

AN OBSERVATIONAL STUDY TO ASSESS THE CHANGES IN *OJAS* AND *DHATU SAARATA* AMONG INDIVIDUALS ON LOW CARBOHYDRATE DIET

¹Dr.Sahad T, ²Dr.Anjali Sivaram

¹Assistant Professor, ²Associate Professor & Head

¹Dept of Kriya Sharira, V. P. S. V Ayurveda College, Kottakkal

²Dept of Kriya Sharira, Govt Ayurveda College Tripunithura

Abstract

Introduction: A restricted diet is more often pursued by those wanting to lose weight. Nowadays, the number of people following diet plans have increased and many of them choose low carbohydrate diet for the purpose of their diet plan. A low carbohydrate diet limits intake of carbohydrates and emphasizes on food high in protein and fat. Among different fundamental concepts of Ayurveda, *ojas* is a unique concept that explains about the basic status of physical and mental health in an individual. *Saara* is the purest form of a *dhatu* and *ojas* is its vital part. It is responsible for health, immunity, consciousness, longevity and memory. So, a reflection of the health status of an individual can be made by assessing the status of *ojas*. *Dhatu saarata* means the excellence of each *dhatu* and *saara pareeksha* can determine the qualitative excellence of each *dhatu*. Since *ojas* and *dhatu saarata* can reflect the strength or health of an individual, assessing them on individuals taking low carbohydrate diet can give an idea about their health status. **Materials & methods:** The present study was an observational study. Individuals taking low carbohydrate diet were selected for the study in a consecutive fashion from the Nutrition Centre, Naripparambu, Tirur, Kerala as per the inclusion criteria. After obtaining written informed consent, *Ojas* was assessed using the validated tool for the assessment of status of *ojas*, which was developed in the department of *Kriyasareera*, Government Ayurveda College, Kannur, Kerala by Dr Amrutha Elamon. *Dhatu saarata* was assessed using the validated computer assisted questionnaire software, Ayusoft developed by C-DAC Pune. The participants were observed for a period of one month. To examine the changes, the assessments were repeated after one month. **Result and discussion:** It was observed that, a significant increase in *ojas* was observed. Change in *dhatu saarata* could be observed except in *majja saarata*. *Rasa*, *asthi*, *shukra satva* and *sarva saarata* showed increase while decrease was noticed in *rakta*, *mamsa* and *medo saarata* the reduction in weight might be the reason for the changes in *saarata* as well as *ojas* as it reduces *medo dhatu* and *avarana* if any, leading to the improvement in overall health. **Conclusion:** Taking low carbohydrate diet for a period of one month can improve the *ojas* and *dhatu saarata* significantly. But the results cannot be extrapolated into a general postulate, as the findings can vary for a different time period of diet intake.

Keywords: Low carbohydrate diet, *dhatu saarata*, *ojas*

Introduction

In the present world, where lifestyle diseases prevail and food pattern is a big ground for that, proper diet practice according to one's need along with avoidance of food articles which have harmful effect is a must. Also, people are more beauty conscious nowadays that they give much importance to how they look. All these make the society to think of new diet practices suitable for the various demands. This gave rise to the development of the practice called 'dieting'. Since higher intake of carbohydrates places a higher load on

metabolism which can lead to different diseases like diabetes and obesity, majority of the diet plans concentrate on a low carbohydrate intake. So, as the carbohydrate intake is restricted, the amount of fat and protein intake should be increased to maintain the daily calorie requirement. This is the primary frame work of low carbohydrate diet. But there is lack of standardisation in the ratio of macronutrients in low carbohydrate diet. According to National Lipid Association USA, a diet containing less than 25% of calories from carbohydrate is considered as low carbohydrate diet and very low carbohydrate diet contains less than 10% of calories from carbohydrates¹, whereas a review article published by Hashimoto Y et al. has classified a diet with less than 50g of carbohydrate per day as low carbohydrate diet and less than 40% of calories from carbohydrates as 'mild' low carbohydrate diet², hence one finds it difficult to get a clear cut definition of low carbohydrate diet. Nowadays, the number of people following these types of diets increases and the low carbohydrate diet, as a concept has become hot topic for discussion in both scientific and non-scientific communities. So it is a necessity to conduct further studies on pros and cons of different diets.

Ojas is a unique concept in Ayurveda. *Acharya Charaka* has explained the living body as the combination of body (*sareera*), sense organs (*indriya*), mind (*satva*) and soul (*atma*)³. *Ojas* is considered as the factor which binds these four (*dehashthithinibandhanam*)⁴ entities in functional harmony. According to *Vagbhata*, *ojas* is the one, which can maintain or destroy life based on its status, i.e., if *ojas* is lost, the life will end, if *ojas* is maintained, then life is sustained⁵. *Acharya Susruta* equated *ojas* to *bala*, as it is the entity which gives strength to body⁶. It is of two types; *para ojas* and *apara ojas*. *Para ojas* is the principal type, which is originated in the intra-uterine life itself, and is not affected easily by simple deviations from normal physiology. Its quantity is measured to be eight drops (*ashtabindhu*)⁷. The main function of *ojas*, which is supporting the life, is carried out by *para ojas*. So, any slight deviation in its quantity will be detrimental to life. The latter one, called *apara ojas*, is generated by the excellence of seven *dhatu*s. The food, which nourishes the *dhatu*s can also nourish the *apara ojas*. Diseases and other etiological factors affect *apara ojas*, not the *para*⁸. If a disease occurs, there are chances of *apara ojas* becoming depleted from its physiological measure, which is half *anjali*⁹. So a proper and wholesome diet can maintain the *apara ojas* in its highest level and purest form.

Dhatu saarata, the excellence of a *dhatu* is the one which assess the qualitative excellence of each *dhatu*¹⁰. The treatment principle in Ayurveda gives prime importance to *bala* or strength of both *roga* (disease) and *rogi* (diseased). So, measuring the *bala* of *rogi* is the first step done before starting the treatment. Hence, *dhatu saarata* can be taken as a tool, since it assesses the quality of *dhatu* and thus health of the individual. The importance of *dhatu saarata* is that, it gives an exact idea about the *bala* of an individual. Assessing the *bala* of an individual by merely assessing the body built can often lead in making wrong conclusions. A lean person need not be weak and a stout person need not be strong. So, a tool must be used to assess the exact strength and *dhatu saarata* can be a choice. *Saarata* is of eight kinds; from *rasa* to *shukra* in the order of seven *dhatu*s, and the eighth one being *satva saara*¹¹. First seven assess the physical health of the individual in terms of seven *dhatu*s and *satva saara* measures the mental strength. So, the examination of *dhatu saarata* can cover both the physical and mental aspects of the individual. Ultimately, both *ojas* and *dhatu saarata* are indicatives of *bala* and can be considered as measures to assess the *bala*. So, in a condition, if any changes in *bala* is to be assessed, these two are the perfect indicators. Many studies have shown the health benefits of low carbohydrate diet, its long-term effects are yet to be studied. Even though many studies have been conducted in low carbohydrate diet to study its effects, health benefits and possible side effects, a study based on Ayurvedic principles regarding low carbohydrate diet is yet to be done. To assess the effect of low carbohydrate diet on body, the best way in an Ayurveda point of view is to assess the changes done in *bala* or body strength by these low carbohydrate diets, and to assess *bala*, the best way is to assess the *ojas* and *dhatu saarata*.

Materials and methods

The study was conducted as an observational study. The plan was to observe the individuals who take low carbohydrate diet. The study was conducted at the Nutrition centre, located at Naripparambu, Tirur, Kerala. Individuals taking a low carbohydrate diet plan from the nutrition centre were enrolled for the study in a consecutive fashion. The study demands a two-time assessment of the individuals which was done just

before starting the diet plan and after the completion of one month of the same. This helps to measure the exact changes occurring in the body because of the diet. Every individual coming afresh to the nutrition centre was interviewed to check whether they satisfy the inclusion criteria. A total of 100 individuals were enrolled for the study based on inclusion and exclusion criteria.

Inclusion criteria

- Individuals following low carbohydrate diet for a minimum period of 30 days, irrespective of gender
- Age: 20-50 years

Exclusion criteria

- Pregnant women and lactating mothers
- Individuals taking medications that affect body weight like steroids, anti-depressants, anti-psychotics etc.
- Individuals on insulin therapy
- Individuals doing additional activities like exercise for accelerating weight loss
- Individuals with mental illness

Written consent was obtained from each subject before conducting the study and confidentiality of the participants were kept safe throughout the study. Before getting consent, the subjects were adequately informed about the exact nature and aim of the study including the way of collecting data from the participants, who would have the right to use data, the role of subjects in the study and how their participation would make a contribution to the current research work and the expected benefits. Also, investigator had informed that their agreement to participate in the study is completely voluntary and they are free to exit from the study at any point of time during the data collection.

After enrolment process, preliminary data of the individuals were collected using a Google form. Then, the *Ojas* and *Dhatu saarata* were assessed. For present study, assessment of *Dhatu saarata* was done using Ayusoft software, developed by Centre for Development of Advanced Computing (C-DAC) Pune, Department of Information Technology MCIT, India. It contains a questionnaire with 79 questions for *Dhatu saarata* assessment. The questions were asked to the participants and the relevant columns were ticked. After finishing the questions, the results are displayed in the software, which can be noted down. The *Ojas* of the individuals were assessed using 'Tool for the clinical evaluation of the status of *Ojas*' developed as a part of research work in the department of Kriyasareera, Government Ayurveda college, Kannur, Pariyaram by Dr Amrutha Elamon. It is a validated tool for the assessment of status of *Ojas*. The tool contains 37 questions.

Along with preliminary data, the tool for *Ojas* assessment was also made into a Google form, and it was conducted in such a way that; the interviewer himself would record the participants' response in the google form. Use of google form made data collection easier and prevented the possible data loss. The assessment of *ojas* and *dhatu saarata* of the participants after taking the low carbohydrate diet was done when they completed 30 days of using the same materials and methods.

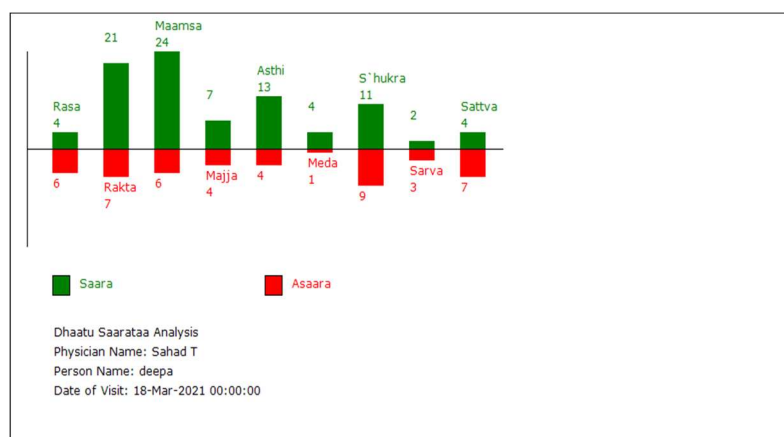


Figure 1 - A Sample bar diagram of dhatu saratha assessment in Ayusoft

Observation and analysis

The study, which included 100 participants with a majority being male (59%), found significant changes in *Ojas* and *Dhatu Saarata* after a month of low-carbohydrate diet. The mean *Ojas* score increased from 57.39 to 60.41, with 71% of participants showing an improvement. *Rasa Saarata* also showed improvement in 58% of participants, with a mean score increase from 10.76 to 11.72. However, *Raktha Saarata* decreased in 68% of participants, with a mean score change from 24.10 to 23.10. *Mamsa Saarata* decreased in all participants, with a mean score drop from 31.69 to 26.57. In contrast, *Asthi Saarata* increased, with a mean score change from 14.56 to 16.11. *Majja Saarata* increased in 33% of participants, decreased in 29%, and remained unchanged in 38%. *Shukra Saarata* improved in 60% of participants, with a mean score increase from 19.56 to 20.37. Finally, *Satva Saarata* increased in 53% of participants.

The data was tabulated using statistical package, SPSS (version 22.0.0.0) and analysed using appropriate statistical tests. As the parameters assessed were of subjective nature with pre and post assessment scores, statistical analysis was done using Wilcoxon signed rank test. In all analyses, significance level was taken to be 0.05 (i.e., if the p-value is less than 0.05, it can be concluded that the hypothesis is statistically significant).

Results

The study, which included 100 participants with a majority being male (59%), found significant changes in *Ojas* and *Dhatu Saarata* after a month of low-carbohydrate diet. The mean *Ojas* score increased from 57.39 to 60.41, with 71% of participants showing an improvement. *Rasa Saarata* also showed improvement in 58% of participants, with a mean score increase from 10.76 to 11.72. However, *Raktha Saarata* decreased in 68% of participants, with a mean score change from 24.10 to 23.10. *Mamsa Saarata* decreased in all participants, with a mean score drop from 31.69 to 26.57. In contrast, *Asthi Saarata* increased, with a mean score change from 14.56 to 16.11. *Majja Saarata* increased in 33% of participants, decreased in 29%, and remained unchanged in 38%. *Shukra Saarata* improved in 60% of participants, with a mean score increase from 19.56 to 20.37. Finally, *Satva Saarata* increased in 53% of participants.

Table 1 - Rank of different parameters in Wilcoxon signed rank test

Parameter		N	Mean Rank	Sum of Ranks	Z	Asymp. Sig. (2-tailed)
<i>Ojas</i> _After- <i>Ojas</i> _Before	Negative Ranks	18 ^a	30.69	552.50	-5.947	0.001
	Positive Ranks	71 ^b	48.63	3452.50		
	Ties	11 ^c				
	Total	100				
<i>Rasa_saara</i> _after - <i>Rasa_saara</i> _before	Negative Ranks	17 ^a	22.56	383.50	-5.604	0.001
	Positive Ranks	58 ^b	42.53	2466.50		
	Ties	25 ^c				
	Total	100				
<i>Raktha_saara</i> _after - <i>Raktha_saara</i> _before	Negative Ranks	68 ^a	44.38	3018.00	-5.318	0.001
	Positive Ranks	17 ^b	37.47	637.00		
	Ties	15 ^c				
	Total	100				
<i>Mamsa_saara</i> _after - <i>Mamsa_saara</i> _before	Negative Ranks	100 ^a	50.50	5050.00	-8.723	0.001
	Positive Ranks	0 ^b	.00	.00		
	Ties	0 ^c				
	Total	100				
<i>Meda_saara</i> _after - <i>Meda_saara</i> _before	Negative Ranks	82 ^a	42.67	3499.00		
	Positive Ranks	2 ^b	35.50	71.00		

	Ties	16 ^c			-7.733	0.001
	Total	100				
<i>Asthi_saara_after</i> - <i>Asthi_saara_before</i>	Negative Ranks	1 ^a	69.50	69.50	-6.983	0.001
	Positive Ranks	70 ^b	35.52	2486.5		
	Ties	29 ^c				
	Total	100				
<i>Majja_saara_after</i> - <i>Majja_saara_before</i>	Negative Ranks	29 ^a	28.50	826.50	-1.117	0.264
	Positive Ranks	33 ^b	34.14	1126.5		
	Ties	38 ^c				
	Total	100				
<i>Shukra_saara_after</i> - <i>Shukra_saara_before</i>	Negative Ranks	28 ^a	31.93	894.00	-4.505	0.001
	Positive Ranks	60 ^b	50.37	3022.00		
	Ties	12 ^c				
	Total	100				
<i>Sattva_saara_after</i> - <i>Sattva_saara_before</i>	Negative Ranks	29 ^a	41.88	1214.50	-2.322	0.020
	Positive Ranks	53 ^b	41.29	2188.50		
	Ties	18 ^c				
	Total	100				

a

a-After < Before

b-After > Before

c-After = Before

Discussion

Ojas

Ojas is the factor which sustains the life. According to Susrutha, *ojas* is *bala* itself. So, it can be interpreted that, *ojas* is the entity which accounts for activities of the body since *bala* is the *karma sadhanasakthi*. In the present study, it was seen that, the body weight, BMI and body fat % decreased after taking the low carbohydrate diet, which was the result of increased burning of fat after switching to low carbohydrate diet. The decrease in these parameters make the body more compact than before. The body fat, which can be correlated with *medo dhatu*, when decreased makes the individual more active and the *avarana* of *medo dhatu* if any, might have cleared. Also, the correction at the level of *medodhatu* brings about *laghava* [lightness] to the body and also this makes the person more active. Ease of movement is a feature denoting better *ojas* and hence it can be considered that improvement in *ojas* might have been brought about by the practice of low carbohydrate diet. All these lead to the improvement of *ojas* in the body.

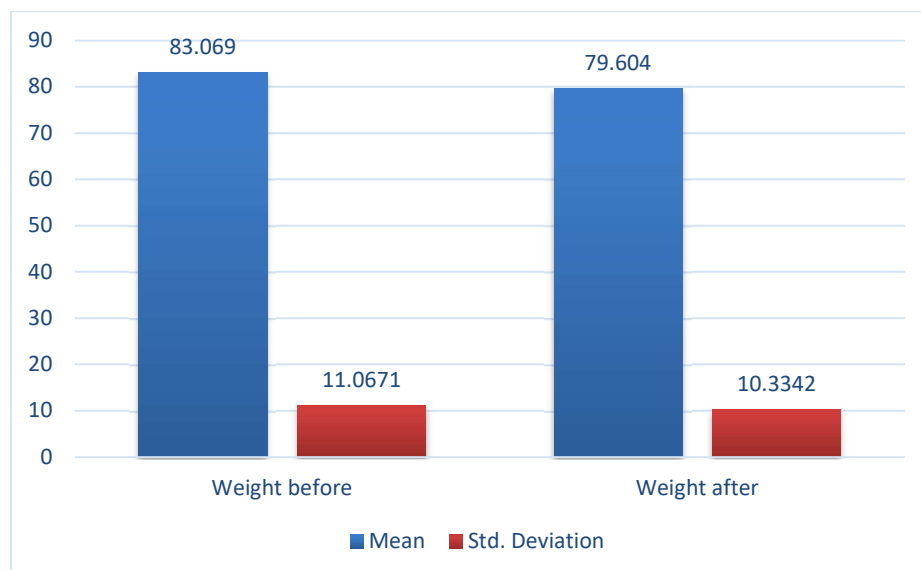


Figure 2 - Mean body weight – before & after (showing decrease in mean body weight after the diet plan)

When a low carbohydrate diet is adopted by an individual who follows a conventional diet, the time of intake (*kala*), quantity of food (*rasi*) and method of preparation of food (*karana*) become proper. This leads to the correction of *agni*. *Charaka* has explained in *grahani chikitsa* that managing proper *agni* can facilitate *ojas*. So, in the present study, this might have added a fraction for the improvement of *ojas*.

Ojas is the caretaker of both physical and mental health. So, any changes in *ojas* can manifest physically or mentally as a disease of the body or a psychological disturbance. In the present study, all the subjects had increased body weight, most of them were 'obese' with BMI >30kg/m². Obese people are always bullied socially and become a victim for body shaming. They become a laughing stock in places where people gather. All the individuals with obesity or over weight might have faced this issue at some point in their life. This is a factor which can reduce their self-confidence and also can lead to psychological disturbances like stress, anxiety etc. which may affect their productivity and *ojas*. When the body weight decreases, they will become confident and mentally more stable. This also can contribute to increase in *ojas*. So, the increase in *ojas* of the individuals took part in the study can be due to these multiple factors, rather than a single reason.

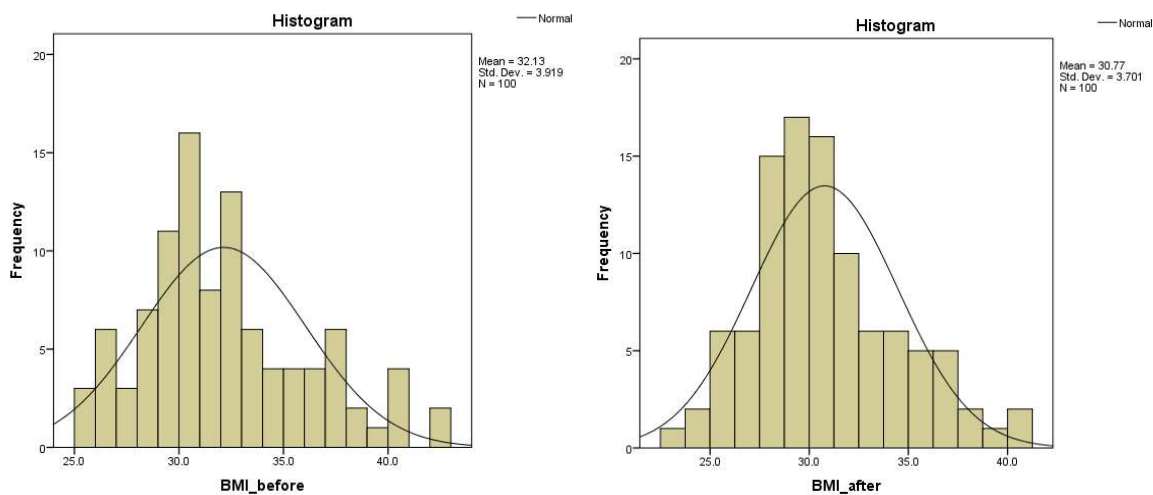


Figure 3 - Frequency Distribution of BMI (frequencies are gradually decreased towards right from the left, showing the distribution of the BMI is positively skewed)

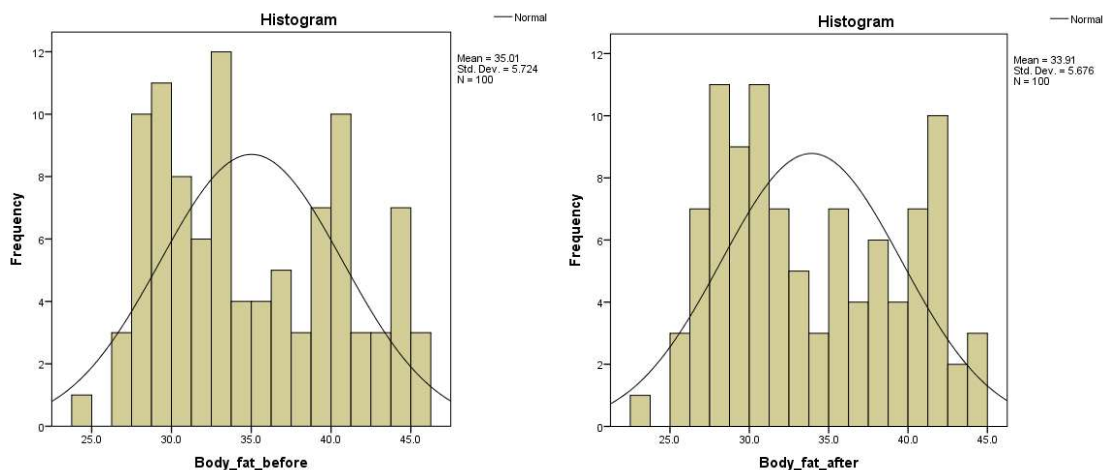


Figure 4 - Frequency Distribution of Body fat % (frequencies are gradually decreased towards right from the left, showing the distribution of the body fat % is positively skewed)

Dhatu saarata

When the data was analysed, it was observed that among the eight *saara*, *rasa saara*, *asthi saara*, *shukra saara* and *satva saara* showed increase after following the low carbohydrate diet, whereas *raktha saara*, *mamsa saara* and *medo saara* were shown a declination. The one which showed no significant change in this course was the *majja saara*.

Rasa Saarata

Rasa is the first *dhatu* formed by the action of *agni* on *ahara*. So, *rasa dhatu* is the prime factor affected, if *agni* is impaired. It can lead to a condition called '*ama*' which can act as the substratum for different diseases. In *Ashtangahridaya sutrasthana*, *Vagbhata* has explained that, due to the weakness of digestive fire, the *rasa dhatu* forms improperly, which is called *ama*¹². So proper *agni* can make proper *rasa dhatu*. When a low carbohydrate diet is adopted by an individual, there is a correction of *agni* due to modifications in the intake of food. The improvement in *agni* will be reflected as improvement in the formation of *rasa dhatu*, thus *rasa saara*.

Raktha Saarata

When *rasa dhatu* increases, *raktha* also has to increase as *rasa dhatu* is the entity which nourishes *raktha dhatu*¹³. On contrary to that, the observation was against this postulation. But this can be compared with the findings from the study which was published as an article in the 'Journal of nutrition and metabolism', which assessed the effect of low carbohydrate diet on blood gas, haematological profiles and organ functions in a rat model¹⁴. The study was conducted for a period of 60 days. For the study, a total of 15 male Wistar rats were divided into control (n=8) and ketogenic group (n=9). Controls received a standard diet contained 52.20% of carbohydrates, meanwhile, the ketogenic group received a diet which contains only 5.66% of carbohydrates. When the result was analysed, it was observed that, there is a significant alteration in blood gas homeostasis. There was a very significant decrease in blood pH of rats following low carbohydrate diet compared to those fed with a standard diet. The result of haematological analysis revealed that, the low carb diet group appeared to have slightly lower RBC counts, significantly lower haemoglobin, and haematocrit, as well as significantly smaller mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) indices. All these abnormalities indicate that rats fed with low carbohydrate diet were anaemic. This result shows similarity with the observations in the present study. So, it can be assumed that each *dhatu* requires its own specific nutrients which along with the *poshakamsha* derived from the previous *dhatu*. This proclaims the importance of *khale kapota nyaya* to explain *dhatuposhana*.

Mamsa Saarata

The decrease in the *mamsa saara* is may be due to the fact that low carbohydrate diets are often associated with muscle loss, where the muscles are the structures which can be correlated with *mamsa dhatu*. When body is running out of carbohydrates, the first compensatory mechanism starts from liver and skeletal muscles. Body starts to utilize the glucose stored in both these structures for its glucose needs, which can result in the reduction of muscle mass. Also, carbohydrates have a protein sparing effect, which reduces muscle catabolism or breakdown. So, in a situation like consumption of low carb diet, this protein sparing effect becomes minimal, as carbohydrate intake is less. This too can substantiate the observation found in the present study.

Medo Saarata

Medo saara of the individuals in the present study was significantly decreased after taking the low carbohydrate diet. 82 participants out of 100 showed a decrease with mean score varying from 11.12 to 9.26. Since body fat is the most appropriate correlation for *medas*, the reason for decrease in *medo saarata* is clear. Almost all the studies quoted above have stated the fact that, the first thing that happens on switching to low carb diet is the increase in fat burn. As carbohydrates are unavailable to meet the body needs due to restricted intake, fats are burned more for body metabolism. Also, the high fat, low carbohydrate diet are more satiating, reducing hunger and overall calorie intake without conscious effort, leading to body fat burn. All these leads to the decrease in body fat and thus *medo dhatu*. When the definition of *medo dhatu* was explained, *Shabdakalpadruma* has quoted that *medas* is the one causing *athibrimhana* or the factor making the body stout, simply means the factor causing obesity. It was clearly observed that, all the participants in the study were stout and they have reduced their body weight during the course of low carb diet. This provides direct evidence for the declination in *medo saarata*.

Asthi Saarata

The visible change occurring in individuals on low carbohydrate diet is in the body weight. Their body becomes more compact (*saara sthira sareera*) and body proportion comes to a normal level. This makes the individual more active (*kriyavantha*). All these are in favour of observable *asthi saara* purusha lakshana explained in *Caraka samhitha vimana sthana*¹⁵. This can be the reason for improvement of *asthi saara*.

Low carbohydrate diets have high amount of fat, protein, saturated fats and omega-6 fatty acids in them. This diet can also lead to a ketogenic state in the body. These factors could lead to an increased bone turnover. But a study conducted by J D Carter et al. states the opposite in a controlled clinical trial to find out the effect of a low carbohydrate diet on bone turnover¹⁶. The study was done in 30 subjects for a period of 3 months with 15 individuals in study group and control group each. The study group received a low carbohydrate diet and control group had no restriction on their diet. The result obtained was, although individuals with low carbohydrate diet did lose what significantly more than the controls did, the diet did not increase bone turnover markers compared with controls at any time point. This study is supportive to the observations in the present study that *asthi saara* was improved, as increase in bone turnover occurs in conditions with deterioration of bone microarchitecture, which leads to decrease in bone health.

Majja Saarata

Majja saara was the only *dhatu saara*, which showed no change in the present study. No significant difference was there before and after taking the low carbohydrate diet. *Asthi dhatu* has a function of nourishing *majja*¹⁷. So, in a condition where *asthi saara* is increased, *majja saara* also have to increase. But in the present study, no change was seen in *majja*. This can be due to the fact that, there are comparatively lesser number of questions to assess the *majja saara*, so that the subtle changes might not have detected by them

Shukra Saarata

While explaining the *ashta doshas* of *sthoola*, Acharya Charaka has explained that, the *sthoola* (obese) individuals will be having *krichravyavayatha* (difficulty in sexual intercourse) due to less quantity of semen and obstruction of channels of semen by *medas*¹⁸. Obesity has been found to be associated with mild to moderate symptoms of erectile dysfunction¹⁹. Obesity changes the metabolism of sex hormones and its impacts can cause loss of libido or low sexual drive. In the present study, since majority were obese, the decreased sex drive might have reflected as a decrease in their *shukra saara*. After following the low carb diet, due to decrease in *medo dhatu*, the obstruction might have cleared and due to proper nourishment, correction of metabolism of androgen also might have resulted, which all together could have contributed to an improvement in *shukra saarata*.

Sattva Saarata

Sattva saara is the instrument which can measure the mental health of an individual. The increase of *sattva saara* in the present study might be associated with the increase in *ojas*, as *ojas* sustains both physical and mental faculties. The increase in self-confidence and ability to do more activity by reduction in the body weight may have improved their mental health. A strong will power is needed to restrict the diet and to avoid the favourite foods one likes most. So, if one could practice a low carb diet for a month by sacrificing his/her likes and cravings, the *sattva bala* might have increased.

A study published in the 'Nutrition journal' titled 'Adherence to low carbohydrate diet and prevalence of psychological disorders in adults' was done to find if there exist any association between the adherence to a low carbohydrate diet and psychological disorders among Iranian adults²⁰. It was done as cross-sectional study on 3362 adult men and women; their dietary intakes were examined by the use of a validated semi-quantitative food frequency questionnaire and the psychological distress was assessed by validated Iranian versions of the hospital anxiety and depression scale and general health questionnaire. At the end of the study, it was found that, there was no association between low carbohydrate diet intake and psychological disorders. This study is supportive of low carbohydrate diet, that, it does not cause any psychological disturbance.

Conclusion

In conclusion, the use of low carbohydrate diet for a period of one month was found to be able to increase the level of *ojas* significantly. Among *dhatu saarata*, a miscellaneous result was obtained. The status of *rasa saara*, *asthi saara*, *shukra saara* and *sattva saara* have shown an increase, whereas *rakta saara*, *mamsa saara* and *medo saara* were decreased significantly. Apart from all these, the level of *majja saara* didn't vary much. But the results obtained in the present study cannot be extrapolated into a general postulate, as the findings can vary for different time period of low carbohydrate diet intake. Extensive use of low carb diet to manage obesity can't be promoted without conducting multiple rigorous studies.

Reproducing the same study with more samples in multiple settings for a longer period may yield more conclusive results.

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