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Physiological, histological, anatomical and biochemical effects of a 2-months marked carbohydrate deprivation dietary program in obese male albino rats

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Abstract

Marked-carbohydrate deprivation diets are commonly used for weight loss based on the belief that carbohydrates cause weight gain.

This work aimed to study the effect of marked carbohydrate deprivation (for two months) on the body weight, body mass index, protein, fat metabolism and insulin and leptin levels, and to clarify the mechanism by which marked carbohydrate deprivation can cause reduction of body weight and to clear possible side effects for this type of food regimen used increasingly to reduce weight.

Twenty obese male albino rats were included in this study. All rats were chosen obese and were fed for one month each on 150 grams normal chow containing high carbohydrates, proteins and fats to make this content their normal daily intake and assure obesity

Rats were divided into two groups. The first group was the control group. It contained ten rats, and they were fed on (half the daily regular intake) 75 grams normal chow containing carbohydrates, proteins and fats for two months. The second group contained ten rats and they were fed on 150 grams of food containing proteins and fats with minimal carbohydrates for two months. Rat groups were weighed and body mass index was calculated at the beginning and end of the study. Insulin, leptin levels and pH of blood were measured at the beginning and end of the study. Change in values in weight, BMI, pH of blood, insulin, and leptin levels were compared at the beginning of the study and at its end. Rate of change for all parameters in group two was compared to that of group one.

There were significant decrease in weight, and BMI, in both groups at end of the study compared to the results obtained at the beginning. The rate of change decreased more significantly for the previously mentioned parameters in group two compared to group one.

There were significant increase in insulin level in group one at end of the study compared to its level at the beginning of the study. While there were significant decrease in the insulin level in group two at end of the study compared to the level recorded at the beginning. There were significant decrease in the rate of change for the insulin in group two compared to that for group one at end of the study.

Non-significant change in the pH at the end of the study was recorded in both groups compared to the results obtained at the beginning, The rate of change for pH in group two showed significant decrease compared to that of group one at end of the study.

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Leptin level showed non-significant decrease at the end of the study in group one compared to the results at the beginning. While it showed significant increase in group two at end of the study compared to the results obtained at the beginning. There was significant increase in the rate of change for leptin in group two compared to that in group one at end of the study.

Keywords: BMI (Body mass index); Marked Carbohydrate deprivation; Insulin; Leptin

1. Introduction

The worldwide prevalence of obesity was nearly tripled between 1975 and 2016. About 13% of the world's adult population (11% of men and 15% of women) were obese in 2016. This number increased to 39% in 2019 most of them suffer obesity and try hardly to decrease their weight.

Prevalence of obesity has increased in Egypt to reach 40% according to 100million health survey (2019) compared to the 36% estimate of 2017 STEPwise survey (1). According to the World Health Organization (WHO), Egypt ranks 18th with the highest prevalence of obesity worldwide (2).

Obesity is a major contributor to the development of diabetes mellitus, hypertension, obstructive sleep apnea and fatty liver, in addition to several serious diseases. The estimated annual deaths due to obesity was about 115 thousand (19.08% of the total estimated deaths in 2020) (3).

Body Mass Index (B.M.I.) is a measurement of a person's weight with respect to his or her height. It is more of an indicator than a direct measurement of a person's total body fat. BMI, more often than not, correlates with total body fat. This means that as the BMI score increases, so does a person's total body fat (4).

The WHO defines an adult who has a BMI between 25 and 29.9 as overweight - an adult who has a BMI of 30 or higher is considered obese - a BMI below 18.5 is considered underweight, and between 18.5 to 24.9 a healthy weight (5).

Consuming carbohydrates may cause obesity because the high glycemic index in carbohydrates causes sudden increase in blood sugar, which if not utilized by the body may build up fats (6).

Carbohydrates are essential for well-balanced diet and healthy body. They are considered the only fuel source for many vital organs, as the, nervous system and kidneys. They are broken into glucose then the pancreas secretes insulin is to help the glucose move from the blood into the cells.

The most common source for carbohydrates is grain foods such as bread, rice, and pastas. Low-carb diets recommend limitation of the amounts consumed of these foods (7).

Very low-carbohydrate diets are unlikely to meet your daily nutritional needs. because they are low in thiamine, folate, vitamins A, E and B6, calcium, magnesium, iron and potassium, fiber and missing important antioxidants and phytochemicals (8)

Advocates of these diets advise people to consume more protein and fats, present in beef, chicken, bacon, fish, eggs and non-starchy vegetables, as well as fats such as oils, butter and mayonnaise. Few fruits but recommend eating less than 100 g of carbohydrate per day (9).

Foods that are restricted include many types of fruit, bread, cereals and other grains, starchy vegetables and dairy products other than cheese, cream or butter (10).

When carbohydrate intake is very low -- between 20 and 50 grams per day -- the body's metabolism changes. Rather than relying on glucose for energy, alternate substances can be burned. These include fatty acids, which are harvested from fat cells, and ketones, which are made from proteins.

These diets are sometimes called Karatay diet or ketogenic diet because they can lead to the accumulation of ketones in the blood and urine. Tissues that require glucose can have their energy needs filled by the small amounts of carbohydrates from the diet or from gluconeogenesis, in which glucose is manufactured from other substances (11).

In short term, low-carbohydrate diets may cause you to lose weight because they restrict energy. The body begins to use body stores of glucose and glycogen (from the liver and muscles) to replace the carbohydrates it is not getting from food. Around 3 g of water is needed to release 1 g of glycogen, so the rapid initial weight loss on a low-carbohydrate diet is mostly water, not body fat (12).

As carbohydrate stores are used up, the body begins to rely on other sources of fuel such as fat. This can lead to the development of ketones in the body, which can make the body acidic. This can lead to metabolic changes, which may be dangerous for some people, such as those with diabetes (13).

Some people may experience problems with a low-carbohydrate diet, including: nausea, dizziness, constipation, lethargy, dehydration, bad breath and loss of appetite.

The long-term safety of a diet very low in carbohydrates but high in saturated fat is still uncertain, and the potential effects on a person's health are not known. Follow-up studies are needed over years to determine its safety (14).

Possible long-term effects of very low carb diets may include, weight gain – when a normal diet is resumed, as some muscle tissue is rebuilt, and water is restored and weight quickly returns. Bowel problems may occur due to restricted intake of antioxidants and fibre from fruits and vegetables that can increase the risk of constipation. Also among the long term effects are dieting problems such as the 'yoyo' effect where people lose and regain weight many times over a long period of time, and not sustaining weight loss (15).

Diets that are high in protein and fats are associated with hazardous conditions, including heart disease, diabetes and cancer. This can occur if the diet is very high in fat, particularly from high-fat meats such as salami, sausages and bacon. Also Kidney problems – can occur in people with impaired kidney function or diabetes as well as osteoporosis and related conditions due to loss of calcium from the bones (16).

Very-low carbohydrate diets may have effects beyond weight as they may cause fatigue, muscle weakness, headache and constipation/diarrhea also there are concerns about their effects on the cardiovascular system as they are associated with increased risk of atherosclerosis (17).

On following low-carbohydrate diet, it is better not to avoid carbohydrates completely as they are needed to metabolise fat. It is better to choose carbohydrate-rich foods that are unrefined or unprocessed, including whole grains and fruit, rather than the more refined and energy-dense forms such as cakes, sweets and soft drinks. It is preferred to consume protein-rich foods that are also low in saturated fat, as lean cuts of red meat, fish (including fatty fish), and lean chicken (18).

Protein-rich foods that are plant based, can be also used as nuts, legumes beans and soy products. It is better to choose fats from plant sources (such as olives, olive oil, canola oil, peanuts, peanut oil, soy or soy oil) rather than from animal sources (butter or meat fat) (19).

Other studies consider carbohydrate deprivation diets to have beneficial effect on heart as they lead to increase high-density lipoprotein cholesterol and decrease in blood triglyceride levels. These lipid changes are associated with a decreased risk of heart disease (20). Diets low in carbohydrates may help lower blood glucose levels in people with type 2 diabetes and may be able to prevent or reverse some of the complications of diabetes, such as nerve damage (21).

Leptin hormone is secreted mainly by the white adipose tissue, and its level is correlated with the amount of body fat. Circulating leptin level reflects the amount of energy stored in fat and acute changes in caloric intake. It acts on the brain to regulate food intake, energy expenditure and suppress appetite (22).

2. Material and methods

Twenty white male albino obese rats weighed (430-450 grams) were included in the present study which began by the end of January 2021. They were obtained from the animal house in the October 6 University. They were fed on high fat diet 150 grams per day and contained also proteins and carbohydrates for one month to be the daily amount fed for each of them.

They were housed in wire mesh cages in groups at room temperature and had free access to water.

The rats were divided into two groups, each group contained ten rats

2.1. Group one

Control group after the first month they were subjected to decrease their daily food intake by 50%.

2.2. Group two

Fed on same high fat diet but with minimal carbohydrates to decrease the content of the carbohydrates to minimal (23).

At the beginning and end of the experiment all rats were weighed and, B.M.I. was calculated, Ph of blood was detected, and insulin and leptin levels were estimated (24).

2.3. Measurement of pH

Animals were anaesthesized using Ketamine at a dose of (50-100mg/kg body weight intraperitoneally. Immediately after cannulation 0.25 ml of blood was withdrawn from the carotid artery (25).

2.4. Statistical analysis

The data were encoded and entered using the statistical package SPSS version 15. The data were summarized using mean, comparison between studied groups was done using unpaired t-test. P value < 0.05 were considered statistically significant.

3. Results

Table 1 Mean weight, B.M.I., pH, insulin and leptin levels before and after marked carbohydrate deprivation

		Mean weight in grams	**Mean B.M.I. g/cm**	**Mean Ph**	**Mean insulin level µU/mL**	**Mean Leptin level ng/ml**
Decrease daily requirement group	(beginning of experiment)	441	0.68 ± 0.05	7.45	15.66	6.8
Decrease daily requirement group	(End of experiment)	376	0.59 ± 0.02	7.43	21.33	6.6
Marked carbohydrate deprivation group	(Beginning of experiment)	482	0.63 ± 0.55	7.43	16.47	6.4
Marked carbohydrate deprivation group	(End of experiment)	366	0.44 ± 0.25	7.38	10.22	8.1
Rate of change in the Decrease daily requirement group		-14.70%	-13.20%	-0.26%	36%	-2.90%
Rate of change in the marked carbohydrate deprivation group		-24%	-30.10%	-0.67%	-37.90%	26.50%

The obtained results showed significant decrease in the weight, and B.M.I. in the first group that decreased the daily requirements by 50% at the end of the experiment by about 14.7% and 13.2% respectively compared to the results obtained at the beginning of the experiment.

The obtained results showed significant decrease in the weight, and B.M.I. in the second group of maximal carbohydrate deprivation at the end of the experiment by about 24% and 30% respectively compared to the results obtained at the beginning of the experiment.

The obtained results at the end of the experiment showed significant decrease in the rate of change for the weight, and B.M.I. in the second group compared to their rate of change in the first group.

The obtained results at the end of the study showed non-significant change in the pH in the first group compared to the results obtained at the beginning of the study while the results obtained at the end of the study for the second group showed significant decrease in the pH compared to the results obtained at the beginning.

The obtained results for group one showed significant increase in the insulin level at the end of the study compared to the results obtained at the beginning of the study. While the results obtained at the end of the study for the same group showed non-significant decrease for leptin hormone compared to the results obtained at the beginning.

The obtained results for group two showed significant decrease in the insulin level at the end of the study compared to the results obtained at the beginning. While the results obtained at the end of the study for the same group showed significant increase for leptin hormone compared to the results obtained at the beginning.

The results obtained at the end of experiment showed significant decrease in the rate of change for the insulin level for group two compared to that for group one while there were significant increase in the rate of change for leptin level for group two compared to that for group one.

4. Discussion

The objective of the present study was to find the effect of marked deprivation of carbohydrates on body weight, B.M.I., insulin, leptin levels and pH, and to clear the possible hazardous effects of such diet on body. We aimed also to compare its effect to another dietary regimen in which the daily regular intake is decreased by 50%.

The results of the study showed significant decreased body weight by 14% compared to the initial weight in group one which was subjected to decrease its daily intake by 50%, while the B.M.I. was found decreased significantly by 13% compared to the initial value for the same group. This indicates the effect of decreased daily food intake on body weight through increasing consumption and burn of the stored fats to correct the deficit between the caloric intake and caloric needs.

The results of the study showed non-significant decrease in the pH of the blood by 0.26% in group one compared to the initial value before reduction of daily intake indicating that this type of regimen nearly not affect the pH of the blood and hence its hazards through its change is negligible.

The results of the study showed significant increase in insulin level by about 36% in group one at the end of the study compared to its level at the beginning of the study. This may be attributed to the stress occurred in the rats on decreasing the daily intake to half the usual content. While the results for the same group at the end of the experiment showed non-significant decrease by 2.9% in the leptin level compared to the initial value at the beginning of the study indicating that this type of regimen does not act through increasing leptin level and that it acts only through increasing consumption of stored fat by increasing the deficit between the calories needed each day and that really taken.

The results of the study showed significant decreased body weight by 24% compared to the initial weight in group two which was given the normal daily content of food without reduction but with marked carbohydrate deprivation, while the B.M.I. was found to be decreased significantly by 33% compared to the initial value for the same group. This indicates the powerful effect of marked deprivation of carbohydrates on body weight through increasing consumption and burn of stored fats which was easier to be burnt than proteins to release energy and correct the deficit between the caloric intake and caloric needs. More over the calories resultant from the breakdown of fats and proteins were not that much as that released from consumption of carbohydrates, this forced the body to burn large amount of fats to release suitable amount of calories to correct the deficit between energy needs and that was taken.

The results of the study showed non-significant decrease in the pH of the blood by 0.67% in group two compared to the initial value before marked deprivation of carbohydrates indicating that this type of regimen although the change it made was non-significant yet it is not minimal and may increase by time so those people on this type of regimen should not continue on it for long time and should hold on in between the trials not to affect the PH of blood markedly and reach acidosis.

The results of the study showed significant decrease in insulin level by about 38% in group two at the end of the experiment compared to its level at the beginning of the experiment. This may be attributed to decreasing the stimulus

for the release of insulin by the marked deprivation of carbohydrates and hence decreasing the ability to take glucose inside the cells. This forced the body to release energy through consuming fats and proteins and hence decrease body weight and decrease in pH through formation of fatty acids and amino acids. While the results of the study showed significant increase of leptin level by about 27% at end of the study compared to the level recorded at the beginning.

The Leptin circulates in blood and acts on the brain to regulate food intake and energy expenditure and suppress appetite until weight is lost.

Leptin mediates its effects by binding to specific leptin receptors (ObRs) expressed in the brain as well as in peripHeral tissues. The ObRa isoform (the short leptin receptor isoform) is thought to play an important role in transporting leptin across the blood–brain barrier (26). The ObRb isoform (the long leptin receptor isoform) mediates signal transduction and is strongly expressed in the hypothalamus, an important site for the regulation of energy homeostasis and neuroendocrine function (27).

The binding of leptin to the ObRb receptor activates several signal transduction pathways, including Janus Kinase-Signal Transducer and Activator of Transcription-3 (JAK-STAT3), which is important for regulation of energy homeostasis, and PHospHatidylinositol 3-Kinase (PI3K), which is important for regulation of both food intake and glucose homeostasis (28).

Other effects of leptin involving regulation of immune function, and bone metabolism are under intense investigations. This raises further studies to find out the other effects and hazards of such diet regimen that includes increased leptin and possible side effects due to such increase for long time (29).

Leptin activates a complex neural circuit comprising of anorexigenic (i.e. appetite-diminishing) neuropeptides to control food intake. Outside of the hypothalamus, it interacts with the mesolimbic dopamine system, which is involved in motivation for and reward of feeding, and the nucleus of the solitary tract of the brainstem to contribute to satiety (30).

The results of the study showed that group two showed significant decrease in the rate of change in weight and B.M.I. by about 24% and 30% respectively at end of the experiment compared to that attained by group one (14%, 13%) respectively, indicating that maximal carbohydrate deprivation may be much more effective in decreasing body weight than decreasing the daily amount of food consumed as the rats of group two were given the whole daily amount of food but with no carbohydrates yet they lost more weight and their B.M.I. decreased more than the group decreased its amount of by by 50%.

The results of the study showed that the rate of change for the pH for group two at end of the study showed significant decrease in pH compared to that for group one. Indicating some hazard of acidosis that may occur on the long use of the regimen of maximal carbohydrate deprivation.

The results of the study showed that insulin level at the end of the study showed marked significant decrease compared to the level at the beginning of the study on the contrary the level of insulin in group one showed marked significant increase compared to the level at the beginning of the study indicating that marked carbohydrate deprivation even on high amount of food consumed per day may be much more effective in forcing body to find other sources for energy to burn as fats and proteins other than carbohydrates and thus its more effectiveness in decreasing body weight than decreasing the daily food consumed even by 50%. On the other hand the rate of change for leptin at end of the study for group two showed significant increase compared to that for group one indicating that the mechanism by which the regimen of marked carbohydrate deprivation causes decrease in weight and B.M.I. is through the increased leptin level.

5. Conclusion

Marked carbohydrate deprivation may be much more effective regimen to decrease body weight in short time yet it should not be continued on for long time for fear of hazardous acidosis, and also further studies are needed to clear other side effects of the increased leptin level occurring in such regimen.

Compliance with ethical standards

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Disclosure of conflict of interest

There was no conflict of interest between authors.

Statement of ethical approval

This work was approved by the ethical commity of October 6 university.

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