Title

The effects of different high-protein-low-carbohydrates proprietary foods on blood sugar in healthy subjects

Running Title

The effects of proprietary foods in healthy subjects

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Abstract

The aim of our work was to analyze the effects on blood sugar through the calculation of the glycemic score (GS) of 10 different high-protein-low-carbohydrates proprietary foods which are commonly used as meals during very low carbohydrate ketogenic diet or during low carbohydrate diet. 14 healthy female tested the glycemic response curve elicited by 1000 Kj of glucose three times within 3 weeks period (1 test each week) compared to each of the 10 test-foods once on separate days twice a week. After determining the GS of each food in each individual, the mean GS of each test food was calculated. All test foods, compared to glucose, produced a significantly lower glycemic response . The GS of all test foods resulted lower than 25 and the difference between the mean glycemia after the intake of glucose (mean 122 ±15 mg/dl) and after the intake of the sweet test foods (mean 89 ± 7 mg/dl) was of 33 mg/dl ($P<0.001$), whereas that after the intake of glucose and the savory test foods (mean 91 ± 8 mg/dl) was of 31mg/dl ($P<0.001$). We can conclude that the reformulation of ultra-processed ready-to-consume foods in a low carbohydrate, high protein version can produce a significant low glycemic response while maintaining the valued ready-to-use format and high palatability demanded by consumers. The low impact on postprandial glycemia and the nutritional characteristics of these proprietary foods make them useful both in weight control and in diabetes care dietary management.

Key Words

Glycemic index; diets; diabetes

INTRODUCTION

Despite being considered a “preventible disease” by the World Health Organization, obesity is rising in both, high income and low income countries.[1] Moreover overweightness and obesity are one of the majour causes of the woldwide burden of the four main non-communicable diseases (NCDs): cardiovascular (CV) diseases, chronic respiratory diseases, cancers and diabetes melitus (DM). [2]

Amongst multiple reasons involved in the onset of overweight and obesity, nutrition appears to be the most important one.[3] However, research has yet to produce a generally accepted nutritional approach.[4] Populations experiencing an increase in obesity and CV diseases show common eating and drinking habits, notably a general decrease in minimally processed foods intake and in an increase in the consumption of ultra-processed ready-to-consume products. These foods, based on the accepted definition acknowledged by the Pan American Health Organization[5], are “*industrial formulations manufactured from substances derived from foods or synthesized from other organic sources. (…) Most of these products contain little or no whole food. They are ready-to-consume or ready-to heat, and thus require little or no culinary preparation*”. Examples of ultra-processed foods are savory and sweet snacks, ice-cream, frozen and chilled ready meals and soft drinks[5] and they seem to be the cause of the extra daily diet calory intake of both, the young and the older populations.[6] Ultra-processed ready-to-consume products present particular characteristics, which make them extremely profitable for producers and retailers and highly attractive for consumers. For example, consumers purchase them because they commonly require a minimal culinary action, they are flavoursome and relatively inexpensive. However, when analyzing ultra-processed products less protein, potassium and dietary fiber and more free sugar, total saturated and trans-unsaturated fats and sodium are generally evident when compared to traditional foods.[7,8]

All these characteristics appear to be linked to the burden of obesity and metabolic syndrome (MetS).[6] A potential solution to this scenario could be to review ultra-processed, ready-to-consume products by reduction of their sugar and fat contents. A particular kind of these new ultra-processed ready-to-consume products are proprietary foods which are high in proteins and fibers and low in sugar and saturated fats. These are specifically designed for particular diets such as the ketogenic regimen, but are successfully used also in more easy low carbohydrate diet as snacks or meal replacements.[9] During ketosis, carbohydrate (CHO) intake must be under 30g/day[10,11] and in previous studies [4,9], we demonstrated that these special foods, which mimic the taste and aspect of high content CHO foods but are low in sugar and high in protein content were able to increase the compliance of subjects to the ketogenic diet. Moreover, after the termination of a very low CHO ketogenic diet (VLCKD) intervention, patients tended to maintain the consumption of those proprietary foods during the day (usually at breakfast or during breaks). This can be considered a postive change of behaviour, since it is known that meal replacement during the maintenance phase is useful to prevent weight regain.[12] During a VLCKD it is mandatory to maintain a low level of glycemia (about 80-90 mg/dL) to avoid insulin spikes[13]. This condition allows subjects to improve their fat oxidation as demonstrated by Paoli et al.[14] and by Tagliabue et al.[15]

Another important aspect of a VLCKD is the influence of such dietary regime on the perception of hunger.[16] It has been suggested that ketone bodies reduce hunger through different and complex mechanisms [17]; on the other side it is known that post prandial glucose and insuline spikes, tipically produced after the intake of traditional ultra-processed products which usually show a high glycemic index (GI)[5] elicit food craving and overeating, with a preference for high-GI carbohydrates[18], a phenomenon defined as the CHO-craving effect.[19]. Conversely, the consumption of non-processed foods low in simple sugars may ameliorate overeating and facilitate the maintenance of a healthy weight.[18]

The above positive changes necassitates the need to analyze the effect of different high-protein-low-CHO proprietary foods which are commonly used during VLCKD or low CHO diet (LCD) on glycemia compared to glucose.

MATERIAL AND METHODS

Subjects were recruited via advertisement placed in two pharmacies located in the province of Vicenza (Veneto, Italy). Exclusion criteria for this study were the presence of diabetes or pre-diabetes, being on a food diet and females who where either pregnant or breast-feeding. After a pre-selectin process of 32 participats, 14 females were eligible to participate in the present study (mean age: 42±13, mean weight: 72±21kg, mean BMI: 26±7). Participants were required to report any change of daily habits, such as engaging in a new exercise program, new pharmaceutical interventions or engaging in other than the present diets during the experimental phase which would have resulted in a study exclusion. The study was approved by the Ethical Board of the University of Padova, Department of Biomedical Sciences and conformed to standards for the use of human subjects in research as outlined in the Declaration of Helsinki. Investigators explained the purpose of the study, the protocol to be followed and the experimental procedures to be used prior to the start of the study. Subjects were required to sign a participation consent form and did not receive any monetary compensation.

Subjects were tested for indivdual glycemic response curves elicited by the ingestion of 1000 Kj of glucose three times within a 3 week period (1 test per week) and that of each of 10 high-protein-low-CHO proprietary test foods once on separate days twice a week. Tests were performed in the morning after a 10-12 hours overnight-fast. Subjects were asked to have a regular meal, not to consume any alcohol and to avoid any unaccustomed exercise the night before tests. During the study period, participants maintained a constant foods supply, without changing their usual eating habits.

*** FIGURE 1 HERE***

Fingertip capillary blood samples were collected in the fasted state and after 15, 30, 45, 60, 90, and 120 min after starting to eat, changing the finger each time to avoid traumatization of the skin. The puncture was performed with the lancet Accu-Check Safe-T-Pro Plus (Roche Diagnostics, Basel, Switzerland) and blood was collected directly and immediately analysed using test strip *Reflotron® Glucose.*[20-22] Postprandial effect of sugar content on glycemia is commonly defined through three methods: the glycemic index (GI), the glycemic load (GL) and the glycemic score (GS).

The GI method was developed to rank foods according to the extent to which they increase blood sugar concentrations [23] and it is a number which ranges from 0 to 100, where 100 represents the GI of the reference food glucose. To calculate the GI of a particular food, the area under the curve (AUC) of the rise in blood sugar for a 2 hour postprandial period is calculated. This value is consequently expressed as a percentage of the incremental AUC following the consumption of a reference food (commonly white bread or glucose) consumed by the same person on a different day. [24]

The test food and the reference food must contain the same amount of available CHO (25g or 50g) and the individual has to perform the test under standardized conditions.

$$GI = \frac{\text{120 min iAUC(blood sugar) for portion size of test foods containing 50g (or 25g) of available carbohydrate}}{\text{120 min iAUC (blood sugar) for portion size of reference food containing 50g (or 25g) of available carbohydrate}} \times 100$$

$$\frac{\text{120 min iAUC(blood sugar) for portion size of test foods containing 50g (or 25g) of available carbohydrate}}{\text{120 min iAUC (blood sugar) for portion size of reference food containing 50g (or 25g) of available carbohydrate}}$$

The GL method takes into account not only the magnitude of the glucose blood spike, but also the content (grams) of CHO in the portion of food consumed and it is calculated as the mathematical product of the GI for the available CHO content of the food. [25]

$$GL = \frac{\text{GI (test food) X available grams of CHO in the quantity of test food consumed}}{100}$$

$\frac{\text{GI (test food) X available grams of CHO in the quantity of test food consumed}}{100}$ The GS method tests the glycemic response after the ingestion of low CHO foods and differs from the GI as it does not compare a standard amount of available CHO.

However it compares the effect on glycemia of the same amount of a 1000 KJ portion of the test food to the reference food. [25]

$$GS= \frac{\text{120 min iAUC(blood sugar) for 1000 kJ test food}}{\text{120 min i AUC(blood sugar)for 1000 KJ reference glucose}} \text{ x } 100$$

$\frac{\text{120 min iAUC(blood sugar) for 1000 kJ test food}}{\text{120 min i AUC(blood sugar)for 1000 KJ reference glucose}}$Due to the very low CHO content of the tested foods and due to the quantity of food required to reach the 25g of available CHO for the calculation of GI being too large, the present study utilised the GS method. [25]

Each tested food was served as a 1000 KJ portion with 220 ml warm (no sugar) tea for a better compliance of subjects in cold winter mornings after an overnight fast (tea doesn't alter the incremental area under the glycaemic response curve [26]) and consumed within a period of 10 minute. The present study tested 10 proprietary foods selected from the product range of Tisanoreica® snacks and meals (Gianluca Mech S.p.A., Asigliano Veneto, Vicenza, Italy). These are ready-to-consume foods high in protein and fiber content and low in CHO content designed to be consumed during a VLCKD or a LCD regimen.[4, 9, 27].

Among the products selected, six of them were sweet [chocolate biscuits CB (Cioco-Mech); chocolate and hazelnut balls CHB (Bon Mech); apple-cinnamon biscuits ACB (T-Biscuit); chocolate-almonds-pistachio bar CAPB (T-Smart); nuts and chocolate muffin NCM (T-Muffin); chocolate drink CD (Cocoa Drink)]. The other four products tested were savory [two different types of pasta P1 (Original Tisanopast) and P2 (Tisanopast Style), the rosemary breadsticks RB (T-Smech) and the pizza dough PD (Pizza Dough)] (Table 1). Glucose was used as reference food. This was dissolved in 220 ml of water and served as 1000 kj portions (15.68 kJ/g)[28] and had to be consumed within a 10 minute period.

*** table 1 here ***

All statistical analyses were performed using package GraphPad Prism version 6.00 for Mac, GraphPad Software, San Diego, CA, USA.The AUC values above the fasting glucose concentration for each test food and for the reference food were used to calculate the GS of each test food and assessed using a XY data table by selecting the AUC analysis. The effect of each test food on glycemia compared to that of the reference food over time was assessed using a mixed model ANOVA (time *x treatment*). A post hoc Sidak's multiple comparison test was performed.

In order to select those test foods with a significant difference of blood sugar values compared to the other test foods, a two-way repeated measure ANOVA (time *vs* nominal variables test foods *vs* measures) was performed. Each raw represented a different time point, so matched values were stucked into a subcolum. Tukey's multiple comparison test was chosen in order to compare columns within each raw.

A bivariate analysis was used to test, through a linear regression analysis, the significance of the associations between GS and sugars, protein and fiber in the 10 foods tested. An alpha level of $p < 0.05$ was used to denote a significant effect.

RESULTS

Mean GS, mean glycemia and mean glycemia in the different time points of the ten test foods and the reference food among the subjects tested are listed in Table 2.

Mean glycemia after taking the reference food glucose resulted being 122 ±15 mg/dl, that after taking the sweet test foods was 89 ± 7 mg/dl and that after ingestion of the savory test foods was 91 ± 8 mg/dl.

Figure 2 shows the mean blood sugar trend comparison at the different time points between glucose and sweet test foods, whereas Figure 3 shows that between glucose and savory test foods. After the ingestion of all sweet and savory test foods, the blood sugar showed

always a significantly lower trend compared to that after the intake of the reference food glucose after 15, 30, 45, 60 and 90 minutes, though several test foods (CHB, CAPB, NCM, ACB, PD and RB) were able to maintain this significance even after 120 minutes.

***Figure 2 here**

Figure 3 here

Figure 4 shows the blood sugar trend comparison between sweet test foods and savory test foods. The mean blood sugar trends comparison at the seven different time points highlighted a significant higher increase of glycemia particularly 15 and 30 minutes after taking the chocolate and hazelnut balls CHB and the two kind of pasta P1 and P2 compared to the other test foods (Figure 4).

Figure 4 here

In particular, the mean glycemia increased significantly 15 min and 30 min after the intake of CHB compared to the mean glycemia after the ingestion of the chocolate biscuits CB, the chocolate-almonds-pistachio bar CAPB, the rosemary breadsticks RB and the pizza dough PD (Figure 5).

Figure 5 here

After the intake of P2 the mean glycemia increased significantly after 15 min and 30 min. compared to the sweet test foods CB, CAPB and NCM and to the savory test foods PD and SB (Figure 6).

Figure 6 here

After the intake of P1 the mean glycemia increased significantly after 15 min and 30 min. compared to the sweet test foods CB and CAPB and to the savory test foods PD and SB (Figure 7).

Figure 7 here

The statistical two way anova analysis of the trend of blood sugar from before starting to eat up to two hours after the intake of the reference food or of the test foods shows that, on

average, the 40% of the total variation observed is due to the difference between the foods eaten (glucose or test foods). This result shows that, among all the "Sources of Variation" analized (Time, Food, Subjects), the variable “Food” appears to be the one that explains most of the variation observed between the blood sugar trends after the intake of test foods and the blood sugar trend after the intake of the reference food.

The results didn’t show any correlation between GS and fiber content (r=-0.08; P=0.37), neither between GS and sugar content (r= 0.17; P=0.09), nor between GS and protein content (Figure 8).

Figure 8 here

The average GS of each test food, calculated as the mean of GS values of each test food resulted from every subjects, was always less than 25 compared to the GS reference value of glucose which is 100 (Table 2). The test food with the highest GS is the sweet test food Bon Mech with a GS of 23. The test food with the lowest GS are the sweet test food Cioco Mech and T-Smart, with a GS of 14.

*** Table 2 here ***

DISCUSSION

In this study the GS of 10 proprietary foods high in proteins and fibers and low in sugars and saturated fats were tested. These proprietary foods claim to replicate the taste and aspect of high CHO foods and are commonly used as meals during VLCKD regimes. In our study the products tested showed a significant lower blood sugar response and lower GS compared to an isoenergetic amount of glucose. Amongst the six sweet and four savory test foods, the sweet little balls with chocolate and hazelnut CHB showed the highest GS (GS= 23). This result is consistent with the higher quantity of available sugars of CHB compared to the other test foods. The chocolate biscuits CB and the bar with chocolate, almonds and pistachio CAPB had the lowest GS of 14. This GS value is, according to the data avalable [25], similar to the GS of a low fat processed cheese.

The macronutrient composition is important for glucose response, with carbohydrate as the food component which acts directly on glycemia, rising it and stimulating insulin secretion. However, even if carbohydrate counting is still the basis for insulin dose adjustment in diabetes care management,[29] data show [25] that sugar content could be a stronger predictor of the observed glucose response compared to carbohydrate. Other studies show that the structure of carbohydrates should also be keep in consideration: a disrupted structure, typical of processed whole grains, has a different effect on glycemia compared to intact grains. [30].

Considering fibers, even if there is a strong evidence supporting their beneficial effect in reducing disease risk [25], only soluble fibers with gel-forming properties show a distinguishable effect for glycemic control.[31, 32]

The present study, according to these data, doesn't show any significant relation between GS and fiber content, but, differently from them, it doesn't show any correlations between GS and sugar content (Figure 8). This conflicting result might be due to the very low quantity of available sugars in the test foods. Finally, protein content, despite being considered predictive for the GS[25], did not show such correlation in this study.

The low postprandial glycemia produced by the proprietary foods tested is an important factor, since ultra-processed ready to consume products are commonly high in simple sugars that negatively affect a number of health parameters. Postprandial hyperglycemia and compensatory hypoglycemia are factors linked to the development of diabetes and cardiovascular diseases [33]. Furthermore, the consumption of high-sugar snacks seems to be the main cause for the increase in intrahepatic triglyceride content (IHTG)[34]. Finally, the usual rapid and high glycemic peak caused by ultra-processed products, together with their lack in fiber, proteins and water, triggers an excessive consumption[19, 35].

Sugar is rapidly absorbed and produces a consequent high blood sugar spike which acts centrally, increasing the production and utilization of dopamine, imitating the typical

neuromodulation of addictive substances.[36]

The abuse of high sugary ultra-processed foods leads to the synthesis and the accumulation of fat and weight gain [8], which increase the risk for obesity and MetS. The MetS is, according to the World Health Organization definition, the symultaneous presence of insulin resistance along with two other risk factors from high blood pressure, raised triglycerides, low HDL and increased BMI (or increased waist:hip ratio) and microalbuminuria.[37] The prevalence of this desease, once suggested to be exclusive of adulthood, is becoming a major worldwide concern among both children and adolescents [38, 39] and the consumption of ultra-processed foods is considered an important risk factor for its development.[40] Bielemann et al.[41] recently demonstrated that ultra-processed foods were responsible for the 50% of the daily caloric intake among a cohort of 23 year old participants in Brazil. Interestingly, the household availability of ultra-processed ready-to-eat foods was associated with a low percentage of proteins and fibers intake.

Appetite control isn't related only to glucose content and post prandial glycemia, but also to othere factors[42] among which the reward system in the brain, aside from the homeostatic control by the hypothalamus, has been the focus of recent interest, since food rewards is a goal that drives both appetite and eating.[43, 44]

Stated that the larger the portion size the more food is eaten [44] and that eating is only indirectly related to energy balancing since it seems that we eat essentially for pleasure,[44] these low calorie proprietary foods could help to reduce energy intake, useful for a better weight maintenance or a more successfully weight loss. Moreover, since high energy dense foods have the lowest satiating capacity even if they usually have a high palatability,[44] the high level of proteins and fibers and the high palatability, despite the low sugar content, of these new ultra-processed foods, are important features which contribute to both food reward and satiety.[45]

The tendency to prefer sugary fatty foods over savory foods is considered innate and universal and finds its roots in very important adaptive processes: a bitter taste is considered predictive of toxicity and then avoided (alkaloids, glycosides and other toxins have a bitter taste), whilst the sweet taste is associated with energy and nourishment [46]. Although it is recognized that the VLCKDs lead to greater weight losses compared to a low-calorie balanced diet at least in the short term,[47] subjects with a sweet food preference may not adhere to this diet because of the lack of their prefered taste.[48, 49] A VLCKD that includes these proprietary foods which imitate taste and aspects of high CHO food but have a low glucose content can consequently produce a higher level of adherence and a reduced drop-out rate.[4, 50]

Sweet foods are usually rich in refined CHO, have a high GI and are related with an increased risk for overweight, obesity[51] and Type 2 DM.[52] Type 2 DM (T2DM) is increasing among young people [53] and a dietary management is the most important factor to be considered to prevent the progression of impaired glucose tolerance to clinical DM. The dietary management is also important to minimize the glycemic variability, which is the measure of the of blood sugar concentration changes over time.[54] An uncontrolled blood sugar concentration is the major risk factor in the development of T2DM complications such as retinopaty, neuropathy, nephropathy and cardiovascular diseases.[55-58] It is important to make healthier nutritional choices to prevent these complications, which are associated with high economic, social and personal costs. Low-CHO high-protein diets help to normalize glycemic fluctuations in T2DM management.[59] As suggested by the European Association for the Study of Diabetes (EASD) dietary fibers can further positively influence blood sugar variability. The EASD consequently recommends the the consumption of high-fiber, low-GI foods as CHO source.[54]

Dietary amino acids contribute to the de novo synthesis of glucose through gluconeogenesis and participate in the re-cycling of glucose carbon via the glucose-alanine cycle.[60] However,

dietary proteins have a minimal impact on glycemia and insulin secretion compared to CHOs [61] and a high-quality protein supplementation has been suggested during a weight loss program to preserve muscle mass, to improve glycemic regulation and to maintenaneuglycemia.[62, 63] The ten proprietary food tested in the present study are formulated with whey proteins. These, due to their high content of leucine which promotes muscle mass synthesis and their fast digestion and delivery of aminoacids in the circulation, are consequently considered the best type of proteins. [62]

Moreover, whey protein decrease appetite better than other types of proteins [64] and increase satiety through an increase of the release of of CCK and GLP-1 and a reduction of ghrelin levels.[65]

The sweet proprietary foods tested in the present study also contained low-calorie sweeteners. These are compounds able to stimulate, in the same way as sugar does, the sweet taste receptors. [66]

Unlike sugar, low-calorie sweeteners do not release energy and for this reason they are used in weight loss programs, even though perceived as controversial by the scientific community with respect to their possible adverse metabolic effects, such as increase of appetite, weight gain and metabolic disorders.[67-69] However, more studies are required to confirm these negative suggestions, since a recent review shows that there is no evidence for a limitation of their use to reduce energy intake.[70] The same author states that our congenitally liking for sweetness implies that the reward value from sugar and low-calorie sweeteners is the same, but low calorie sweeteners should be preferred, since they avoid the high-calorie-intake side effect of sugar.[44] These compounds could be useful in the prevention of overweightness and obesity in populations which are less sensitive to sweetness, predisposing them to consume more sugar in order to have the same ‘taste sensation’ as people more sensitive to sweetness.[71] Nowadays low-calorie sweeteners are important tools in DM management, where the dietary adherence is amongst the most

difficult cornerstones[72], especially for children and adolescents with T2DM who suffer from the perceived lack of normality in their diet and consequenlty desire non-recommended sweet foods.[73]

Ready-to-consume proprietary foods, high in good quality proteins and fibers, could improve both, the diet of young people, and the diet of T2DM patients. In the former population this could prevent them from eating high sugary fatty foods, predisposing them to the development of T2DM and in the latter to minimize blood sugar variability that often complicates the pathology. The ten proprietary foods tested showed a significant lower glycemia compared to the standard food glucose and their GS resulted always lower than 25. This low glycemic response, together with their valuable ready-to-use format, make these proprietary foods a valid tool both during weight management and weight loss programs, facilitating the adherence to a low CHO diet of people who tend to have a high preference for sweet foods.

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