Clinical Hemorheology and Microcirculation 21 (1999) 147–153
IOS Press

Conference communication

Hemorheologic effects of a short-term ketogenetic diet

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Received 12 September 1998
Accepted 4 March 1999

Keywords: Blood viscosity, hemorheology, erythrocyte deformability, protein-sparing diet, obesity, ketone bodies

1. Introduction

The interest of treating overweight is underlined by the increase in cardiovascular risk associated with obesity [1–3]. It is clear that even small degrees of weight loss are associated with improvements in lipid and carbohydrate homeostasis [4,5], as well as marked reduction in mortality [6]. However, some slimming procedures may induce deleterious effects on cardiovascular risk factors [7]. Recently, the use of protein-sparing diet procedures with very reduced carbohydrate quantities has been largely extended in the treatment of obese patients [8]. This procedure is based upon the concept that during carbohydrate starvation, the glucose requirements are met by gluconeogenesis while ketone bodies formation from fatty acids is enhanced, thus increasing lipid catabolism [9]. However, ketogenesis results in acidosis [10], a metabolic situation that has been reported to experimentally impair red cell deformability [11]. Whether commonly used ketogenetic diets modify blood rheology has not been, to our knowledge, reported. Therefore, we investigated the hemorheologic effects of a 3-day ketogenetic diet protocol.

2. Subjects and methods

Subjects used in this study (see Table 1) were 10 women with isolated obesity without any additional medical problem (51.1 ± 3.2 yr body mass index: 26 ± 0.6 kg/m^2) who underwent a 3-day slimming-inducing diet (470 kcal/day including 1.2 g/kg/day of proteins and 200 g of vegetables).

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Body composition was assessed with a four terminal impedance plethismograph BIA 101/s from Akern RJL Systems (Detroit, MI, USA). The four electrode method minimizes contact impedance and skin–electrode interactions. Measurements were made in fasting subjects after 15 min resting in a supine position. A current of 800 μA and 50 kHz is introduced into the subject and the measurement of the voltage drop allows the determination of total body reactance and impedance. These values are then introduced into a software provided by the manufacturer for calculating body water, fat mass, fat-free mass, and body cell mass [12].

Blood samples for hemorheological measurements (7 ml) were drawn with potassium EDTA as the anticoagulant in a vacuum tube (Vacutainer). Viscometric measurements were done at very high shear rate (1000 s^{-1}) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [13,14]. Accuracy of the measurements was regularly controlled with the Carrimed Rheometer 'CS' (purchased from Rhéo, 91120 Palaiseau, France) [15]. The coefficient of variation of this method ranged between 0.6 and 0.8% [16]. With this device we measured apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (0.45) according to the equation of Quemada [17]. Dintenfass' 'Tk' index of erythrocyte rigidity was calculated [18]. RBC aggregation was assessed with the Myrenne aggregometer [19] which gives two indices of RBC aggregation: 'M' (aggregation during stasis after shearing at 600 s^{-1}) and 'M1' (facilitated aggregation at low shear rate after shearing at 600 s^{-1}). The hematocrit/viscosity (h/η) ratio, an index of oxygen supply to tissues, was calculated according to Chien [20] and Stoltz [21], with hematocrit (as percentage) divided by viscosity at high shear rate determined as described above. Insulin, Glucagon and C-peptide were measured by radioimmunoassay with well standardized commercial kits from Sorin Biomedica, Pharmacia and Amersham.

2.1. *Statistics*

Results are presented as mean $\pm$ the SE of the mean. A value of $p < 0.05$ was considered as significant. Comparisons were made with nonparametric tests [22]. Correlations were tested by least square fitting for linear, exponential, logarithmic and power relationships.

3. Results

This diet resulted in a weight loss 1.8 $\pm$ 0.2 kg (Table 1). Changes in metabolic and hemorheologic parameters are reported in Table 2. This diet results in a very significant increase in blood ketone bodies ($p < 0.01$ for both acetoacetate and β OH butyrate). However, serum bicarbonate remained unchanged suggesting that there was no metabolic acidosis. Whole blood viscosity increased (+9%, $p < 0.05$). When looking at the individual factors of blood viscosity there was no change in either plasma viscosity or hematocrit. By contrast, red cell rigidity significantly increased (+9%, $p < 0.05$).

4. Discussion

Results of this study show that such a protein-sparing, very low calorie diet (VLCD) induces a very significant increase in blood ketone bodies, while there is no metabolic acidosis as reflected by the unchanged levels of plasma bicarbonate. Concerning blood rheology, there is a +9% increase in whole blood viscosity explained by a 9% increase in red cell rigidity, while neither plasma viscosity nor hematocrit changed.

Table 1

Characteristics and weight loss of 10 overweight women underwent a three day protein sparing, very low calorie diet

Patient	Age (years)	Weight (kg)	BMI (kg/m^2)	Fat mass (%)	Weight loss after diet (kg)
1	43	72.0	26.4	41.20	1.3
2	43	74.3	27.3	61.35	3.1
3	57	67.1	29.6	56.93	0.8
4	59	68.4	25.1	53.04	1.6
5	59	62.5	23.2	26.80	1.3
6	27	75.8	26.9	37.43	1.9
7	55	83.5	29.2	40.34	2.6
8	57	65.4	24.0	29.64	1.9
9	42	66.6	24.2	28.24	1.2
10	59	55.0	24.1	26.35	1.9
mean ± SEM	50.1 ± 3.2	69 ± 2.4	26.0 ± 0.7	40.13 ± 3.90	1.8 ± 0.2

Table 2

Changes in metabolic and hemorheologic parameters in ten overweight women submitted to a three day protein sparing, very low calorie diet

Mean ± SEM	Before diet	After diet
glycemia (mmol/l)	4.66 ± 0.21	3.97 ± 0.20*
bicarbonates (mmol/ml)	24.30 ± 0.60	23.2 ± 0.54 NS
blood ketone bodies:		
acetoacetate (μmol/ml)	180.90 ± 20.50	656.77 ± 66.41**
β OH butyrate (μmol/ml)	69.40 ± 11.38	566.67 ± 89.03**
urea (g/24 h)	26.48 ± 2.03	26.18 ± 1.56 NS
urinary C Peptide (μg/24 h)	54.54 ± 5.70	27.80 ± 4.26**
glucagon (pg/ml)	192.38 ± 41.79	154.125 ± 31.16**
insulin (μU/ml)	12.22 ± 3.00	10.11 ± 1.61 NS
blood viscosity (mPa s)	3.05 ± 0.10	3.32 ± 0.14*
plasma viscosity (mPa s)	1.38 ± 0.02	1.34 ± 0.02 NS
hematocrit (%)	38.56 ± 1.00	40.00 ± 0.80 NS
Tk	0.70 ± 0.01	0.76 ± 0.02*

*$p < 0.05$; **$p < 0.01$.

Long term follow-up studies have demonstrated that weight loss improves blood fluidity, beside other risk factors, in most obese patients submitted to a slimming procedure [23]. Such an improvement is potentially important when one keeps in mind the well known cardiovascular risk associated with obesity [1–3] as well as the involvement of blood viscosity factors in most situations of cardiovascular risk [24–30].

It is clear on the other hand that some slimming procedures are at risk and that their benefit/risk ratio should be evaluated [7]. This is likely to be true for protein-sparing diet procedures with very reduced carbohydrate quantities [8]. Some years ago, long-term diet with such programs was shown to be extremely dangerous, with a significant percentage of fatalities. In fact, current diets based on this concept employ protein preparations with a high biological value, and for a restricted period of time, so that they can be considered as reasonably safe [8]. However, the effect of these diets on risk

factors remains important to investigate, if one considers that they induce marked modifications of fuel metabolism which keep it very far from a physiological state [8,9].

One potential deleterious effect of these diets could be to induce a shift in the acid-base balance [9,10], a situation that is known to experimentally decrease red cell deformability [11]. The diet studied here does not appear to induce a metabolic acidosis, since it fails to significantly modify bicarbonate levels. However, it clearly impairs blood fluidity.

The mechanism for this rheological change is a reduction of red cell deformability, as evidenced by a rise in 'Tk'. Whether ketone bodies could influence red cell deformability has not been, as far as we know, investigated. However, there is a report of a reduction of erythrocyte Na/K ATPase after a long period of VLCD [31]. The function of this enzyme seems to be proportional to the caloric level, with an increased activity after overfeeding and a decreased activity after calorie reduction [31]. On the other hand, erythrocyte Na/K ATPase function has been reported to be regulated by insulin in the surrounding medium [32]. While the reduction of baseline plasma insulin is not significant in our study, the decrease in urinary C Peptide demonstrates a sharp decrease in insulin secretion after diet, as shown in Table 2. It should be reminded that insulin is a well-demonstrated physiological regulator of red cell deformability [33,34] which acts on the red cell membrane microviscosity [35]. Although *in vitro* experiments with supraphysiological insulin concentrations suggested that this hormone may reduce red cell deformability [34] the bulk of evidence supports the concept that insulin at physiological levels improves deformability [11,33,34]. Therefore, diet-induced hypoinsulinemia may in some cases be expected to decrease red cell deformability. However, there is no correlation in our data to support this assumption. On the other hand, glucagon, which is involved in the metabolic adaptation to to a diet including a high percentage of proteins [36], has been reported to impair red cell deformability [37]. Theoretically, high glucagon levels may play a role in this reduction of red cell deformability. However, as shown in Table 2, there is no increase in fasting glucagon values, but rather a moderate decrease. This finding, which contrasts with experimental animal data [36], may be due to the fact that the diet used here is not purely proteic but includes also a small amount of carbohydrates, so that the "ketogenetic" hormonal response is less pronounced. Another potential explanation can be found in a recent paper by Beguin and coworkers [38]. These authors show that a 2-week very-low-energy all-protein diet (70 g protein, total daily energy intake being progressively reduced to 1.26 MJ) results in a 50% decrease in serum iron and in a 30% decrease in transferrin saturation. Thus, there appears to be a certain degree of iron deficiency. In the conditions of that work, changes in iron metabolism did not translate into changes in erythropoiesis or red cell indexes, but the white blood cell count decreased by 16%. The authors concluded that such a 2-wk very-low-energy all-protein diet induces a functional tissue iron deficiency, that is likely to result from alterations in iron storage and release behavior of the reticuloendothelial cell due to energy deprivation. The authors assume that this deficiency is too short in duration to produce alterations in red blood cell indexes. However, iron deficiency has been shown to result in alterations in blood rheology [39] that may account for the explanation of short-term rheologic changes after very-low-energy all-protein diets. Presumably, the short duration of our protocol rules out such an explanation, but this point would require some investigation. On the whole, we think that the pathophysiological mechanism of the increased red cell rigidity we observe after this protein-sparing diet remains to be explained by further studies.

It should be pointed out that recent literature mostly emphasizes the beneficial effects of VLCDs on cardiovascular risk factors, rather than a putative negative effect. For instance, Pekkarinen et al. [40] investigated 24 h ambulatory blood pressure, lipids, glucose and insulin during a prospective study of a 17-week weight loss programme containing an eight-week VLCD period in twenty-nine obese women.

After one-year follow-up they evidenced a decrease in blood pressure as well as beneficial changes in serum glucose, triglyceride and HDL cholesterol. Despite some regain in weight during follow-up, the beneficial effects were overall maintained over the year. Another recent study by Markman and coworkers [41] investigated the effects of major weight loss (average 13.6 kg) in obese subjects. Results show after 24 weeks of weight maintenance, irrespective of the tested protocol (VLCD, low fat ad libitum, or more conventional), a lowering of plasma total cholesterol, triglycerides and factor VIIc, while high density lipoprotein cholesterol is increased. Thus, currently available information supports the assumption that the benefits of VLCD largely overcomes its hazards. Moreover, new procedures, e.g., intermittent protocols of VLCD treatment havee been proposed, resulting in less side effects that would favour a better tolerance of treatment [42].

In this picture, blood rheology, which appears more and more as a determinant of microvascular endothelial disfunction in obese subjects [43], has been studied by several investigators during slimming regimens. It is clear that a 20 kg loss in very obese people improves the initially very high plasma and blood viscosity [44]. If this weight reduction is obtain with a VLCD, this improvement is still observed, as shown by Poggi [45] who described the effects of a 3 months duration VLCD (about 500 kcal/day) on blood rheology. He observed an decrease in red cell aggregation and plasma viscosity, while fibrinogen remained surprisingly unchanged. Actually, although a recent study of Pekkarinen [40] shows a decrease in both fibrinogen (−6%) and PAI-1 antigen (−34%) after major weight loss, there seems to be a general agreement that slimming exerts only a little or marginal effect on plasma fibrinogen levels [45, 46]. Given the effect of weight reduction on red cell aggregability [45], this findings rises the question of the determinants of red cell aggregability others than fibrinogen, that may explain strong correlations between aggregability and fat mass that have been previously observed even within a physiological range [47,48].

Notwithstanding, our current study focuses on a quite short-term adaptation period to a protein-sparing, VLCD, regardless of the long term benefits discussed above. Thus, we are unable to provide any information on what would occur after a longer duration of time. Moreover, the clinical characteristics of the subjects, with the overweight being very slight in several cases, suggest that this study is more representative of what occurs in control subjects submitted to a ketogenetic diet than in markedly obese people. Presumably, very obese subjects could exhibit a different metabolic response which could be associated to a different hemorheologic pattern. In fact, the situation investigated in this study is probably relevant to the frequent use of drastic diets in people who are not markedly overweight, but who want to slim for cosmetic reasons.

On the whole, the hemorheological change that is observed here is moderate and does not seem likely to reflect a risk for otherwise healthy people submitted to such diets. Given the well-known risks of obesity [1–3], treatment, although it is generally difficult and frequently deceiving [42,49], is obviously required. In this perspective, the clinical relevance of moderate changes of red cell rheology at the beginning of a vigorous slimming procedure remains to be further evaluated. It is clear that similar diets, when used by the patient alone with inadequate protein sources, or for a long time period, could result in some biological disturbances that are potentially harmful [49]. To our knowledge, whether rheological alterations are part of this mechanism is still unknown.

In conclusion, these data lead us to think that a ketogenetic diet, even without any measurable acidosis, induces a moderate decrease in RBC deformability which is responsible for a moderate but significant increase in whole blood viscosity. The mechanism as well as the relevance of this process remain to be clarified.

Acknowledgements

We are grateful to Sylvie Palous from Servier International and to Frank Bonlieu from BIO_2 Laboratories for their help with the work presented in this paper.

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