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Effect of a Ketogenic Diet on Some Biochemical Parameters in Obese People

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Abstract

This study examined the effects of a few biochemical variables on obese Iraqi males and females with a 30.5 body mass index (BMI) when they were fed a ketogenic diet. The present study demonstrates how an individual who follows a ketogenic diet has an increase in low-density lipoprotein (LDL-cholesterol). This research's objective was to assess the levels of some biochemical variables in obese people who were eating a ketogenic diet. Following 35 days on a ketogenic diet, the results show a significantly higher $P \leq 0.05$ level of low-density lipoprotein (LDL) and total cholesterol (TC). Additionally, insulin, fasting blood sugar (FBS), cortisol, HOMA-IR, urea, BMI, and creatinine all show a considerable reduction, $P \leq 0.05$.

Keywords: Ketogenic diet, lipid profile, biochemical parameters.

تأثير غذاء الكيتو على بعض الاختبارات البايوكيميائية مع الاشخاص البدنيين

مهند سلام مجيد الفياض

قسم التقنيات الاحيائية، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

تم إجراء هذا البحث على تأثير بعض الاختبارات البايوكيميائية على الذكور والاناث العراقيين البدنيين مع مؤشر كتلة الجسم 30.5 مع غذاء الكيتو. تشير الدراسة الى زيادة في البروتينات الدهنية واطنة الكثافة في الاشخاص المتبعين لحمية الكيتو. هدفت هذه الدراسة هي قياس مستوى بعض المؤشرات البايوكيميائية في الاشخاص البدنيين المتبعين لحمية الكيتو. النتائج تدل على زيادة معنوية في الكوليسترول الكلي و البروتينات الدهنية الواطنة الكثافة بعد 35 يوم من حمية الكيتو. ايضا هنالك نقصان معنوي في سكر الدم، الانسولين، مقاومة الانسولين، الكورتيزول، مؤشر كتلة الجسم، يوريا و الكرياتينين.

Introduction

One of the main issues of the twenty-first century is obesity, which has spread globally. Also, obesity can be described as excessive or abnormal fat buildup, which raises health risks [1]. The body mass index (BMI), which measures obesity, is determined by dividing the weight of the body (kg) by the square value of height (m²). Overweight is defined as having a BMI of 25 kg/m² or more, and obesity is characterized as having a BMI of 30 kg/m² or higher. Obesity is further subdivided into obesity classes I (BMI: 30-34.90 kg/m²), II (BMI: 35-39.90 kg/m²), and III (BMI: over 40 kg/m²). Obesity is a health issue as well as an

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aesthetic one. Cardiovascular disease (CVD), Type II diabetes (T2DM), hypertension, and several cancers are all made more likely by obesity [2]. Additionally, common obesity comorbidities include respiratory issues, metabolic syndrome, and poor cognitive abilities, which ultimately increase mortality rates and cause premature death in obese people [3]. Additionally, many people are pointing to psychosocial effects such as discrimination, bullying, and inadequate medical care [2].

Ketones are compounds that include a carbonyl group. Their structure could be indicated as $RC(=O)R'$, in which R' and R could be any number of carbon-containing substituents. Although hepatocyte mitochondria are the primary producers of ketones in the human body, the majority of body tissues can use ketones as an alternate energy source. Following 3 days of starvation, the number of circulating ketones increases to 30–40%, providing 2–6% of the energy during overnight fasting [4]. Acetone, acetoacetate, and 3 β -hydroxybutyrate are the three primary ketone bodies produced by mitochondria; technically, the last is a hydroxyfatty acid rather than a ketone. Whereas acetone, which is a volatile compound primarily eliminated through respiration, shouldn't be present in the bloodstream because it is a volatile compound, acetoacetate and 3 β -hydroxybutyrate are the two main ketone bodies found in the bloodstream. The production of ketones (ketogenesis) and their degradation (ketolysis), regulated by the secretion of glucagon and insulin, respectively, control levels of ketone bodies in the blood (Figure 1). Ketogenesis is regulated more favorably by glucagon than by insulin, which can inhibit ketone production [5].

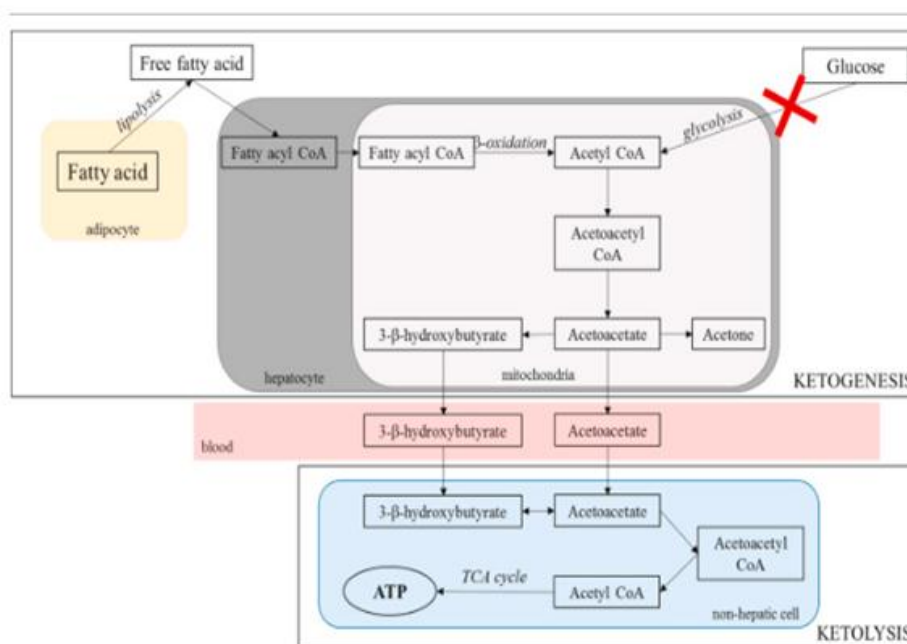


Figure 1: Ketolysis and Ketogenesis [5]

The process of ketogenesis begins when fatty acids are broken down into free fatty acids. The free fatty acids are then moved from adipocytes to hepatocytes and oxidized to make acetyl coenzyme A (CoA) [5]. Acetyl CoA is collected in low-glucose environments and, after that, transformed into acetoacetyl CoA by the enzyme thiolase. After that, the HMG CoA synthase catalyzes the synthesis of the β -hydroxy- β -methyl-glutaryl CoA (HMG CoA) from the acetoacetyl CoA. HMG CoA is then converted by HMG CoA lyase into acetoacetate and acetoacetyl CoA. Acetoacetate could be further converted into acetone via non-enzymatic de-carboxylation or 3 β -hydroxybutyrate through the β -hydroxybutyrate dehydrogenase. Acetone is no longer used and is thus eliminated through the lungs or urine. 3-

hydroxybutyrate and acetoacetate can both diffuse into the circulation simultaneously and reach non-hepatic tissues. Ketolysis is required for using the ketone bodies as an energy source. In ketolysis, 3 β -hydroxybutyrate and acetoacetate are converted back into acetyl CoA through succinyl CoA: 3-oxoacid CoA transferase (SCOT), followed by acetyl CoA acetyltransferase (ACAT-1). After completing the Krebs cycle, acetyl CoA is oxidized further, producing 22 molecules of ATP [5].

According to the findings of all previously reviewed studies, following a ketogenic diet (KD) significantly decreased BMI, fat mass content, and body weight. Yet, it is uncertain whether the extremely low calorie intake or the ketosis condition are to blame. Most research on the utilization of the ketogenic diet (KD) for managing obesity used the very low caloric ketogenic diet (VLCKD), which had a daily caloric intake of only 500–800 kcal. Since most of the research didn't use control groups to compare the effects of different nutrient compositions of diets, which has been cited by authors as a weakness of the research [6], it's possible that the changes found are caused by less food intake and not by the nutrient composition of the diet. Adherence to KD could have negative effects along with weight loss. Long-term KD was linked to elevated circulating uric acid levels, kidney stone risk, and osteoporosis due to inadequate calcium consumption. To minimize the frequency of seizures, patients with refractory epilepsy generally follow a long-term KD diet, the negative effects of which have been thoroughly covered in the literature [7].

Although the diet isn't followed for over a few weeks, a KD for obesity management is typically completed for the purpose of achieving a considerable decrease in body weight. As a result, the adverse effects are minor and include constipation, headaches, irritability, low mood, and hunger. According to the findings of recent research, obese patients scheduled for bariatric surgery who followed the VLCKD regime for 25 days experienced no negative side effects [8]. The aim of this study is to evaluate some biochemical parameters, especially low-density lipoprotein (LDL), because it is considered bad cholesterol in obese people on a ketogenic diet.

Material and methods

Study design

The study that is being presented was completed at the University of Baghdad, College of Sciences, Department of Biotechnology. An Iraqi female and male aged 32 who are obese have a BMI of 30.5 on average. They stated that they had been strictly following a KD and engaging in daily, consistent exercise for around 30-35 days. Seafood, low-carb vegetables, eggs, avocados, coconut oil, and olive oil made up their diet. In this study, researchers looked into serum insulin, fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL), as well as creatinine, urea, and the homeostatic model evaluation for insulin resistance (HOMA-IR). Samples were gathered, and the Chemical Laboratory at the University of Baghdad College of Science, Department of Biotechnology, is where they are being analyzed. In the morning, a 16-hour fast was followed by the collection of blood samples. 5 ml of blood samples were drawn from individuals using a syringe and needle.

Determination of serum total cholesterol (T. Chol):

Utilizing a commercially available kit (bio-Merieux) (France), the TC concentration has been assessed using the enzymatic method [9]. At 500 nm, the T. Chol value is specified spectrophotometrically.

Determining serum HDL-c:

The bio-Merieux kit was used for measuring the level of HDL-cholesterol using an

enzymatic method [10]. The main goal of this method is to get the LDL and VLDL lipoproteins and chylomicrons to stick together by adding phosphotungstic acid while magnesium ions are present. The HDL, which contains cholesterol and phospholipids, was included in the supernatant that was created after centrifuging. At 500 nm, HDL was spectrophotometrically defined.

Determining the serum triglycerides (TG):

The enzymatic approach suggested by Fossati and Prencipel was utilized with the Bio-Merieux kit for measuring the total concentration of serum TG [11]. The total serum concentration of TG was denoted as 500 nm.

Determining the serum very density lipoprotein (VLDL-C):

The VLDL has been determined based on the classical equation [12]. $VLDL\text{-cholesterol (mg/dl)} = 0.20 \times TG \text{ (mg/dl)}$.

Determination of serum LDL-c:

LDL was specified depending on Friedewald's equation: $LDL\text{-cholesterol} = TC - (HDL\text{-cholesterol} + VLDL\text{-cholesterol})$.

FBS Determination

FBS is enzymatically estimated using glucose oxidase GOD PAP (kit) (liquid) GL-2624.

Determining serum insulin

The German company's AESKULISA ELISA kit measures serum insulin levels. The specific antigen-coated microplates are incubated with diluted serum samples (1:101). If present in the specimen, the patient's antibodies bind to the antigen. The next step involves washing off the unbound fraction. Anti-human immunoglobulins that have been attached to horseradish peroxidase (called a "conjugate") are mixed with samples in microplates, where they react with the antigen-antibody complex. The next step is washing out any unbound conjugates. A colorimetric (blue) enzymatic reaction is produced by adding TMB-substrate and is stopped by dilute acid (the color changes to yellow). The intensity of the color made by the chromogen is determined by the amount of conjugate that is bound to the antigen-antibody complex. This amount is proportional to the concentration of the relevant antibodies in the patient sample.

Assay procedure:

- 1- Pipetting 10 μ l of standards 1 to 6 to duplicate wells has been carried out.
- 2- Pipetting 10 μ l of each diluted sample 1:101 to duplicate wells has been done.
- 3- Microplate has been covered with plate sealer and, after that, incubated at a temperature of 37 °C for 30 minutes.
- 4- Wash 3X with 300 μ l washing buffer (diluted 1:50).
- 5- Pipette 100.0 μ l of conjugates into every well.
- 6- Incubate for 30 min at a temperature of 37 °C.
- 7- Wash 3X with 300 μ l washing buffer (diluted 1:50).
- 8- Pipette 100 μ l TMB substrate into every well.
- 9- Incubate for 30 minutes at 37 °C, away from intense light.
- 10- Pipette 100 μ l stop solution into every one of the wells.
- 11- Incubate for 5 minutes.
- 12- Carefully agitate the plate for 5 seconds.
- 13- Read absorbance at 450 nm.
- 14- Calculating the concentration of insulin by standard curve.

Determining BMI

The BMI is calculated by dividing weight (kg) per square meter of height.

Determination of homeostatic model assessments for the insulin resistance (HOMA-IR)

The homeostatic model evaluation for insulin resistance (HOMA-IR) was estimated utilizing the following formula: (fasting glucose (mg/dl) x fasting insulin) / 405 or (fasting glucose (mmol/L) x fasting insulin) /22.5.

Serum Creatinine Determination:

Creatinine levels in the serum were tested colorimetrically using commercially available kits (BIOLABO) in India. When creatinine and alkaline picrate are mixed together in a 1:1 ratio, they form a colored complex that carries ions. The rate of colored complex production is inversely correlated with creatinine content.

Determination of serum urea level:

Based on Scott and Fawcett [13], serum urea was determined using a colorimetric method using a Randox kit (France).

Determination of serum cortisol

Ichroma Kit's (France) competitive immunological detection approach was used to measure serum cortisol.

Statistical analyses:

The SPSS for Windows, version 22, program has been deployed in order to conduct the data analyses. Data were represented as mean \pm SD. The researched parameters were checked to see if they followed the gaussian distribution with the Shapiro-Wilk normality test. After the tests of the ANOVA, the Bonfferoni post hoc test was used for several comparisons. Using Pearson's analysis of correlation, the levels of the association were examined. Significant has been defined as a p value less than 0.05 [14].

Results and discussion

Effect of ketogenic diet on lipid profile

Individuals' total cholesterol and LDL cholesterol levels increased significantly after 30–35 days on a ketogenic diet. Following 35 days on the KD, T.ch. increased significantly ($P \leq 0.05$) (187 mg/dl) compared with the control (133 mg/dl) before the experiment. Additionally, there is a considerable rise in LDL ($P \leq 0.05$) (126.4 mg/dl) in comparison to pre-experiment levels (73.0 mg/dl) after 35 days of following a KD, while other lipid profile measures were unaffected (Table 1).

Table 1: Effect of KD on Lipid Profile

Parameters	Before experiment	After 35 days ketogenic diet	P value
T.ch mg/dl	133 \pm 7.8	187 \pm 11.9	≤ 0.05
TG mg/dl	111 \pm 4.9	113 \pm 9.8	≤ 0.05
HDL-C mg/dl	37 \pm 2.9	38 \pm 6.6	≤ 0.05
VLDL-C mg/dl	22.2 \pm 2.1	22.6 \pm 3.1	≤ 0.05
LDL-C mg/dl	73.0 \pm 4.3	126.4 \pm 11.8	≤ 0.05

When patients are switched to a typical KD, their dietary cholesterol intake rises by over 100%, which causes an increase in both total cholesterol and LDL-C. It's interesting to note

that following six weeks on KD, these two biomarkers were not significantly raised in certain studies [15]. The LDL subfractions, which are regarded as advantageous for preventing CVD, are also changing. On the other hand, the main LDL subfractions can occasionally be small, dense LDL particles, known to raise the risk of CVD. Thus, in patients following a KD, close routine monitoring regarding body weight, lipid panel, including LDL subfractions, and glucose levels must be strongly emphasized. Because LDL-C levels and the type of LDL subfraction that is most common can vary a lot, it is also important to talk to patients about why they shouldn't do KD if they have a lot of CVD. A Mediterranean diet is an alternative for such people, as it has been demonstrated in various research studies to lower the risk of CVD and enhance dyslipidemia [16].

Effect of ketogenic diet on some biochemical parameters

This research looked at how a KD affected insulin, FBS, cortisol, HOMA-IR, and BMI (Table 2). It was found that after 35 days on the diet, there was a considerable reduction $P \leq 0.05$ FBS, HOMA-IR, insulin, cortisol, and BMI compared to before the experiment.

Table 2: Effects of KD on Certain Biochemical Parameters

Parameters	Before experiment	After 35 days ketogenic diet	P value
FBS (mg/dl)	113±7.9	82±11.7	≤ 0.05
Insulin (μIU/ml)	13.2±1.1	10.1±2.1	≤ 0.05
HOMA-IR	3.6±0.6	2.0±0.4	≤ 0.05
Cortisol (ng/ml)	8.7±1.1	7.9±1.8	≤ 0.05
BMI (kg/m ²)	30.5±2.3	27.6±4.5	≤ 0.05

The findings of this work show that the KD is a useful method for glycemic control and weight loss. The hepatic transformation regarding fatty acids created and released the ketone body into circulation in cases where the human body faced acute hunger or very little carbohydrate [17]. In contrast to severe pathological diabetic ketosis, nutritional ketosis has no symptoms and blood ketone levels that are between 0.50 and 3 mmol/L, with lowered blood glucose and a normal pH of the blood. Many studies have shown that KD is effective at controlling glucose metabolism, which may be good for your health because it limits carbs and lowers the absorption of monosaccharides in your gut [18]. These result in lower blood glucose levels and less fluctuation in blood glucose levels. The HOMA-IR test is utilized in order to assess the level of insulin resistance. Insulin resistance and obesity are both clinical traits of type 2 diabetes. One of the main goals of treating diabetes is reducing insulin resistance [19]. On the other hand, there are very few studies examining the significance of KD in reducing insulin resistance in diabetic individuals; the majority of these studies concentrated on the impact on obese people [20]. For example, a controlled clinical investigation examining the impacts of KD consumption in obese individuals who don't have diabetes found that after consuming KD for 6 weeks, patients with insulin resistance in T2DM are more likely to have lipid metabolic disorders than other patient groups [21]. Dyslipidemia causes or worsens insulin resistance because it is lipotoxic to cells. Increases in free fatty acid (FFA) and TG are usual symptoms [22]. Increased FFA could potentially raise the risk of CVD and be an independent pathogenic factor for insulin resistance [23]. As a result, lowering dyslipidemia helps control the development and progression of diabetes problems as well as the regulation of insulin sensitivity [24]. Many studies have demonstrated how KD consumption helps obese patients lose weight [25].

Effect of ketogenic diet on kidney functions

Table 3 displays kidney function parameters following a 35-day KD. Following a 35-day KD, there is a considerable drop in creatinine (0.86 mg/dl) and urea (16.4 mg/dl) when

compared to the pre-experiments (creatinine 1.2 mg/dl) and (20.2 mg/dl), respectively.

Table 3: Effect of the ketogenic diet on kidney functions

Parameters	Before experiment	After 35 days ketogenic diet	P value
Urea (mg/dl)	20.2±5.4	16.4±6.1	≤ 0.05
Creatinine (mg/dl)	1.20±0.30	0.86±0.20	≤ 0.05

Previous research has demonstrated that following a KD can have both short- and long-term impacts. Kidney stones, dyslipidemia, and a lack of carnitine are just a few of the long-term effects [26].

Rosemary and Bibiana [27] found that the results of this work didn't show a big change in the renal parameters of rats that had been given KD. However, the renal parameters (such as serum creatinine, urinary albumin, and urea) were better on the diets than on the control. On the other hand, there were considerable differences between groups when the various KDs (coconut oil, butter, and olive oil) were compared to one another, even if the values were within the range of the control values. The values of urinary albumin and creatinine that have been lower in ketogenic groups compared to the control groups have further supported the findings that a high-fat KD does not harm the kidney [28]. This is in line with results from Bolla et al. [18], who found that participants with type I and type II diabetes who consumed a high-fat KD had their impaired kidney function reversed. In their investigation, they discovered that following 8 weeks of KD intake in mice, genes linked to diabetic-related kidney failure had their expression reversed. This result is comparable to that of our work, in which KD were shown to have increased renal parameters when compared with controls.

Conclusion

A KD can be utilized as part of the integrated management of T2DM because it lowers blood sugar, improves lipid metabolism, improves kidney function, and reduces insulin resistance.

Ethical clearance

This research was ethically approved by the Research Ethical Committee of both the Ministry of Higher Education and Scientific Research and the Ministry of Health and Environment in Iraq.

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