

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 “preventable 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 major causes of the worldwide 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 Organization⁵, 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 drinks⁵ 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 positive 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 spikes¹³. 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 mechanisms¹⁷; on the other side it is known that post prandial glucose and insuline spikes, typically 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 carbohydrates¹⁸, 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 necessitates 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 were either pregnant or breast-feeding. After a pre-selection process of 32 participants, 14 females were eligible to participate in the present study (mean age: 42 ± 13 , mean weight: 72 ± 21 kg, 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 individual 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 food 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.

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

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

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

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

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

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

$$\frac{120 \text{ min iAUC(blood sugar) for 1000 kJ test food}}{120 \text{ min iAUC(blood sugar) for 1000 KJ reference glucose}}$$
 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 \times 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 row represented a different time point, so matched values were stucked into a subcolumn. Tukey's multiple comparison test was chosen in order to compare columns within each row.

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" analyzed (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 available²⁵, 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 show²⁵ 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 kept 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 risk²⁵, 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 GS²⁵, 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 diseases³³. 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 gain⁸, which increase the risk for obesity and MetS. The MetS is, according to the World Health Organization definition, the simultaneous 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 disease, 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 other factors⁴² 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 eaten⁴⁴ 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 preferred 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, obesity⁵¹ 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 retinopathy, 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 CHOs⁶¹ and a high-quality protein supplementation has been suggested during a weight loss program to preserve muscle mass, to improve glycaemic regulation and to maintain euglycemia.^{62, 63} The ten proprietary foods 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 amino acids in the circulation, are consequently considered the best type of proteins.⁶²

Moreover, whey protein decreases appetite better than other types of proteins⁶⁴ and increases satiety through an increase in the release 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 congenital 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 dietary adherence is amongst the most

difficult cornerstones⁷², especially for children and adolescents with T2DM who suffer from the perceived lack of normality in their diet and consequently 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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References

1. Seidell JC, Halberstadt J: The global burden of obesity and the challenges of prevention. *Ann. Nutr. Metab.* 2015;66 Suppl 2:7-12.
2. Kontis V, Mathers CD, Rehm J, et al.: Contribution of six risk factors to achieving the 25x25 non-communicable disease mortality reduction target: a modelling study. *Lancet.* 2014;384(9941):427-437.
3. Mutch DM, Clement K: Unraveling the genetics of human obesity. *PLoS Genet.* 2006;2(12):e188.
4. Paoli A, Bianco A, Grimaldi KA, Lodi A, Bosco G: Long term successful weight loss with a combination biphasic ketogenic mediterranean diet and mediterranean diet maintenance protocol. *Nutrients.* 2013;5(12):5205-5217.
5. Pan American Health Organization WHO: Consumption of ultra-processed food and drink products in Latin America: Trends, impact on obesity, and policy implications: PAHO Washington, DC; 2014.
6. Monteiro CA, Moubarac JC, Cannon G, Ng SW, Popkin B: Ultra-processed products are becoming dominant in the global food system. *Obes. Rev.* 2013;14 Suppl 2:21-28.
7. Monteiro CA, Levy RB, Claro RM, de Castro IR, Cannon G: Increasing consumption of ultra-processed foods and likely impact on human health: evidence from Brazil. *Public Health Nutr.* 2011;14(1):5-13.
8. Moubarac JC, Batal M, Martins AP, et al.: Processed and ultra-processed food products: consumption trends in Canada from 1938 to 2011. *Can. J. Diet. Pract. Res.* 2014;75(1):15-21.
9. Paoli A, Cenci L, Grimaldi KA: Effect of Ketogenic Mediterranean diet with phytoextracts and low carbohydrates/high-protein meals on weight, cardiovascular risk factors, body composition and diet compliance in Italian council employees. *Nutr. J.* 2011;10(1):112.
10. Paoli A: Ketogenic Diet for Obesity: Friend or Foe? *Int J Env Res Pub He.* 2014;11(2):2092-2107.
11. Paoli A, Rubini A, Volek JS, Grimaldi KA: Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *European journal of clinical nutrition.* 2013;67(8):789-796.
12. Vazquez C, Montagna C, Alcaraz F, et al.: Meal replacement with a low-calorie diet formula in weight loss maintenance after weight loss induction with diet alone. *Eur. J. Clin. Nutr.* 2009;63(10):1226-1232.
13. Paoli A, Canato M, Toniolo L, et al.: The ketogenic diet: an underappreciated therapeutic option? *La Clinica terapeutica.* 2011;162(5):e145-e153.
14. Paoli A, Grimaldi K, Bianco A, Lodi A, Cenci L, Parmagnani A: Medium term effects of a ketogenic diet and a Mediterranean diet on resting energy expenditure and respiratory ratio. *BMC Proc.* 2012;6:P37.
15. Tagliabue A, Bertoli S, Trentani C, Borrelli P, Veggiotti P: Effects of the ketogenic diet on nutritional status, resting energy expenditure, and substrate oxidation in patients with medically refractory epilepsy: a 6-month prospective observational study. *Clinical nutrition.* 2012;31(2):246-249.
16. Gibson AA, Seimon RV, Lee CM, et al.: Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obes. Rev.* 2015;16(1):64-76.
17. Paoli A, Bosco G, Camporesi EM, Mangar D: Ketosis, ketogenic diet and food intake control: a complex relationship. *Front. Psychol.* 2015;6:27.
18. Lennerz BS, Alsop DC, Holsen LM, et al.: Effects of dietary glycemic index on brain regions related to reward and craving in men. *Am. J. Clin. Nutr.* 2013;98(3):641-647.

19. Ventura T, Santander J, Torres R, Contreras AM: Neurobiologic basis of craving for carbohydrates. *Nutrition*. 2014;30(3):252-256.
20. Bijster P: A multi-centre evaluation of the measurement of high density lipoprotein cholesterol by the Reflotron assay. *European journal of clinical chemistry and clinical biochemistry : journal of the Forum of European Clinical Chemistry Societies*. 1993;31(3):173-178.
21. Richter V, Rassoul F, Luttge F, Thiery J: Cardiovascular risk factor profile on a population basis: Results from the Lipid Study Leipzig (LSL). *Exp. Clin. Cardiol*. 2007;12(1):51-53.
22. Warnick GR, Boerma GJ, Assmann G, et al.: Multicenter evaluation of Reflotron direct dry-chemistry assay of high-density lipoprotein cholesterol in venous and fingerstick specimens. *Clin. Chem*. 1993;39(2):271-277.
23. Holt SH, Miller JC, Petocz P: An insulin index of foods: the insulin demand generated by 1000-kJ portions of common foods. *Am. J. Clin. Nutr*. 1997;66(5):1264-1276.
24. Fao: *Carbohydrates in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation*1998.
25. Bao J, Atkinson F, Petocz P, Willett WC, Brand-Miller JC: Prediction of postprandial glycemia and insulinemia in lean, young, healthy adults: glycemic load compared with carbohydrate content alone. *Am. J. Clin. Nutr*. 2011;93(5):984-996.
26. Brouns F, Bjorck I, Frayn KN, et al.: Glycaemic index methodology. *Nutr. Res. Rev*. 2005;18(1):145-171.
27. Paoli A, Grimaldi K, D'Agostino D, et al.: Ketogenic diet does not affect strength performance in elite artistic gymnasts. *J. Int. Soc. Sports Nutr*. 2012;9(1):34.
28. Plowman SA, Smith DL: *Exercise physiology for health fitness and performance*: Lippincott Williams & Wilkins; 2013.
29. Campbell MD, Walker M, King D, et al.: Carbohydrate Counting at Meal Time Followed by a Small Secondary Postprandial Bolus Injection at 3 Hours Prevents Late Hyperglycemia, Without Hypoglycemia, After a High-Carbohydrate, High-Fat Meal in Type 1 Diabetes. *Diabetes Care*. 2016.
30. Foster-Powell K, Holt SH, Brand-Miller JC: International table of glycemic index and glycemic load values: 2002. *Am. J. Clin. Nutr*. 2002;76(1):5-56.
31. Venn BJ, Mann JI: Cereal grains, legumes and diabetes. *Eur. J. Clin. Nutr*. 2004;58(11):1443-1461.
32. Gibb RD, McRorie JW, Jr., Russell DA, Hasselblad V, D'Alessio DA: Psyllium fiber improves glycemic control proportional to loss of glycemic control: a meta-analysis of data in euglycemic subjects, patients at risk of type 2 diabetes mellitus, and patients being treated for type 2 diabetes mellitus. *Am. J. Clin. Nutr*. 2015;102(6):1604-1614.
33. Zeevi D, Korem T, Zmora N, et al.: Personalized Nutrition by Prediction of Glycemic Responses. *Cell*. 2015;163(5):1079-1094.
34. Koopman KE, Caan MW, Nederveen AJ, et al.: Hypercaloric diets with increased meal frequency, but not meal size, increase intrahepatic triglycerides: a randomized controlled trial. *Hepatology*. 2014;60(2):545-553.
35. Blum K, Thanos PK, Gold MS: Dopamine and glucose, obesity, and reward deficiency syndrome. *Front. Psychol*. 2014;5:919.
36. Blum K, Braverman ER, Holder JM, et al.: Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. *J. Psychoactive Drugs*. 2000;32 Suppl:i-iv, 1-112.
37. Grundy SM, Brewer HB, Jr., Cleeman JI, et al.: Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Arterioscler. Thromb. Vasc. Biol*. 2004;24(2):e13-18.
38. Taylor AM, Peeters PH, Norat T, Vineis P, Romaguera D: An update on the prevalence of the metabolic syndrome in children and adolescents. *International journal of pediatric obesity :*

IJPO : an official journal of the International Association for the Study of Obesity. 2010;5(3):202-213.

39. Kursawe R, Santoro N: Metabolic syndrome in pediatrics. *Adv. Clin. Chem.* 2014;65:91-142.
40. Tavares LF, Fonseca SC, Garcia Rosa ML, Yokoo EM: Relationship between ultra-processed foods and metabolic syndrome in adolescents from a Brazilian Family Doctor Program. *Public Health Nutr.* 2012;15(1):82-87.
41. Bielemann RM, Motta JV, Minten GC, Horta BL, Gigante DP: Consumption of ultra-processed foods and their impact on the diet of young adults. *Rev. Saude Publica.* 2015;49:1-10.
42. Farr OM, Li CS, Mantzoros CS: Central nervous system regulation of eating: Insights from human brain imaging. *Metabolism.* 2016;65(5):699-713.
43. Rolls ET: Reward Systems in the Brain and Nutrition. *Annu. Rev. Nutr.* 2016;36:435-470.
44. Rogers PJ, Brunstrom JM: Appetite and energy balancing. *Physiol. Behav.* 2016.
45. Tovar AR, Caamano Mdel C, Garcia-Padilla S, Garcia OP, Duarte MA, Rosado JL: The inclusion of a partial meal replacement with or without inulin to a calorie restricted diet contributes to reach recommended intakes of micronutrients and decrease plasma triglycerides: a randomized clinical trial in obese Mexican women. *Nutr. J.* 2012;11:44.
46. Drewnowski A, Krahn DD, Demitrack MA, Nairn K, Gosnell BA: Taste responses and preferences for sweet high-fat foods: evidence for opioid involvement. *Physiol. Behav.* 1992;51(2):371-379.
47. Bueno NB, de Melo IS, de Oliveira SL, da Rocha Ataide T: Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. *Br. J. Nutr.* 2013;110(7):1178-1187.
48. Shai I, Schwarzfuchs D, Henkin Y, et al.: Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *The New England journal of medicine.* 2008;359(3):229-241.
49. McVay MA, Voils CI, Coffman CJ, et al.: Factors associated with choice of a low-fat or low-carbohydrate diet during a behavioral weight loss intervention. *Appetite.* 2014;83:117-124.
50. Paoli A, Cenci L, Fancelli M, et al.: Ketogenic diet and phytoextracts Comparison of the efficacy of Mediterranean, zone and tisanoreica diet on some health risk factors. *Agro Food Ind. Hi Tech.* 2010;21(4):24-29.
51. Schwingshackl L, Hoffmann G: Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. *Nutr. Metab. Cardiovasc. Dis.* 2013;23(8):699-706.
52. Maki KC, Phillips AK: Dietary substitutions for refined carbohydrate that show promise for reducing risk of type 2 diabetes in men and women. *J. Nutr.* 2015;145(1):159S-163S.
53. Reinehr T: Type 2 diabetes mellitus in children and adolescents. *World J. Diabetes.* 2013;4(6):270-281.
54. Tay J, Thompson CH, Brinkworth GD: Glycemic Variability: Assessing Glycemia Differently and the Implications for Dietary Management of Diabetes. *Annu. Rev. Nutr.* 2015.
55. Brownlee M, Hirsch IB: Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. *JAMA : the journal of the American Medical Association.* 2006;295(14):1707-1708.
56. Forbes JM, Cooper ME: Mechanisms of diabetic complications. *Physiol Rev.* 2013;93(1):137-188.
57. Bonora E: Postprandial peaks as a risk factor for cardiovascular disease: epidemiological perspectives. *Int J Clin Pract Suppl.* 2002(129):5-11.
58. Pradeepa R, Mohan V: Postprandial glycaemic excursions and cardiovascular risk. *Journal of the Indian Medical Association.* 2011;109(12):912, 917-920.
59. Feinman RD, Pogozelski WK, Astrup A, et al.: Dietary carbohydrate restriction as the first approach in diabetes management: critical review and evidence base. *Nutrition.* 2015;31(1):1-13.

60. Dashty M: A quick look at biochemistry: carbohydrate metabolism. *Clin Biochem.* 2013;46(15):1339-1352.
61. Layman DK, Baum JI: Dietary protein impact on glycemic control during weight loss. *J. Nutr.* 2004;134(4):968S-973S.
62. Verreijen AM, Verlaan S, Engberink MF, Swinkels S, de Vogel-van den Bosch J, Weijs PJ: A high whey protein-, leucine-, and vitamin D-enriched supplement preserves muscle mass during intentional weight loss in obese older adults: a double-blind randomized controlled trial. *Am. J. Clin. Nutr.* 2015;101(2):279-286.
63. Pasiakos SM: Metabolic advantages of higher protein diets and benefits of dairy foods on weight management, glycemic regulation, and bone. *J. Food Sci.* 2015;80 Suppl 1:A2-7.
64. Pal S, Ellis V: The acute effects of four protein meals on insulin, glucose, appetite and energy intake in lean men. *Br. J. Nutr.* 2010;104(8):1241-1248.
65. Luhovyy BL, Akhavan T, Anderson GH: Whey proteins in the regulation of food intake and satiety. *J. Am. Coll. Nutr.* 2007;26(6):704S-712S.
66. Brown RJ, Rother KI: Non-nutritive sweeteners and their role in the gastrointestinal tract. *J. Clin. Endocrinol. Metab.* 2012;97(8):2597-2605.
67. Yang Q: Gain weight by "going diet?" Artificial sweeteners and the neurobiology of sugar cravings: Neuroscience 2010. *Yale J. Biol. Med.* 2010;83(2):101-108.
68. Swithers SE, Martin AA, Davidson TL: High-intensity sweeteners and energy balance. *Physiol. Behav.* 2010;100(1):55-62.
69. Ludwig DS: Artificially sweetened beverages: cause for concern. *JAMA.* 2009;302(22):2477-2478.
70. Rogers PJ, Hogenkamp PS, de Graaf C, et al.: Does low-energy sweetener consumption affect energy intake and body weight? A systematic review, including meta-analyses, of the evidence from human and animal studies. *Int. J. Obes. (Lond.).* 2016;40(3):381-394.
71. Low YQ, Lacy K, Keast R: The role of sweet taste in satiation and satiety. *Nutrients.* 2014;6(9):3431-3450.
72. Anders S, Schroeter C: Diabetes, diet-health behavior, and obesity. *Front. Endocrinol. (Lausanne).* 2015;6:33.
73. Mulvaney SA, Schlundt DG, Mudasingh E, et al.: Parent perceptions of caring for adolescents with type 2 diabetes. *Diabetes Care.* 2006;29(5):993-997.