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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet

Asian Network for Scientific Information

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.801.806

Research Article
Effects of Ketogenic Diet on Some Immunological Parameters

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Abstract
Background and Objective: A well-established treatment for childhood epilepsy, the Ketogenic Diet (KD) is increasingly gaining acceptance as a preventative tool for obesity. The current study aims to compare the effects of a ketogenic diet on some of the immunological parameters among obese individuals and contrast them with non-obese individuals. This includes finding the absolute numbers of white blood cells and estimating the efficacy of phagocytic cells and serum IgA concentration. **Materials and Methods:** Twenty healthy persons were chosen for the study, with an age range between 18-45 years, a weight range before the ketogenic diet of 80-115 kg and after the ketogenic diet 60-80 kg with a height range between 155-180 cm during a period between 8-16 weeks. Their daily food was composed of high fats (80%), moderate protein (15%) and small amounts of carbohydrates (<5%). The control sample included twenty healthy subjects, their food consisted of different types of food carbohydrates (75-80%), proteins (10%) and fats (<10%). **Results:** The results showed a significant increase in the White Blood Cells (WBC) count in KD individuals. Additionally, no significant difference was noticed in the absolute number of neutrophils and lymphocytes. Conversely, a significant increase in the absolute number of monocytes was observed. Moreover, the study showed a significant decrease in IgA concentration in KD individuals compared with control. **Conclusion:** In the ketogenic system users indicated a rise in the number of monocytes and a decrease in the concentration of IgA. This provides a strong sign that autoimmune disorders are emerging.

Key words: IgA, ketogenic diet, monocytes, WBC count, food carbohydrates, neutrophils, obesity

Citation: Al-Allaff, R.G.M. and F.K. Mohmad, 2021. Effects of ketogenic diet on some immunological parameters. Pak. J. Biol. Sci., 24: 801-806.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obesity is a rising global problem[1]. In both developing and developed countries, obesity has become a serious chronic disease. Furthermore, it is also related to several chronic illnesses[2]. More than 50 million girls, 74 million boys, 390 million women and 281 million men were estimated to have obesity in 2016[2]. Obesity is one of the key risk factors for cardiovascular disease and leads to metabolic syndrome alongside dyslipidaemia, hypertension and diabetes[3]. Several methods to decrease energy consumption (diets, medications and bariatric surgery)[4] and to improve energy production (exercise and non-exercise)[5] have been suggested. But even though there may be a consensus on the basic conceptual basis-increasing energy consumption and physical activity rates-how these targets can be met is less evident. Regarding initiatives on obesity, diet is one of the most divisive topics. Several different diets have been suggested for weight loss, but there is no empirical evidence to support one diet over another[6].

Specific weight reduction strategies that incorporate calorie and fat restriction with exercise have struggled to produce long-term results[7]. A high-fat Ketogenic Diet (KD) is highly effective for weight loss, according to recent research from some laboratories[8,9], including ours.

The KD diet is characterised as a high fat (>60% of energy) (<20% sufficient protein) and low carbohydrate (<10% of energy) diet[10]. The previous KD version was composed of 80% of the daily fat intake, 15% of protein and 5% of carbohydrates[11]. KD has become a popular diet not only for epilepsy, obesity and type 2 diabetes mellitus, etc., but also for body shape and athletic performance[12].

Fat metabolism, known as ketogenesis, creates water-soluble ketone bodies. It triggers ketogenesis in the liver, which breaks fat into fatty acids and ketone bodies. These ketone bodies can cross the blood-brain barrier and provide the brain with energy. Many organ systems may also use ketones as an effective energy source, characterised by D-beta-hydroxybutyrate and acetoacetate elevations. Ketosis happens when the fuel of the body changes from carbohydrates to fat. Incomplete degradation of fatty food by the liver results in the production of ketone bodies in the body. A KD keeps the body in a state of ketosis, which enables the body to use fat instead of carbohydrates as a preferred energy substrate[13].

Several studies focused on following the effects of KD on some haematological and immunological parameters. A study by Pillam[14] surveyed improvements in body weight, lipid profile, glucose, urea, WBC count and creatinine following low-carbohydrate administration and KD in overweight and obese diabetic subjects compared to non-diabetic obese subjects for 56 weeks.

A study by Goldberg[15] recorded that a high-fat, low-carbohydrate (KD) feeding of mice protected against lethal influenza infection. By identifying the immune response in the lungs, the author identified that KD stimulates the expansion of Ƴδ-T cells in the lungs. Using mice lacking ϒδ-T cells, the authors have identified the functional significance of these cells in providing defence.

KDs have been used in the treatment of epilepsy for a long time and are being studied for different diseases. Clinical data suggest KDs affect cytokine release and inflammation. Following previous studies, there has been speculation about significant effects on the immune system in people who follow the KD system. The current study focused on following up on changes in the number of white cells, measuring the phagocytic activity of neutrophils in the peripheral blood and monitoring changes in IgA immunoglobulin concentration in the bloodstream.

MATERIALS AND METHODS

Study area: The study was carried out at Department of Biology, Advanced Immunology Lab, Iraq from October, 2019-April, 2020.

Patient data: Twenty healthy persons were chosen for the study, ten for each sex with an age range between 18-45 years, a weight range before the ketogenic diet of 80-115 kg and a BMI of 34.85-36.30 and after the ketogenic diet, a weight range of 60-80 kg and a BMI of 27.70-32.60 with a height range between 155-180 cm during a period between 8-16 weeks. Their daily food was composed of high fats (80%), moderate protein (15%) and small amounts of carbohydrates (<5%). The control sample included twenty healthy subjects, ten for each sex. Their food consisted of different types of food carbohydrates (75-80%), proteins (10%) and fats (<10%).

White blood cell count:

- Whole blood was collected in EDTA tubes
- A dilution process was performed of whole blood samples with acetic acid to lyse the RBCs and platelets
- The diluted blood was suspended and then placed in a chamber for cell counting
- Counted cells were multiplied with the dilution factor and reported as several cells per microlitre (µL) of whole blood[16]

Differential white blood cell count: Thin films of blood were prepared from EDTA tubes. Slides were covered with Leishman Stain for 1-2 min. The stain was diluted with an equal amount of buffer water for 5-7 min and then flooded off with a buffer. The film was observed under a high lens after smearing oil droplets over the length of the slide. The white blood cell count was estimated by noting the number of white cells per high power field (100X).

The percentage of each cell type in a differential count is the total number per 100[17].

Phagocytic activity:

- About 50 µL of NBT stain was transferred to a vial by a sterile plastic pipette
- About 50 µL of heparinised blood was mixed gently with NBT solution
- The incubation process was performed at 37°C for 30 min
- With a plastic pipette, 5 µL of the mixture was transferred onto a clean glass slide
- A smear was prepared moderately thick to reduce mechanical damage to formazan-containing cells, which become more fragile
- The smear was treated with Leishman Stain

Microscopic examination and counting: A stained smear was scanned using an oil immersion objective and counting a total of 100 or more neutrophils. Neutrophils showing formazan deposits were recorded as positive[18]:

$$\text{Phagocytic cell (\%)} = \frac{\text{Number of neutrophils with formazan}}{\text{Total number of neutrophils}} \times 100$$

Measurement of IgA immunoglobulin concentration: Serum IgA was calculated by a simple linear regression coefficient equation; this equation allows us to calculate the concentration of IgA mathematically depending only on the BMI value[19]:

$$\text{IgA} = \beta_0 + \beta_1 \text{ BMI}$$

$$\text{IgA} = -309.608 + 12.215 * \text{BMI}$$

Statistical analysis: Comparisons of means were analyzed statistically using a one-way analysis of variance (ANOVA) of probability $p \leq 0.05$. All statistical analysis was performed using SPSS 24.0 software.

RESULTS AND DISCUSSION

The results showed a significant difference in the white blood cell count in ketogenic diet subjects compared with the control sample and also suggested no significant difference in the absolute number of neutrophils and lymphocytes between the ketogenic diet subject and the control sample, On the other hand, the results also showed a significant increase in the absolute number of monocytes between the ketogenic diet subject and the control sample as shown in Table 1.

The results showed no significant difference in the absolute number of phagocytic cells in ketogenic diet subjects compared with the control sample; the obtained results are shown in Table 2.

As previously mentioned, KD was used as a dietary guideline for patients with epilepsy, so the majority of studies that study KD's effects on obese people are restricted. This

Table 1: Comparison between the absolute number of WBC, neutrophils, lymphocytes and monocytes of ketogenic groups and control

	Sample	Sample No.	Mean±SD cell mm^{3-1}	Extreme value cell mm^{3-1}	p-value
WBC count	Ketogenic	20	9650.0±3300.6	3800-15600	0.002*
	Control	20	7165.0±27.5	7109-7200	
Neutrophils	Ketogenic	20	5114.5±2486	8856-950	0.06
	Control	20	3964.0±9315	7125-1800	
Lymphocytes	Ketogenic	20	2470.0±1417.9	6500-900	0.5
	Control	20	2236.0±602.6	2586-1090	
Monocytes	Ketogenic	20	2075.0±1292.8	6000-400	0.000*
	Control	20	219.0±71.2	455-164	

*$p \leq 0.05$

Table 2: Comparison between the absolute number of phagocytic cells of ketogenic groups and control

Phagocytic cell	Sample	Mean±SD cell mm^{3-1}	Extreme value cell mm^{3-1}	p-value
Ketogenic	20	5114.5±2486.4	6199-684	0.06
Control	20	3964.0±931.5	2746-1133	

*$p \leq 0.05$

Table 3: Comparison between the concentration of IgA in ketogenic groups and control samples

IgA	Sample	Mean±SD (mg dL^{-1})	Extreme value (mg dL^{-1})	p-value
Ketogenic	20	74.52±39.94	32.7-159.6	0.002*
Control	20	130.43±23.00	97.6-164.8	

*$p \leq 0.05$

study disagrees with the study by Schreck *et al.*[20]. A small but significant reduction in total WBC and neutrophil counts were observed in KD-treated epilepsy patients. The study by Kose *et al.*[21] suggested there was no major effect of KD in patients with epilepsy on haematological parameters. A pilot study Schmidt *et al.*[22] showed that a KD is acceptable even for advanced cancer patients with no significant side effects. In some patients with advanced metastatic tumours, it may improve aspects of the quality of life and blood parameters. Our study findings were not consistent with previous research results, as most participants in previous studies suffered from unusual conditions such as epilepsy and cancer. An elevated white blood cell count may reflect an inflammatory condition in the body and the differential WBC test result supported a significant increase in the absolute numbers of monocytes whose elevation represents the appearance of an acute inflammatory condition.

A study by Maha *et al.*[23] found that monocytes, the key cells of the immune system, can increase tissue damage in organs throughout the body in response to high blood fat levels. Monocytes are part of the immune system and are a heterogeneous white blood cell population responsible for preserving and restoring tissue integrity. The aggregation of fats in the blood with the ketogenic diet creates a hyperlipidaemic environment. Monocytes have been shown to ingest excess lipids that activate them via multiple signalling pathways that culminate in increased pro-inflammatory cytokine secretion. However, it remains poorly understood which lipids cause monocyte alterations and which kind of monocytes respond to this rise in lipids.

A study by Valledor *et al.*[24] observed that as fat accumulates all over the body in organs, monocytes transform into other immune cells. Macrophages and tissue cells accumulate fat and are converted into 'foam cells'. Both foam cells and macrophages secrete higher levels of chemokine CCL_4, promoting the extravasation and aggregation of blood monocytes within the tissues. The continuing high saturated fat levels foster this process. The previous observations confirm what the current study obtained from the results. The significant increase in the number of monocytes reflects the presence of inflammatory conditions in the body tissues in general and fatty tissue in particular. The study estimated the IgA immunoglobulin concentration in the serum in a ketogenic individual because it is the humoral immune component specialised in protecting different tissues, especially secretory tissue.

The results showed a significant decrease in IgA concentration in the serum of ketogenic diet subjects compared with the control sample, as shown in Table 3.

It is especially important to notice that the specific immune system alterations caused by high-fat foods occur most prominently in intestinal IgA, expressed by B cells. Hydrophobic lipid-containing antigens in the intestine are connected with adipose tissues that lead to selecting B cells that produce hydrophobic antibodies at these sites. It is well established in humans that B cells have clearer evidence of past antigenic exposures, such as strongly mutated B-memory cells. There is a proven selection for shorter CDRH3 lengths (genes responsible for antibody structure), although the mechanical reasons for this are not well understood[25].

Another logical explanation for shifts in the B-cell repertory was given in the studies by Karlsson *et al.*[26]. These studies observed the effect of diet on intestinal microbiota in high-fat diet mice. The intestines are the place where food is absorbed and host an enormous community of commensal microbes but are also a major site of the pathogenic attack. Immunoglobulin IgA, the most common antibody found in the secretory tissue, plays an important role in protecting the body from an infectious agent and in regulating metabolism through actions on non-pathogenic microbes in ways that are sometimes interconnected. There is ample clear evidence that abnormal gut microbiota is linked with obesity and type 2 diabetes[27]. The results obtained from the current study showed a decrease in the IgA concentration in people with KD. This decrease may be attributed to the emergence of new fat antigens that stimulate the immune response against it. The biggest evidence for the emergence of new antigens is the beginning of coding by new genes that are partly different from the old ones for antibody production[25]. Eating food that contains a high percentage of fat results in a change in the microbial community in the intestinal tract and leads to the emergence of a new microbial community against which an immune response appears. This requires the intervention of the immune component specialised in secretory tissue IgA. KD has been used as a therapy for epilepsy for almost a century; however, other benefits of KD have been found in recent decades. Amongst these virtues, obesity treatment is the most thoroughly discussed. In this study, the potential applications of KD to metabolic states, such as weight control, energy homeostasis and some immunological effects, were summarized.

CONCLUSION

The current study concluded that there is a defect in the immunological parameters, represented by an increase in the numbers of monocytes. Besides, decreasing IgA concentration may indicate the emergence of new antigens in KD individuals, which indicates the probability of a new immune response against the components of the body and the development of autoimmune diseases. Further investigations should be considered and no widespread application of KD is preferred in the future.

SIGNIFICANCE STATEMENT

This study explores the effects of the ketogenic diet on some of the most important immunological parameters of infection resistance. This study will help researchers uncover the negative effects of the ketogenic diet and how to inhibit the host's defences by introducing large amounts of fats into immune cells thus impeding their action, which many researchers have been unable to shed light on. Thus, this study provides nutritionists and people trying to lose weight a complete picture of the shortcomings of this system despite its high efficiency and speed in losing weight.

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