of visceral fat and a change in the levels of adipokines. A study reported that individuals following LCD had lower leptin levels compared to whom following low fat diet [46]. Here, it seems likely that galactin1, TGF-beta, and PAI adipokines produced by adipose tissue are other factors with energy-mood mediatory effects as well as leptin and adiponectin. Although, this finding are to a certain degree unclear.

Altogether, the present study demonstrated that 47.66% of obese subjects had depression which emphasize the importance of controlling obesity through diet affecting both obesity and depression. Here, for the first time we demonstrated that adipokines galactin1, TGF-beta, and PAI may act as a critical link between obesity and depression, and as a consequence, they may represent a possible novel insight in the disease monitoring and treatment. However, the relatively small number of subjects in the sample size and also investigating subjects with a same gender (256 females) are some limitations of the present study which deserve to be mentioned.

5. Conclusion

In the current cross-sectional study, we found the mediatory role of circulating galactin1, TGF-beta, and PAI adipokines in obesity and depression. Therefore, these adipokines may represent a potential new biomarker for obesity and depression. Here, it seems likely that LCD is associated with depression and obesity through mediating the levels of galactin1, TGF-beta, and PAI adipokines. However, further studies and clinical trials should be performed comprising the cellular and molecular states to clarify the biology

of galactin1, TGF-beta, and PAI and also their effects on the association of obesity and mood disorder through following LCD.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Commission of Tehran University of medical sciences (IR.TUMS.VCR.REC.1395.1234) and all participants signed a written informed consent.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by Tehran University of Medical Sciences (Grant No: :34988,34479,33893,33833).

Authors' contributions

LS and RE wrote the manuscript with support from SM, AB, and FZG. ER and NB performed the experiments. KH and HI fabricated the samples. HY and LS and SP analyzed and interpreted the data and supervised the project. All authors read and approved the final manuscript.

Acknowledgements

This study was supported by Tehran University of Medical Sciences (grant No:34988,34479,33893,33833) and is acknowledged by authors.

References

1. Taylor, V.H. and G.M. MacQueen, *The Role of Adipokines in Understanding the Associations between Obesity and Depression.* Journal of Obesity, 2010. **2010**: p. 6.
2. Chopra, M., A. Siddhu, and N. Tandon, *Effect of Nutritional Regulation on Adipokines in Obesity: A Review.* American Journal of Food and Nutrition, 2014. **2**(4): p. 66-70.

3. Carvalho, A.F., et al., *Adipokines as emerging depression biomarkers: a systematic review and meta-analysis.* J Psychiatr Res, 2014. **59**: p. 28-37.
4. Taylor, V.H. and G.M. MacQueen, *The role of adipokines in understanding the associations between obesity and depression.* Journal of obesity, 2010. **2010**.
5. Rajan, T. and V. Menon, *Psychiatric disorders and obesity: A review of association studies.* Journal of postgraduate medicine, 2017. **63**(3): p. 182.
6. Diniz, B.S., et al., *Reduced serum levels of adiponectin in elderly patients with major depression.* Journal of psychiatric research, 2012. **46**(8): p. 1081-1085.
7. Halton, T.L., et al., *Low-carbohydrate-diet score and the risk of coronary heart disease in women.* N Engl J Med, 2006. **355**(19): p. 1991-2002.
8. Price, D.D., et al., *The validation of visual analogue scales as ratio scale measures for chronic and experimental pain.* Pain, 1983. **17**(1): p. 45-56.
9. Mirmiran, P., et al., *Does dietary intake by Tehranian adults align with the 2005 dietary guidelines for Americans? Observations from the Tehran lipid and glucose study.* Journal of health, population, and nutrition, 2011. **29**(1): p. 39.
10. Eslamian, G., et al., *Low carbohydrate diet score does not predict metabolic syndrome in children and adolescents: Tehran Lipid and Glucose Study.* Arch Iran Med, 2014. **17**(6): p. 417-22.
11. Brown, T.A., et al., *Psychometric properties of the Depression Anxiety Stress Scales (DASS) in clinical samples.* Behaviour research and therapy, 1997. **35**(1): p. 79-89.
12. Craig, C.L., et al., *International physical activity questionnaire: 12-country reliability and validity.* Medicine & science in sports & exercise, 2003. **35**(8): p. 1381-1395.
13. Polyzos, S.A., J. Kountouras, and C.S. Mantzoros, *Adipose tissue, obesity and non-alcoholic fatty liver disease.* Minerva Endocrinol, 2017. **42**(2): p. 92-108.
14. Zoppini, G., et al., *Body mass index and the risk of mortality in type II diabetic patients from Verona.* International Journal Of Obesity, 2003. **27**: p. 281.
15. De Pergola, G. and F. Silvestris, *Obesity as a Major Risk Factor for Cancer.* J Obes, 2013. **2013**.
16. Beuther, D.A., S.T. Weiss, and E.R. Sutherland, *Obesity and Asthma.* Am J Respir Crit Care Med, 2006. **174**(2): p. 112-9.
17. Silva, F.M., J.C. de Almeida, and A.M. Feoli, *Effect of diet on adiponectin levels in blood.* Nutr Rev, 2011. **69**(10): p. 599-612.
18. Galic, S., J.S. Oakhill, and G.R. Steinberg, *Adipose tissue as an endocrine organ.* Molecular and cellular endocrinology, 2010. **316**(2): p. 129-139.
19. de Oliveira Leal, V. and D. Mafra, *Adipokines in obesity.* Clinica Chimica Acta, 2013. **419**: p. 87-94.
20. Taylor, V.H. and G.M. MacQueen, *The Role of Adipokines in Understanding the Associations between Obesity and Depression.* J Obes, 2010. **2010**.
21. Morton, N.M., *Obesity and corticosteroids: 11β-hydroxysteroid type 1 as a cause and therapeutic target in metabolic disease.* Molecular and cellular endocrinology, 2010. **316**(2): p. 154-164.

22. Sonino, N. and G.A. Fava, *Psychiatric disorders associated with Cushing's syndrome.* CNS drugs, 2001. **15**(5): p. 361-373.
23. Bornstein, S.R., et al., *Approaching the shared biology of obesity and depression: the stress axis as the locus of gene-environment interactions.* Mol Psychiatry, 2006. **11**(10): p. 892-902.
24. Lu, X.-Y., *The leptin hypothesis of depression: a potential link between mood disorders and obesity?* Current opinion in pharmacology, 2007. **7**(6): p. 648-652.
25. Weber-Hamann, B., et al., *Resistin and adiponectin in major depression: the association with free cortisol and effects of antidepressant treatment.* Journal of psychiatric research, 2007. **41**(3-4): p. 344-350.
26. Carvalho, A.F., et al., *Adipokines as emerging depression biomarkers: a systematic review and meta-analysis.* Journal of psychiatric research, 2014. **59**: p. 28-37.
27. Antonijevic, I.a., et al., *Elevated nocturnal profiles of serum leptin in patients with depression.* Journal of Psychiatric Research, 1998. **32**(6): p. 403-410.
28. Esel, E., et al., *Effects of antidepressant treatment and of gender on serum leptin levels in patients with major depression.* Progress in Neuro-Psychopharmacology and Biological Psychiatry, 2005. **29**(4): p. 565-570.
29. Pan, A., et al., *The association of depressive symptoms with inflammatory factors and adipokines in middle-aged and older Chinese.* PLoS One, 2008. **3**(1): p. e1392.
30. Schilling, C., et al., *Leptin plasma concentrations increase during antidepressant treatment with amitriptyline and mirtazapine, but not paroxetine and venlafaxine: leptin resistance mediated by antihistaminergic activity?* Journal of clinical psychopharmacology, 2013. **33**(1): p. 99-103.
31. Kraus, T., et al., *Body weight, the tumor necrosis factor system, and leptin production during treatment with mirtazapine or venlafaxine.* Pharmacopsychiatry, 2002. **35**(06): p. 220-225.
32. Rubin, R.T., M.E. Rhodes, and R.K. Czambel, *Sexual diergism of baseline plasma leptin and leptin suppression by arginine vasopressin in major depressives and matched controls.* Psychiatry research, 2002. **113**(3): p. 255-268.
33. Paz, A. and E.M. Berry, *Effect of meal composition on alertness and performance of hospital night-shift workers.* Annals of nutrition and metabolism, 1997. **41**(5): p. 291-298.
34. Spring, B., et al., *Effects of protein and carbohydrate meals on mood and performance: interactions with sex and age.* Journal of Psychiatric Research, 1982. **17**(2): p. 155-167.
35. D'Anci, K.E., et al., *Low-carbohydrate weight-loss diets. Effects on cognition and mood.* Appetite, 2009. **52**(1): p. 96-103.
36. Cantello, R., et al., *Ketogenic diet: electrophysiological effects on the normal human cortex.* Epilepsia, 2007. **48**(9): p. 1756-1763.
37. Freitas, A., et al., *Ketogenic diet for the treatment of refractory epilepsy: a 10 year experience in children.* Arquivos de neuro-psiquiatria, 2007. **65**(2B): p. 381-384.

38. Hartman, A.L., et al., *The neuropharmacology of the ketogenic diet.* Pediatric neurology, 2007. **36**(5): p. 281-292.
39. Samaha, F.F., et al., *A low-carbohydrate as compared with a low-fat diet in severe obesity.* New England Journal of Medicine, 2003. **348**(21): p. 2074-2081.
40. Foster, G.D., et al., *A randomized trial of a low-carbohydrate diet for obesity.* New England Journal of Medicine, 2003. **348**(21): p. 2082-2090.
41. Brinkworth, G.D., et al., *Long-term effects of a very low-carbohydrate diet and a low-fat diet on mood and cognitive function.* Archives of internal medicine, 2009. **169**(20): p. 1873-1880.
42. Stern, L., et al., *The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial.* Annals of internal medicine, 2004. **140**(10): p. 778-785.
43. Boden, G., et al., *Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type 2 diabetes.* Annals of internal medicine, 2005. **142**(6): p. 403-411.
44. Nickols-Richardson, S.M., et al., *Perceived hunger is lower and weight loss is greater in overweight premenopausal women consuming a low-carbohydrate/high-protein vs high-carbohydrate/low-fat diet.* Journal of the American Dietetic Association, 2005. **105**(9): p. 1433-1437.
45. El-Mallakh, R. and M. Paskitti, *The ketogenic diet may have mood-stabilizing properties.* Medical hypotheses, 2001. **57**(6): p. 724-726.
46. Vetter, M.L., et al., *Effect of a low-carbohydrate diet versus a low-fat, calorie-restricted diet on adipokine levels in obese, diabetic participants.* Diabetes, metabolic syndrome and obesity: targets and therapy, 2010. **3**: p. 357.

Tables

Table 1 *Anthropometric and laboratory characteristics of study population.*

Parameters	N	Minimum	Maximum	Mean	Std. Deviation
Age (years)	291	17.00	56.00	36.49	8.38
Weight (kg)	291	59.50	136.60	80.89	12.45
Height (cm)	291	142	179	161.38	5.90
BMI(kg/m2)	291	24.20	49.60	31.04	4.31
Fat percentage (%)	291	15.00	54.30	41.53	5.48
Waist hip ratio (CM)	291	.81	92	1.23	5.24
Visceral fat(%)	291	7.00	208.40	16.64	13.94
Obesity degree percentage(%)	291	29.40	231	143.69	21.51
Body cell mass	291	22.90	43.90	30.41	3.66
FFMI (kg/m2)	291	14.60	147.80	18.37	7.64
FMI (kg/m2)	291	6.90	26.90	13.15	3.37
GLU(mg/dl)	291	67	137	87.49	9.64
TChol (mmol/L)	291	104	344	185.30	35.77
TG (mmol/L)	291	370	512.	122.10	69.29
HDL-C (mg/dL)	291	18	87	46.58	10.86
LDL-C (mg/dL)	291	34	156	95.30	24.12
hs.CRP (mg/dL)	291	.00	22.73	4.34	4.624
Energy (kcal)	291	1028.98	4192.72	2613.03	751.74
LCDS1	291	30	40	35.	3.16
Valid N (listwise)	291				

Tchol total cholesterol, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, TG triglyceride, hs-CRP high-sensitivity C-reactive protein, BMI body mass index, FFM fat free mass, LCDS low carbohydrate diet score

RMR resting metabolic rate, BMI body mass index, FFM fat-free mass, FBS fasting blood sugar, TG triglyceride, Tchol total cholesterol, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, hs-CRP high-sensitivity C-reactive protein, TNF-α tumor necrosis factor alpha, IL interleukin, BMD bone mass density, LCDS low carbohydrate diet, BMR basal metabolic rate. IN order to examine the association of serum low carbohydrate score with variables and depression, the participants were grouped based on LCD score, group 1 (n = 100) and group 2 (n = 100) and group 3(n=100). The three column in table is arrange by the adherence of low carbohydrate diet and the third group(T3) have higher adherence camper to others(T3>T2>T1) *P value resulted by one way ANNOVA

Table2 *Association of adherence Low Carbohydrate Diet and variable*

Variable	T1 n=97	T2 n=97	T3 n=97	p
Age(years)	36.16± 8.40	35.29 ±8.32	37.81 ±8.66	0.11
Weight(kg)	83.59 ±3.40	81.37 ±12.82	78.81 ±10.09	0.03
Height(cm)	161.89 ±5.50	162.12 ±5.91	160.06 ±6.19	0.02
BMI(kg/m2)	31.57 ±4.86	31.00±3.98	31.56±3.94	0.23
Mineral	3.27±0.44	3.16±0.38	3.25±0.40	0.14
Body fat mass(%)	35.35 ±9.71	33.98 ±8.62	32.67 ±7.37	0.08
Fat free mass(%)	47.40 ±5.49	47.82 ±56.10	45.36 ±4.99	0.00
Skeletal.muscle mass(%)	26.00 ±3.20	26.35 ±3.64	24.86 ±2.97	0.00
Soft lean mass(%)	44.55±5.69	43.17±5.04	44.28±5.16	0.15
Bone mineral content	2.70±0.37	2.61±0.32	2.68±0.34	0.16
Fat percentage(%)	41.26 ±5.54	41.62 ±5.51	41.57 ±5.57	0.88
BMR	1391±130.62	1365.86±112.32	1384.56±120.60	0.31
Waist circumference(CM)	98.55±10.33	98.87±9.00	99.25±10.70	0.89
Waist hip ratio(CM)	0.92±0.04	0.93±0.05	0.93±0.05	0.53
Visceral fat area (cm2)	160.12±39.84	164.76±33.98	163.63±41.48	0.68
Visceral fat(%)	15.88 ±3.40	15.53 ±3.44	15. 21 ±3.19	0.35
Obesity degree percentage	146.84 ±22.63	143.10 ±19.97	142.15±18.47	0.22
Body cell fat	30.69±3.90	30.01±3.37	30.49±3.57	0.40
GLU(mg/dl)	87.15 ±11.07	87.40 ±8.48	888.11±9.58	0.80
TChol(mmol/L)	183.82 ±37.52	183.13 ±33.48	187.82 ±38.25	0.66
TG(mmol/L)	127.84 ±75.16	116.54 ±71.07	122.83±65.62	0.59
HDL-C(mg/L)	45.59 ±11.54	45.86 ±9.21	48.70 ±11.67	0.12
LDL-C(mg/L)	92.84 ±21.21	93.77 ±24.33	97.59 ±27.20	0.42
Hs.CRP(mg/L)	4.62 ±4.71	4.09 ±4.52	3.96 ±4.52	0.59
insulin(IU/mL)	15.69±7.51	15.09±5.56	16.07±5.12	0.60
Depression	5.81±4.91	5.89±4.54	4.20±4.48	0.02
PAI.1 (pg/mL)	17.26±26.05	15.54±32.52	15.14±30.36	0.91
TGF (pg/mL)	75.62±33.70	74.54±34.02	83.63±66.34	0.53
Galactin 1	4.91±7.82	2.05±2.22	4.09±7.81	0.42
HOMA	3.45±1.9	3.20±132	3.5±1.28	0.39

Table3 *Association of studied variation and Depression status*

RMR, resting metabolic rate; Dec. RMR, decreased of normal status of resting metabolic rate; Inc. RMR, increased of normal status of resting metabolic rate; BMI, body mass index; VO2, volume of oxygen; VCO2, volume of carbon dioxide; RQ, respiratory quotient; FFM, fat-free mass; FBS, fasting blood sugar; TG, triglyceride; T-chol, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; GOT, Aspartate Aminotransferase; GPT, Alanine Aminotransferase; hs-CRP, high-sensitivity C-reactive protein; HOMA, homeostasis model assessment; ISQUICKI, insulin sensitivity quantitative insulin sensitivity check index.

we diagnosed 47.66% our participant had depression, we arranged them in two group: (mild and moderate) and (major and severe). In our study we analyzed the association of normal and two group of depression with all variables and LCD score by ANOVA test. (Analysis of variance).

ISQUICKI	0.54±0.05	0.55±0.04	0.53±0.04	0.12	**Table4** *Association of LCDS*
TAC	435.05±93.03	455.58±88.04	429.09±101.20	0.21	*and Depression score*

parameters	Normal	Mild & Moderate depression	Major & Severe depression	p value2
	N=157	N=93	N=50	
age(years)	36.20± 8.31	36.86 ±8.09	36.82 ±9.48	0.82
weight(kg)	81.59 ±13.15	78.98 ±11.65	83.12 ±12.42	0.14
height(cm)	161.40 ±5.92	161.32 ±6.33	161.13 ±5.59	0.96
SBP(mm/hg)	113.45 ±13	109.8 ±13.8	111.02 ±13.27	0.14
DBP(mm/hg)	79.37 ±9.87[b,c]	7.33 ±8.95[a]	75.70±8.40[a]	0.01
Body fat mass(%)	34.66 ±9.23	32.45 ±7.93	35.57 ±8.70	0.08
Fat free mass(%)	46.87 ±6.02	46.61 ±5.44	47.31 ±5.30	0.79
Skeletal muscle mass(%)	25.73 ±3.57	25.61 ±3.19	25.98 ±3.15	0.83
BMI(kg/m2)	31.29±4.51	30.34 ±3.82[c]	32.00 ±4.74[b]	0.08
Percent body fat(%)	41.76 ±6.08	40.70 ±4.76	42.44 ±5.07	0.18
Visceral fat level(%)	17.03 ±16.25	16.50 ±14.82	16.17 ±3.12	0.92
Obesity degree percentage	145.54 ±21.04[b]	138.79 ±22.37[ac]	148.77 ±22.08[b]	0.02
FFMI(kg/m2)	18.83 ±10.78	17.8 ±1.31	18.2 ±1.64	0.66
FMI(kg/m2)	13.34 ±3.52	12.54 ±2.97[c]	13.96 ±3.74[b]	0.05
GLU (mmol/L	87.41±8.75	87.43 ±9.77	87.67 ±11.76	0.98
CHOLE(mmol/L)	187.19 ±35.53	182.75 ±39.66	184.46 ±29.51	0.69
TG (mg/dl)	118.95 ±65.47[c]	108.89 ±55.02[c]	149.23 ±96.49[ab]	0.01

		95 % CI of the difference	beta ±SE	P value
crude	LCDS	5.491 to 9.448	-0.134±0.027	0.025
adjusted	LCDS	-0.129 to -0.08	-0.143±0.031	0.026

low carbohydrate diet(LCDS).

We performed ANOVA test to define the relationship between depression and LCD score, then we used LCD score as a covariant and depression as an independent variable which revealed a significant association (P= 0.02). After adjustment for age, BMI, energy, and the International Physical Activity Questionnaire (IPAQ) the association between LCD score and depression remained significant (P= 0.02)

Table5 Mediatory effect of Adipokeines on the association of Depression and LCDS

	beta ±SE	T value	pv
Galactin1(pg/mL)	0.049±0.062	0.401	0.690
TGF- beta (pg/mL)	-0.69±0.041	-0.831	0.407
PAI 1 (pg/mL)	-0.070±0.040	-0.853	0.395

transforming growth factor beta (TGF- beta). endothelial plasminogen activator inhibitor (PAI-1)

The adipokines we used in our study are galactin1, TGF-β, and PAI. We used these adipokines as covariates according to correlation method. In another words, the regression model linear analysis revealed that after adjustment for age, IPAQ, BMI, and energy, these mediatory effects of galactin1, TGF-β, and PAI did not remain significant as provided. According to our measurements, LCD with mediatory effects on galactin1, TGF-β, and PAI may alter the probability of depression.