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EFFECTS OF A KETOGENIC MEDITERRANEAN DIET ON PHYSIOLOGICAL AND PSYCHOLOGICAL VARIABLES

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COMPENDIUM (Italian/English)

(Italian)

Le diete chetogeniche sono diete in cui l'introito netto di carboidrati, calcolato sottraendo la quantità di fibre dai carboidrati totali, è tra 20 e 50 g/gg (<10% dell'apporto energetico totale) con una proporzione variabile di proteine e grassi (Noakes, Windt 2017). In queste condizioni le riserve di glicogeno sono esaurite (Paoli, Canato et al. 2011), il livello di insulina è basso e il metabolismo energetico dipende prevalentemente dall'ossidazione dei grassi. Le diete chetogeniche portano un aumento significativo dei livelli circolanti dei corpi chetonici β-idrossibutirrato, acetoacetato e acetone (Veldhorst, Westerterp et al. 2010). Mentre sia l'acetoacetato che il β-idrossibutirrato vengono utilizzati come energia, l'acetone è volatile ed è eliminato attraverso l'espirazione, dando all'alito quella nota "fruttata" tipica della chetosi, oppure attraverso i reni (Paoli, Canato et al. 2011). La concentrazione ematica dei corpi chetonici in individui sani che seguono una dieta costituita prevalentemente da carboidrati è 0,1 mmol/L e può salire fino a 0,3 mmol/L dopo il digiuno notturno, ma dopo venti giorni di digiuno il livello di corpi chetonici può salire oltre 10 mmol/L. Una dieta è considerata "chetogenica" quando produce un aumento del livello di β-idrossibutirrato superiore a 0,6 mmol/L (Wiggam, O'Kane et al. 1997) oppure se il rapporto molare tra il glucosio e il β-idrossibutirrato ematici è uguale o minore di 1 (Meidenbauer, Mukherjee et al. 2015). Dato che i chetoni acetoacetato e β-idrossibutirrato sono acidi, lo stato di chetosi implica una condizione di acidosi. Siccome il pH del sangue è 7,4 e la pKa dell'acetoacetato è 3,8 e quella del β-idrossibutirrato è 4,8, questi acidi circolano nel sangue in forma dissociata e sono eliminati insieme agli ioni sodio e potassio (Siliprandi & Tettamanti 2011) . Questa perdita di cationi porta una diminuzione del pH che viene normalmente tamponata dal corpo tranne quando l'assunzione di sodio e potassio è impedita (Phinney 2004) oppure in caso di diabete scompensato, quando c'è una sovrapproduzione di corpi chetonici con livelli superiori a 20 mmol/L e conseguente riduzione del pH. Il biochimico Hans Krebs fu il primo a distinguere la chetosi fisiologica da quella patologica (Krebs 1966). Per i muscoli scheletrici e cardiaco, che utilizzano normalmente i grassi, l'utilizzo dei corpi chetonici a scopo energetico è un vantaggio relativo, mentre per il sistema nervoso centrale, in cui l'accesso degli acidi grassi è impedito dalla barriera ematoencefalica, la disponibilità dei corpi chetonici è un importante surrogato del glucosio, che è il substrato abituale dei neuroni. Durante il digiuno, in dieta chetogenica e nei neonati, il cervello utilizza i corpi chetonici come combustibili principali al posto del glucosio (Laeger, Metges et al. 2010), proporzionalmente al grado di chetosi (Hartman, Gasior et al.). Il β-idrossibutirrato è il principale corpo chetonico circolante e il suo trasporto attraverso la barriera ematoencefalica avviene sia mediante diffusione che attraverso i trasportatori MCT1 e MCT2, dei quali i primi aumentano durante una dieta chetogenica (Newman, Verdin 2014). Quest'azione complementare tra il fegato, che produce i corpi chetonici in assenza di carboidrati, e il sistema nervoso centrale che li può utilizzare, è un evento molto importante che fu determinante per la sopravvivenza della specie umana nei millenni.

La mia ricerca si è focalizzata su tre importanti aspetti delle diete chetogeniche - connesse alla perdita di peso - che richiedevano di essere approfonditi:

1. il mantenimento del peso perso dopo una dieta chetogenica: il mantenimento del peso perso a lungo nel tempo è impegnativo e la paura di ritornare velocemente al peso iniziale è comune, tanto che questo fenomeno viene chiamato "effetto yo-yo". A questo proposito, le diete a basso contenuto di carboidrati sono

note per portare risultati migliori rispetto alle diete a basso contenuto di grassi in termini di perdita di peso (Shai, Schwarzfuchs et al. 2008c), ma non di "compliance" (adesione al protocollo) (Greenberg, Stampfer et al. 2009). Recentemente, Sumithran e collaboratori hanno dimostrato che l'aumento dei livelli circolanti di grelina e del livello di appetito tipici di una dieta ipocalorica erano minori durante un protocollo chetogenico (Sumithran, Prendergast et al. 2013). Abbiamo quindi ipotizzato che alcuni aspetti della dieta chetogenica come il mantenimento della massa muscolare, del metabolismo energetico basale e la stabilità del principale ormone oressigenico (grelina) combinati con gli effetti benefici della nutrizione tradizionale mediterranea, potessero favorire la perdita di peso a lungo nel tempo. Lo scopo del nostro studio è stato quindi quello di indagare l'effetto sul peso e sulla composizione corporea di due brevi periodi di una dieta chetogenica modificata, cioè una dieta fitochetogenica mediterranea (KEMEPHY) (Paoli, Cenci et al. 2010a, Paoli 2011, Paoli 2012) intervallata da 2 periodi più lunghi di dieta di mantenimento basata sulla dieta mediterranea tradizionale per un periodo totale di 12 mesi. I soggetti reclutati erano obesi o in sovrappeso e lo studio è stato retrospettivo. Abbiamo analizzato 89 soggetti (uomini e donne) di età compresa tra i 25 e i 65 anni che erano in uno stato di buona salute generale benchè fossero obesi (IMC medio 35.82 ± 4.11 kg/m^2). I risultati di questo studio hanno dimostrato che la maggioranza dei soggetti ha ottenuto una significativa perdita di peso (10%) a seguito delle due fasi di dieta chetogenica e l'aderenza al protocollo è stata alta sia durante i sei mesi di perdita di peso sia nei successivi sei mesi di mantenimento, senza riacquisto del peso. Inoltre, il protocollo proposto ha portato miglioramenti nella maggior parte dei soggetti dei livelli di parametri importanti per la salute (colesterolo totale, colesterolo LDL, trigliceridi e livelli di glucosio). L'alta "compliance" è stato un fattore determinante per i risultati ottenuti;

2. <u>la formulazione di nuovi prodotti a basso contenuto di carboidrati per sopperire alla mancanza del sapore dolce durante una dieta chetogenica</u>: un aspetto delle diete chetogeniche difficile da tollerare nel lungo tempo, soprattutto per chi ha una spiccata preferenza per i dolci, è la mancanza di questo sapore. In dieta chetogenica è necessario mantenere un basso livello di glicemia (circa 80-90 mg/dL) per evitare i picchi di insulina (Paoli, Canato et al. 2011) e permettere così ai soggetti di migliorare l'ossidazione dei grassi come dimostrato da Paoli et al. (Paoli, Grimaldi et al. 2012) e da Tagliabue et al. (Tagliabue, Bertoli et al. 2012). Oggi la nuova tecnologia alimentare, che è in grado di costruire prodotti ultra-processati con un contenuto di zucchero molto basso e un alto contenuto di proteine e fibre, può aiutare a risolvere questo problema, formulando prodotti di elevata appetibilità in un formato pronto per il consumo, utili sia in chetosi che in diete ipoglucidiche più moderate. Di solito i prodotti ultra-processati mancano di proteine e fibre e producono picchi post-prandiali di glucosio e insulina (OPS WHO 2015) . Questo effetto provoca un forte desiderio di cibo con una preferenza per i carboidrati ad alto indice glicemico (Lennerz, Alsop et al. 2013), fenomeno definito come "carb-craving" (Ventura, Santander et al. 2014). Al fine di analizzare l'effetto di 10 diversi alimenti ultra-processati ad alto contenuto proteico e basso contento di carboidrati sulla glicemia, abbiamo reclutato 14 donne sane e abbiamo testato la loro risposta glicemica attraverso il metodo del punteggio glicemico ("glucose-score", GS). Tutti gli alimenti testati hanno prodotto, rispetto al glucosio, una risposta glicemica significativamente inferiore e il loro GS è risultato inferiore a 25 (rispetto al valore di riferimento del GS del glucosio che è 100). Abbiamo quindi concluso che la riformulazione di prodotti ultra-processati pronti al consumo in una versione ad alto contenuto proteico e basso contenuto di carboidrati è in grado di produrre una risposta glicemica significativamente più bassa, pur mantenendo l'alto valore del

pratico formato pronto per l'uso e l'alta appetibilità richiesta dai consumatori, facilitando quindi l'adesione a una dieta chetogenica di individui che tendono ad avere una forte preferenza per i cibi dolci;

3. l'effetto delle diete chetogeniche sulle funzioni cognitive: il range di variazione della glicemia o dei corpi chetonici nel sangue di soggetti non diabetici è ampia e ciascuno di essi può essere utilizzato come energia dal cervello. I dati sugli effetti della variazione dei livelli di glicemia e chetonemia sulle funzioni cognitive di esseri umani sani dopo diversi tipi di dieta sono scarsi. Lo scopo di questo studio è stato confrontare gli effetti della variazione di glicemia e chetonemia dopo dieci giorni di due differenti diete chetogeniche e di una dieta mediterranea ipocalorica (MD) sulla memoria di lavoro e sulle funzioni esecutive in 63 giovani donne sovrappeso, sedentarie e in buona salute (IMC> 25, età: 20-35) che sono state reclutate nella zona universitaria. I soggetti sono stati divisi in gruppi in base al giorno di inizio della loro fase follicolare per minimizzare gli effetti ormonali sull'umore e le misurazioni basali sono state effettuate cinque giorni prima dell'inizio del protocollo dietetico. I seguenti controlli sono stati fissati al giorno di inizio della dieta (t1), al terzo (t3), al quinto (t5), al settimo (t7) e all'ultimo giorno (t10). Al controllo iniziale è stato misurato il peso dei soggetti ed è stata eseguita un'analisi impedenziometrica. I soggetti hanno poi assunto una colazione ad alto contenuto di carboidrati e hanno completato i test psicologici. Al t1, T3, T5, T7 e t10 sono stati misurati il livello dei corpi chetonici e la glicemia, così come i livelli di appetito. Nel giorno dell'ultimo controllo (T10) i soggetti hanno ripetuto l'analisi impedenziometrica, la misura del peso corporeo e, dopo la colazione (ogni gruppo ha assunto una colazione diversa a seconda della dieta prescritta), hanno completato i test psicologici. I test psicologici consistevano in un test sull'umore, due compiti cognitivi, uno per indagare la memoria di lavoro ("visuo-spatial n back") e uno per analizzare le funzioni esecutive ("inhibitory control task") e in una scala VAS per testare il livello di appetito. 45 soggetti hanno completato lo studio. Considerando tutti i partecipanti insieme, i livelli di glucosio pre-dieta correlavano positivamente con il tempo di reazione nel "go-trial" del test delle funzioni esecutive ($r(43) = 0,358$, $p = 0,018$), ma questa relazione non è stata trovata nel post-dieta, sia quando i soggetti sono stati analizzati tutti insieme che quando i soggetti sono stati divisi in base al tipo di dieta seguita. Nello stesso test psicologico, nel post-dieta la misura della chetonemia ha mostrato una correlazione negativa con l'accuratezza ai compiti "no-go" ($r(29) = -0,455$, $p = 0,027$). Possiamo quindi concludere che giovani soggetti in sovrappeso con livelli di glicemia inferiori al livello di pre-diabete sono stati influenzati negativamente da una colazione ad alto contenuto di carboidrati nel corso di un test di funzioni esecutive. Inoltre, l'effetto di moderati livelli di corpi chetonici ($2 \pm 1,3$ mmol / L) ha nfluenzato negativamente l'accuratezza nelle prove "no-go" del test sulle funzioni esecutive.

Nella prima delle tre ricerche sopra descritte, che era retrospettiva, ho analizzato i dati e ho lavorato alla stesura dell'articolo. Nel secondo e terzo studio, che ho progettato e attivamente condotto sotto la supervisione del Prof. Antonio Paoli, ho gestito con cura tutti i passaggi, dal reclutamento e selezione dei soggetti all'analisi dei risultati e stesura degli articoli. Nel terzo studio, che è stato eseguito tra aprile 2015 e giugno 2016, Lisa Zarantonello, dottoranda in psicologia, si è occupata dei test psicologici.

(English)

Ketogenic diets (KDs) are diets in which the net carbohydrate intake, calculated by subtracting fibres from total carbohydrates, is between 20 and 50 g/day (<10% of total energy intake) with a variable proportion of proteins and fats (Noakes, Windt 2017). In these conditions, glycogen stores are depleted (Paoli, Canato et al. 2011), insulin level is low and energy metabolism is mainly dependent from fat oxidation. KDs lead a significant increase in circulating levels of ketone bodies (KBs) β-hydroxybutyrate (βOHB), acetoacetate (AcAc) and acetone (Veldhorst, Westerterp et al. 2010). While AcAc and βOHB are used as energy, acetone is a volatile compound and is eliminated through expiration, giving the "sweet" breath odour typical of ketosis, or via renal excretion (Paoli, Canato et al. 2011). The concentration of KBs in the blood of healthy individuals during the carbohydrate fed state is about 0.1 mmol/L and increases to about 0.3 mmol/L after an overnight fast, but after prolonged fasting up to 20 days KBs can increase to more than 10 mmol/L. A diet is considered "ketogenic" when produces a stable increase in the level of βOHB higher than 0.6 mmol/L (Wiggam, O'Kane et al. 1997) or when the molar ratio of blood glucose to blood ketone body βOHB is less than or equal to 1 (Meidenbauer, Mukherjee et al. 2015). Since KBs AcAc and βOHB are acids, the ketosis state implies a condition of acidosis. Given the fact that the pH of the blood is 7.4 and that the pKa of AcAc is 3.8 and that of βOHB is 4.8, these acids circulate in the blood in a completely dissociated form and are eliminated together with sodium and potassium ions (Siliprandi & Tettamanti 2011). This loss of cations implies a decrease of pH, which is normally balanced from the body apart when potassium and sodium intake are impaired (Phinney 2004) or in pathological overproduction of KBs during untreated diabetes type 1 which leads to diabetic ketoacidosis, characterized by a KBs level higher than 20 mmol/L with a decrease of pH. Biochemistry Hans Krebs was the first who diversified physiologic from pathologic ketosis (Krebs 1966). For skeletal and cardiac muscle, which usually oxidize fats, the use of KBs is a relative advantage, while for the central nervous system, in which the entrance of fatty acids is prevented from the blood-brain-barrier (BBB), the availability of KBs is an important surrogate of glucose, which is the habitual substrate of nervous tissue. During starvation, under a ketogenic diet or in new-born infants, the brain can utilize KBs as primary fuel instead of glucose (Laeger, Metges et al. 2010) in proportion to the degree of ketosis (Hartman, Gasior et al.). βOHB is the most abundant circulating ketone body and its transport across the blood-brain barrier is mediated both by diffusion and by several monocarboxylic acid transporters as MCT1 and MCT2, the former being upregulated during a ketogenic diet (Newman, Verdin 2014). This complementary action between the liver, which produces KBs in periods of shortage of carbs, and the CNS which use them, it's a very important event which was determinant for the survival of the human species over the millennia.

My research focused on three important aspects of KDs and weight loss, which needed further investigation:

1. long-term successful weight loss after a KD: the maintenance of weight loss over long time is challenging and the fear of weight regain is common, so that this phenomenon is named "yo-yo" effect. In this regard, low-carbohydrate diets are known to bring better results compared to low-fat diets in terms of weight loss (Shai, Schwarzfuchs et al. 2008) but not of compliance (Greenberg, Stampfer et al. 2009). Recently, Sumithran and colleagues have demonstrated that the increase in circulating ghrelin and in subjective appetite, which accompanied a hypocaloric diet, was reduced with a ketogenic approach (Sumithran, Prendergast et al. 2013). Thus, we hypothesized that certain aspects of the KD such as muscle mass retention, RMR (resting metabolic rate) and orexigenic hormone stability combined with the acknowledged

health benefits of traditional Mediterranean nutrition may favour long-term weight loss. The aim of our study was to investigate the effect on weight and body composition of two short periods of a modified KD, i.e., a very low carbohydrate ketogenic diet with phytoextracts (KEMEPHY) (Paoli, Cenci et al. 2010, Paoli 2011, Paoli 2012) interspersed between longer periods of maintenance nutrition, based on the traditional Mediterranean diet, over a total period of 12 months in obese/overweight healthy subjects and was designed as a retrospective study. We analysed 89 male and female subjects, aged between 25 and 65 years who were overall healthy apart from being obese (mean BMI 35.82 ± 4.11 kg/m^2). Data from this study demonstrate that the majority of subjects showed significant weight loss (10%) as a result of a two-phase KD and were compliant both during the six month weight loss phase and the six month normocaloric maintenance phase, with no weight regain. Moreover, the proposed protocol led improvements in health risk factors (total cholesterol, LDL cholesterol, triglycerides and glucose levels) in the majority of subjects. Compliance was very high which was a key determinant of the results seen;

2. formulation of new low-carbohydrate ultraprocessed foods to overcome the lack of sweet taste during a KD: a point of interest, which has always been a detrimental aspect of KDs, is the lack of sweet taste, which could be difficult to sustain for long periods, especially for people with a high sweet food preference. During consumption of a KD, it is mandatory to maintain a low level of glycaemia (about 80–90 mg/dL) to avoid insulin spikes (Paoli, Canato et al. 2011). This condition allows subjects to improve their fat oxidation as demonstrated by Paoli et al. (Paoli, Grimaldi et al. 2012) and by Tagliabue et al. (Tagliabue, Bertoli et al. 2012). Today the new food technology, which is able to build ultra-processed products very low in sugar content and high in protein and fibres, can help to solve this problem, formulating products with a high palatability and ready-to-consume format, useful both in ketosis and in easier low carb diets. Usually, ultra-processed products lack in proteins and fibres and produce postprandial glucose and insulin spikes (PAHO WHO 2015). This effect is known to elicit food craving and overeating, with a preference for high-glycaemic index carbohydrates (high-GI CHO) (Lennerz, Alsop et al. 2013), a phenomenon defined as CHO-craving effect (Ventura, Santander et al. 2014). In order to analyse the effect of 10 different high-protein low-CHO proprietary foods on glycaemia, we recruited 14 healthy females, which were tested for their glycaemic response through the glycaemic score (GS) method. All test foods, compared with glucose, produced a significantly lower glycaemic response and their GS resulted lower than 25 (compared to the reference GS value of glucose which is 100). We concluded that the reformulation of ultraprocessed ready-to-consume foods in a low-CHO, high-protein version can produce a significantly lower glycaemic response whilst maintaining the valued ready-to-use format and high palatability demanded by consumers, facilitating the adherence to a KD of individuals who tend to have a high preference for sweet foods;
3. effect of KDs on cognitive functions: the range of variation of glucose and ketone bodies (KBs) in the blood of non-diabetic individuals is wide and both of them can be used as energy from the brain. Data on glycaemia and ketonemia effects on cognitive functions on healthy humans following different diets are scarce. The purpose of this study was then to compare the effects of glycaemia and ketonemia variation after ten days of two different ketogenic diets and a calorie-restricted Mediterranean diet (MD) on working memory and executive functions in 63 sedentary healthy overweight (BMI>25) young women (age: 20-35), which were recruited in the university area. Subjects were divided in groups according to the day of the beginning of their follicular phase in order to minimize hormonal effects on mood and came for the basal measurements five days before the start of the dietary protocol. The following controls were set on the

starting day of the diet (t1), on the third (t3), on the fifth (t5), on the seventh (t7) and on the last day (t10). On the basal control day, the weight of the subjects was measured and a body impedance analysis was performed. Subjects took a standard high carb breakfast and afterwards they completed the psychological tests. At t1, t3, t5, t7 and t10 ketone bodies levels and glycaemia were measured, as well as appetite levels. On the last control day (t10) subjects repeated the body impedance analysis, the body weight measure and, after breakfast (each group had a different breakfast according to the prescribed diet), the psychological tests. Psychological tests consisted in a mood test, two cognitive tasks, one to investigate working memory (visuo-spatial n back) and the second to stress executive functions (inhibitory control task) and in a VAS scale to test the appetite level. 45 subjects completed the study. Considering all participants together, pre-diet glucose levels were positively correlated with reaction time in the go-trial of the executive function test ($r(43) = 0.358$, $p = 0.018$), but this relation was not found in the post-diet measure both when subjects were analysed all together and when subjects were divided according to the type of diet followed. In the same psychological test, in the post-diet measure ketonemia showed a negative correlation with accuracy of the no-go trials ($r(29) = -0.455$, $p = 0.027$). We can conclude that healthy young overweight subjects with fasting glycaemia below prediabetes level were negatively affected by a high-carb breakfast during an executive function test. Moreover, the effect of mild KBs levels (2 ± 1.3 mmol/L) negatively affected accuracy of the no-go trials of the executive functions test.

In the first of the three above-described researches, which was retrospective, I analysed the data and worked on the drafting of the article. In the second and the third scientific works, which I designed and actively conducted under the supervision of Prof. Antonio Paoli, I carefully managed all the procedures, from the selection and recruitment of subjects to the analysis of the results and the writing of the articles. In the third study, which has been conducted between April 2015 and June 2016, Lisa Zarantonello, PhD student of psychology, guided the psychological tests.

INTRODUCTION

Facing globesity

The World Health Organization defines overweight and obesity as “abnormal or excessive fat accumulation that presents a risk to health” (WHO 2016).

Overweight and obesity are determined through several methods of which three are the most used during normal clinical practice: body mass index (BMI), plicometry and bioelectrical impedance (Deurenberg, Yap 1999).
BMI, also known as “Quetelet index” is the most widely used since it is the same for both sexes and for all ages of adults and it is calculated by dividing the weight of the subject in kilograms for the square of his height in meters. A BMI greater than 25 Kg/m^2 is a sign of overweight, whereas a BMI greater than 30 Kg/m^2 of obesity.

Despite the interest of the scientific world for obesity began to germinate in the early 70s in both Europe and United States, we have to wait the 1995 and the birth of the International Obesity Task Force (IOTF) by Professor Philip James for a global awareness on the gravity and consequences of this phenomenon (World Obesity Federation 2015). The IOTF was born with the purpose of conducting the first scientific research on this subject, involving experts from around the world and drew the first global report on the epidemic of obesity.
In 1997, following the path traced by this first initiative, it was organized in Geneva the first meeting of World Health Organization experts on this issue and in 2000, with the publication of the WHO Technical Report Series 894 entitled Preventing and Managing the Global Epidemic, obesity was defined as a "worldwide epidemic disease" associated with serious health consequences (Consultation 2000).
Today overweight and obesity are social issues, spreading in both high income and low income countries and have been analysed by two opposing views: one individualistic which considers the individual the responsible and one systemic, which discharges the responsibility on the environment and social factors. Both points of view can, however, be useful to understand the evolution of the obesity epidemic. On one hand the individual is responsible for his health, on the other hand the environmental factors may influence the individual's ability to be so. The individual and the environment interact reciprocally: if the environment provides copious amounts of unhealthy food, the individual will modify his food choices eating a higher amount of it.
Food companies manipulate the ingredients of food products generating ultra-processed products to maximize the pleasure of consumers. Salt, sugar, fats and various food additives become the main ingredients of these foods, causing, in those eating them, neurobiological changes similar to those of psychoactive substances (Davis 2014). Such foods are also very low in fibres and proteins, essential elements of healthy nutrition, which help to increase the feeling of satiety (Pesta, Samuel 2014, Clark, Slavin 2013).
Exposure to these "altered" tastes is of particular concern for children, because childhood is the period of life in which there is a greater preference for sweet taste (Ventura, Mennella 2011). The child, easily impressed by the food marketing, develops a preference for ultra-processed foods due to both the strong image component that accompanies these products and to the pleasant taste and particular brain neurochemistry generated by their repetitive intake. From the sale of ultra-processed foods, high in energy but lacking in nutrients, industries earn huge profits. This creates a vicious cycle that could be broken only by government intervention, but such intervention are impeded by great obstacles like the lobby of the industries, the inability of governments to

implement these policies and the lack of social pressure for a political action against it (Roberto, Swinburn et al. 2015).

Published data (Ng, Fleming et al. 2014) report that the global percentage of adults with a BMI greater than 25 has increased from 1980 to 2013 from 28.8% to 36.9% in men and from 29.8% to 38.0% in women. Children and teenagers are not immune to this phenomenon and recorded an increase in obesity with a prevalence of 23% in developed countries and 13% in developing countries. Despite the awareness of the gravity of this epidemic and of its impact on health and welfare, no country has been able, over the last 30 years, to address and reverse this phenomenon (Kleinert, Horton 2015).

Regarding Europe, the prevalence of obesity and overweight has tripled from 1980 to 2015 and continues to increase, especially among children. The European Office of the World Health Organization has therefore established an initiative (COSI, Childhood Obesity Surveillance Initiative) to monitor the weight trends in children between 6 and 10 years (Wijnhoven, van Raaij et al. 2014).

Overweight and obesity are also important modifiable risk factors to prevent the development of both non-communicable diseases (NCDs) and metabolic syndrome (MetS).

NCDs, such as diabetes, cardiovascular disease, various cancers and chronic lung disease, account for 63% of annual global deaths, equivalent to more than 36 million deaths per year of which 17.5 million are caused by cardiovascular disease, 8.2 million by cancer and 1.5 million by diabetes (WHO Fact Sheet on Noncommunicable diseases 2015). Regarding health care costs, NCDs have a global cost of 6.3 trillion dollars (US $), which is expected to rise to 13 trillion (US $) by 2030 (Arena, Guazzi et al. 2015). For this reason, the World Health Organization Plan for the Prevention and Control of Non-communicable Diseases 2013-2020 targets to avoid any increase in obesity prevalence between 2010 and 2025.

The MetS is the simultaneous presence of at least 3 of the following 5 risk factors: a large waistline, a high triglyceride level, a low HDL cholesterol level, high blood pressure and high fasting blood sugar (U.S. Department of Health and Human Services 2016). Even if reading the above current definition could seem that the risk factors are separated from each other, Jeff Volek and colleagues showed that there is an underlying effect in MetS, which is the resistance to the action of insulin in peripheral tissues that manifests itself as hyperglycaemia, hyperinsulinemia, and atherogenic dyslipidemia (high TG, low HDL-C, and small LDL-C) (Volek, Fernandez et al. 2008).

The World Health Organization defines overweight and obesity as “largely preventable” but, in order to implement an effective prevention plan, it is essential to understand their origin (WHO 2016). Until now this has not be established, since the aetiology of these disorders is complicated by multiple links to genetic, social, economic as well environmental factors (Mutch, Clement 2006). However, based on most recent evidence, overweight and obesity could be seen principally as a marker of a high carbohydrate diet in someone who developed a disorder of carbohydrate metabolism (namely insulin resistance) (Reaven 2012, Mark, Du Toit et al. 2016) and an effective strategy to prevent weight gain should then be that diet which is effective in minimizing insulin secretion at all times in those with insulin resistance (Prof. Tim Noakes, Jonno Proudfoot et al. 2015).

Nutritional approaches to overweight and obesity

Besides preventing the onset of overweight and obesity, it is also important to have the right tools to manage weight loss. Literature data show that the combination of diet plus exercise is more effective than both diet-only and exercise-only interventions to achieve weight loss in overweight and obese people (Chin, Kahathuduwa et al. 2016). However, despite the science of modern nutrition developed in the early 20th Century and several comparative studies were undertaken to determine the differences between diets (Phinney 2004), there is still no definitive data about which is the best dietary protocol in both the short and long term (Paoli 2014). An ideal weight loss diet would assure a high level of satiety, positive mood, the loss of fat mass together with the subsistence of metabolically active fat-free mass and the maintenance of weight loss over long time.

Before analysing the different types of weight loss diets, let's face two important aspects of modern nutrition:

1. current nutritional guidelines profess that carbohydrates are the essential energy nutrient in the modern diet and should provide between 45 and 60% of the daily energy intake in all humans at all ages (Società Italiana di Nutrizione Umana - SINU 2014, WHO Regional Office for Europe). However, it is interesting to note that humans have no essential requirement for carbohydrate and there is no known human carbohydrate deficiency disease (CDC Centers for Disease Control and Prevention 2014). Moreover, pure hunting cultures of indigenous people like the Inuit of the Canadian Alaskan Artic regions made at most seasonal use of this nutrient class, thus maintaining a functional well-being (Phinney 2004);
2. for over a century the application of the first law of thermodynamics, also known as the law of conservation of energy, has governed nutrition. Observing this concept, the US Department of Agriculture concluded that all low-calorie diets lead an effective weight loss, regardless of macronutrient composition (Paoli, Rubini et al. 2013).

   But calories are really all the same? In laboratory conditions yes: 100 calories of spinach are equivalent from an energy point of view to 100 calories of sugar, but in the human body has been shown that it is not like this and that the law of conservation of energy is not suitable to describe the metabolism of nutrients in our bodies (Fine, 2004). The effects of same caloric intakes on weight and metabolism are different depending on the quantity and quality of protein, carbohydrates, fat, fibres and nutrients contained in the food we ingest (Li, Song et al. 2010, Wien, Sabate et al. 2003). This has led to the formulation of a new paradigm in nutrition based on the metabolic effect of carbohydrates: since it is known that they trigger the release of insulin and that this hormone has an anabolic effect leading to fat accumulation and weight gain, this theory has been named "carbohydrate-insulin model of obesity". As a results, the use of low-carbohydrate diets has been suggested in order to avoid this effect and stimulate fat loss through the increase of the mobilization of free-fatty acids from adipose tissue and their subsequent oxidation. This theory has recently been challenged in studies conducted in metabolic wards, which tested isocaloric diets with different ratio between fat and carbohydrates but with equal amounts of proteins. Interestingly, results failed to show any increase in body fat loss following the low-carbohydrate compared to the high-carbohydrate diets, with variable results on total body weight loss (Hall, Chen et al. 2016, Hall, Bemis et al. 2015). These data, disrupting both the "old" paradigm of nutrition governed by the first law of thermodynamics and the "new" one governed by insulin, add new

material to the hot topic of nutrition "is a calorie a calorie?" and could suggest that, in nutrition, just one paradigm doesn't fit all. Ad-hoc modifications of the new insulin-model of obesity could lead the chance to apply it only on certain groups of people, for example on those who show carbohydrate intolerance (Prof. Tim Noakes, Jonno Proudfoot et al. 2015, Hall 2017).

In free living conditions, where diet adherence is the main determinant for fat loss, the most used weight loss diets are the energy restricted "balanced diets" (i.e. the Mediterranean diet), the low-fat/high-carbohydrate diets (FAT < 30 % of energy intake) where the reduction of total fat should lead to an appreciable effect of total caloric intake (Tobias, Chen et al. 2015) and the high-protein low-carbohydrate diets (HPLCDs) (4% < CHO < 50% of energy intake; 20% < P < 40% of energy intake) (Veldhorst, Westerterp et al. 2010, Soenen, Bonomi et al. 2012) which can be built with or without total energy restriction (Soenen, Bonomi et al. 2012, Veldhorst, Westerterp et al. 2010, Shai, Schwarzfuchs et al. 2008, Shai, Schwarzfuchs et al. 2008). Among HPLCDs there are the ketogenic diets (KDs), which will be discussed extensively in the next chapters.
Nowadays there is a strong scientific support showing that HPLCDs lead to better results in both short and long term compared to higher carb or lower fat diets (Kris Gunnars 2014), while that favouring the low-fat diet is in rapid retreat (Nina Teicholz 2014).

"Americans have dutifully followed official dietary advice to restrict fat and animal products for more than sixty years now, ever since the AHA first recommended this diet in 1961 as the best way to avoid heart disease and obesity. (...) Every reliable indicator of good health is worsened by a low-fat diet. (...) In the end what we believe to be true – our conventional wisdom – is really nothing more than sixty years of misconceived nutritional research. Before 1961, there were our ancestor, with their recipes. And before them were their ancestors with their hunting bows or traps or livestock – but like lost languages, lost skills and lost songs, it takes only a few generations to forget" (Nina Teicholz 2014, pp.326-330)

However, recent data show that it could be the higher-protein and not the low-carb content the main determinant of weight and body fat loss (Soenen, Bonomi et al. 2012) of HPLCDs, probably because of both the high thermogenic action (Leidy, Clifton et al. 2015, Wycherley, Moran et al. 2012) and the good satiating effect of this macronutrient. Studies report three main mechanisms involved in the latter effect:

1. the increase of anorexigenic neuropeptides secreted by the intestinal tracts such as incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), cholecystokinin (CCK), peptide YY (PYY) and pro-opiomelanocortin (POMC) (Pesta, Samuel 2014) ;
2. the decrease of the orexigenic neuropeptide ghrelin (Pesta, Samuel 2014b, Wyka, Malczyk et al. 2015);
3. the increase of circulating levels of aminoacids that, according to the "aminostatic hypotesis" of 1956, decrease hunger (Pesta, Samuel 2014).

Behind the scenes of ketogenic diets

Among the low-carb/high-protein diets are found the ketogenic diets (KDs) in which the net carbohydrate intake, calculated by subtracting fibres from total carbohydrates, is between 20 and 50 g/day (<10% of total energy intake) (Noakes, Windt 2017). KDs lead, compared to HPLCDs, a greater reduction in appetite, a greater reduction in respiratory quotient, which is indicative of increased fat consumption at rest, and a significant increase in circulating levels of ketone bodies (KBs) β-hydroxybutyrate (βOHB), acetoacetate (AcAc) and acetone (Veldhorst, Westerterp et al. 2010). A diet is considered "ketogenic" when produces a stable increase in the level of βOHB higher than 0.6 mmol/L (Wiggam, O'Kane et al. 1997) or when the molar ratio of blood glucose to blood ketone body βOHB is less than or equal to 1 (Meidenbauer, Mukherjee et al. 2015). Based on this definition, diets with less than 38% of energy intake from carbohydrates can be considered ketogenic, since they can bring a mild elevation of KBs (Veldhorst, Westerterp et al. 2010).

Originally, the classic KD was introduced at the Mayo Clinic in 1921 by Dr. Wilder as a starvation-mimicking diet to treat epilepsy (Hartman, Gasior et al. 2007), since starvation showed antiepileptic activity known from ancient time and described also in the New Testament of the Christian biblical canon (*Matteo 17:14-21*) (Paoli, Rubini et al. 2013). Dr. Wilder first described the most studied KD for epilepsy, which had a 4:1 ratio between long-chain fat content and carbohydrate plus protein combined (Hartman, Gasior et al. 2007). In 1925 Peterman at the Mayo Clinic designed the first KD for children, composed by 1g of protein per kilogram of body weight, 10-15 g of carbohydrates per day and the reminder calories in fat (Wheless 2008).
Even there isn't any "official" formulation for the KD (Zupec-Kania, Zupanc 2008), in the classic antiepileptic KD calories are restricted (-25%) and fat, protein and carbohydrates are the 85%-90%, 15% and 5% respectively of total calories intake and the level of ketonemia can reach levels of 12 mmol/L (Bouteldja, Andersen et al. 2014). In this diet the consumption of low carbohydrate proprietary foods is not common (Kossoff, Cervenka et al. 2013), whereas the intake of high fat foods such as whipping cream, butter and vegetables oil is recommended (Zupec-Kania, Zupanc 2008). Several concerns are related to the long term management of this diet despite the high level of ketosis achieved and the high antiepileptic effect, since the micronutrient content of the classic KD has proved to be deficient for calcium, vitamin D, phosphorus and magnesium (Zupec-Kania, Zupanc 2008). Moreover, the volume of fluid intake must be adapted to avoid dehydration, which could enhance adverse effects such as constipation and kidney stones (Zupec-Kania, Zupanc 2008). After the discover of antiepileptic drugs, the ketogenic strategy was abandoned to be re-discovered only in recent years after the first multicentre prospective study of the efficacy of the KD supported by The Charlie Foundation in 1998 (Wheless 2008).

Meanwhile, KDs had been used for weight loss purposes since the 19th Century, when the surgeon Mr. William Harvey cured the obesity of the prosperous London undertaker William Banting with a diet very low in carbohydrate and high in fat with moderate protein content. Mr. Banting, thrilled with the results, published in 1863 the *Letter on Corpulence, Addressed to the Public* to describe the miraculous diet (Figure 1).
Subsequently, the german Dr. Wilhelm Ebstein became the principal advocate of the original Harvey/Banting diet, since Harvey, shunned by the medical community as he was unable to explain why its treatment was effective, changed the original diet, including more protein and less fat, an edit which Banting considered less effective than the original (fatter) version. Then, the Banting/Ebstein diet spread throughout Europe and was

initially promoted in the United State by Dr. William Osler, Professor of Medicine at John Hopkins University in Baltimore, Maryland, USA. He, in his iconic medical textbook published in 1982 *The Principles and Practice of Medicine* prescribed the Banting/Ebstein diet as the treatment for obesity (Prof. Tim Noakes, Jonno Proudfoot et al. 2015).

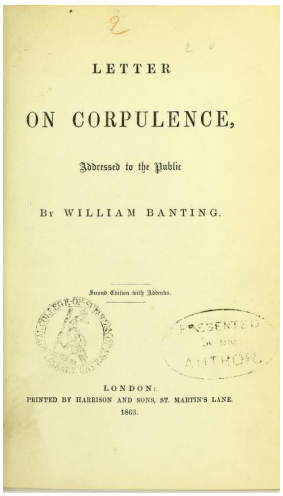
KDs were then used for weight loss under the name of "high fat diets" even if the "high fat" definition was misleading, since the actual fat intake was comparable or lower than the usual daily fat intake, probably because a higher fat intake was a common suggestion for thin people to gain weight and for this reason avoided by subjects aiming for weight loss. Protein content didn't change too, so the total caloric intake during "high fat diets" was commonly reduced (YUDKIN, CAREY 1960). "The inevitable effect of caloric reduction" was taken as the explanation of their efficacy, crushing any hypothesis of particular metabolic mechanisms related to this type of diets. The low-caloric intake couldn't, however, explain the decrease of appetite related to high fat diets, which was debated and put in relation with a supposed directly proportional effect between diet-carbohydrate content, fat intake and weight gain (YUDKIN, CAREY 1960).

However, the main idea of the new-born nutritional science was that carbohydrate was a necessary nutrient for optimum human health and function and this was determined through studies as that of 1939 conducted by two Danish scientists, Christensen and Hansen (Christensen, Hansen 1939) and that conducted by Kark *et al.* during the Second World War (Kark, Johnson et al. 1945) which proved the higher efficiency of a high carb diet in short-term tests of high intensity exercise. Moreover, with the discovery of muscle glycogen as the limiting fuel for high intensity exercise, common consensus was that humans were impaired if nourished with a low carb diet. Despite these data, others were favourable to the use of KDs and were supported by demographic evidence that whole populations of people lived for millennia with virtually zero carbohydrate intake without any apparent impediment (Phinney 2004). In this regard one of the first document was that of Lt. Frederick Schwatka's expedition in 1878-1880 to search the lost Royal Navy Franklin. In his travel diary, found only 85 years later, Schwatka described the transition state to a diet without carbohydrate intake using these words (Stackpole 1965): "When first thrown wholly upon a diet of reindeer meat, it seems inadequate to properly nourish the system, and there is an apparent weakness and inability to perform severe exertive fatiguing journeys. But this soon passes away in the course of two or three weeks." This described for the first time the issue of the "keto-adaptation", deepened later by the elegant studies of Cahill (Phinney 2004). Around 1910 the anthropologist Vilhjalmur Stefansson, ignoring previous works of Schwatka, entered the Artic to study the Inuit culture. He adapted to their nomadic lifestyle and to their diet consisting solely of the products of hunting and fishing. Back to the US, Stefansson wrote a book entitled *Not by Bread Alone* where he enthusiastically reported his experience and became the subjects of a year-long laboratory trial during which he avoided all forms of carbohydrates. Against expectations of scientists, who thought that he got sick from scurvy, Stefansson, despite a 4 kg of weight loss during the first month, completed the trial in perfect health. The fact that humans were capable of surviving eating only one food group, led to the misunderstanding that the Inuit's diet was high in protein, while it was only a modest intake of protein, deriving between 80-05% of their dietary energy intake from fat (Phinney 2004). Stefansson's experience inspired the birth of various diets low in carbohydrates, of which the most influential was that of Dr. Robert Atkins, published in 1972 (Prof. Tim Noakes, Jonno Proudfoot et al. 2015). In Dr. Atkins' *New Diet Revolution* in the first phase of the protocol was a KD very low in carbohydrate (< 20/50 g/day) without any restriction for carbs and proteins (Miller, Bertino et al. 2003) and without the mandatory consumption of fatty foods typical of the original KD (Kossoff, Cervenka et al. 2013). Atkins's hypothesis to

explain the mechanism involved in the success of KDs for weight loss was that the body lost energy through excretion of ketone bodies (Atkins 1972). This book made KDs famous to lose weight and after that many others commercial protocols succeeded. Together with the lower intake of fats compared to the original KD, these protocols, among which the Dukan diet, focused on protein-component, since diets high in protein showed rapid weight loss (Wyka, Malczyk et al. 2015). KDs diets became therefore known as "protein diets", loosing in their definition the term "ketogenic diets", since this made concern among physicians because of a lack of knowledge about the physiological mechanisms involved (Paoli 2014). In these diets the level of ketosis was mild (around 4-6 mmol/L (Bouteldja, Andersen et al. 2014)) compared with that following the classic high fat KD (Kossoff, Cervenka et al. 2013). However, also the "protein diets" definition for KDs was misleading, since *the state of the art* KDs are only *relatively* high in proteins with a daily amount of protein of about 1.2-1.5 g of protein per Kg of body weight (Paoli 2014). A diet is considered a "normal protein diet" with 1.00 g of protein per Kg of body weight (Schiavo, Scalera et al. 2016), whereas it is considered a "high protein diet" if protein content is higher than 1.6 g per Kg of body weight (Wiegmann, Zlomke et al. 1990).
However, even if KDs were commonly not "high protein" diets, negative opinions were related to their long-term adoption for fear of common hyper-proteins side–effects. The use and the success of KDs have therefore always been very linked to the common opinion, which has always lacked of precise information and was commonly against their use.

Another point of interest, which has always been a detrimental aspect of KDs, is the lack of sweet taste, which could be difficult to sustain for long periods, especially for people with a high sweet food preference (Lodi, Karsten et al. 2016). Today the new food technology, which is able to build ultra-processed products very low in sugar content and high in protein and fibres, can help to solve this problem, formulating products with a high palatability and ready-to-consume format, useful both in ketosis and in easier low carb diets (Lodi, Karsten et al. 2016).

Modern era of KDs should then focus on both the quantity and type of proteins and fats as well as of fibres, water and micronutrients, not excluding the advantages brought by the new ultra-processed-very-low-carbohydrates foods in order to avoid detrimental aspects of original KDs.


LETTER

ON CORPULENCE,

Addressed to the Public

By WILLIAM BANTING.

Second Edition with Addenda.

PRESENTED BY THE AUTHOR.

LONDON:
PRINTED BY HARRISON AND SONS, ST. MARTIN'S LANE.
1863.

Figure 1: Second edition of the *Letter on Corpulence, Addressed to the Public* (Banting 1863) located in the archive of the Royal College of Surgeons of England.

What is ketosis?

Ketosis is a physiological process, which allowed our ancestors to survive and to stay efficient even in case of famine or when the consumption of dietary carbohydrate was only opportunistic (Phinney 2004, Paoli, Canato et al. 2011).

After 2-3 days of fasting or drastically reduction of carbohydrate intake (under 20/50 g/day) glycogen stores are depleted (Paoli, Canato et al. 2011) and insulin level is low. In these conditions, energy metabolism is mainly dependent from fat oxidation. This implies an acceleration of the rate of production of Acetyl-CoA and its mitochondrial concentration becomes too high compared to that of its use in the Krebs cycle. In normal carbohydrate-fed conditions, in which the glycolytic pathway provides adequate amounts of pyruvate, this would be converting in oxaloacetate from pyruvate carboxylase activity (Figure 2). The activation of this enzyme by Acetyl-CoA would prevent its accumulation, favoring its conversion to citrate. In the liver and in condition of carbohydrate deficiency, pyruvate and oxaloacetate are essentially used in the gluconeogenesis for the maintenance of blood glucose, and then the excess of acetyl-CoA, coming from increased β-oxidation of fatty acids, is not properly placed in the Krebs cycle. In addition, the β-oxidation of fatty acids requires the availability of CoA, whose content in cells is extremely limited. The set of these metabolic events is responsible for the accumulation of acetyl-CoA in liver mitochondria and causes that the molecules of this metabolite react with each other to make available the CoA required to maintain an adequate rate of β-oxidation (Siliprandi & Tettamanti 2011) .

This marks the beginning of the metabolic pathway of ketosis, which leads to the formation of ketone bodies (KBs) β-hydroxybutyrate (βOHB), acetoacetate (AcAc) and acetone (Figure 3).

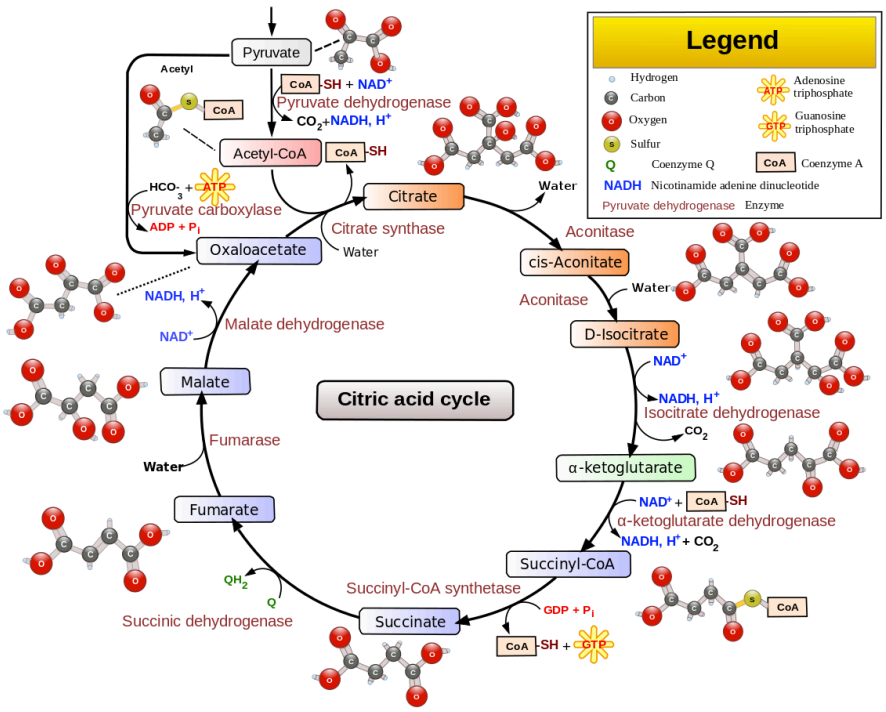


Figure 2. Krebs cycle (Wikipedia 2008).

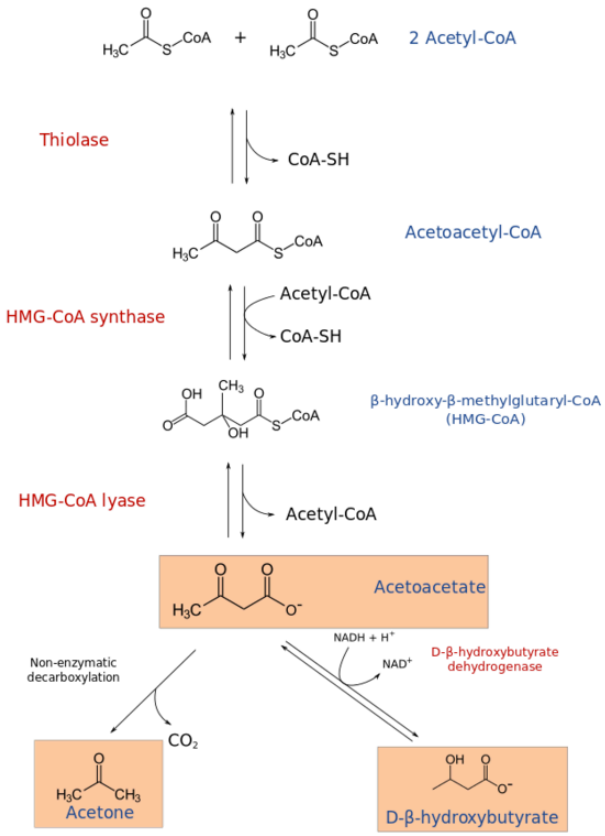


Figure 3. Ketogenesis pathway. The three ketone bodies (acetoacetate, acetone and β-hydroxybutyrate) are marked within an orange box (Wikipedia 2012).

The second reaction of the ketogenic pathway is catalysed by 3-hydroxy-3metylglutaryl-CoA (HMG-CoA) synthesis and it is precisely for its abundance in the liver that ketogenesis happens there. This reaction leads to the synthesis of HMG-CoA and is also present in the cytosol of liver cells, where it is used for the biosynthesis of cholesterol (Paoli, Canato et al. 2011). However, in hepatic mitochondria there is HMG-CoA lyase, which is not present in the cytosol and permits the synthesis of the firs keton body (AcAc).

A little part of AcAc is also formed from direct hydrolysis of acetoacetyl-CoA. This deacylation process happens mainly in the kidneys which can, although in size far below the liver, build ketones, while the liver produces KBs only through the standard pathway (Figure 3).

AcAc, which is the primary product of ketogenesis, is then transformed in βOHB and in a little amount of acetone, the third KB. βOHB needs to be converted to AcAc to be metabolized by tissues through the Krebs cycle and this process is not possible in the liver, which can't use KBs due to a lack of a specific enzyme (Paoli, Canato et al. 2011). From hepatic cells KBs diffuse in the blood, which deliver them to the muscles, heart and brain where they are used as energy instead of glucose, and to the kidney which partially use them and partially eliminate them in the urine.

While AcAc and βOHB are used as energy, acetone is a volatile compound and is eliminated through expiration, giving the "sweet" breath odour typical of ketosis, or via renal excretion (Paoli, Canato et al. 2011).

The concentration of KBs in the blood of healthy individuals during the carbohydrate fed state is about 0.1 mmol/L and increases to about 0.3 mmol/L after an overnight fast, but after prolonged fasting up to 20 days KBs can increase to more than 10 mmol/L.

Since KBs AcAc and βOHB are acids, the ketosis state implies a condition of acidosis. Given the fact that the pH of the blood is 7.4 and that the pKa of AcAc is 3.8 and that of βOHB is 4.8, these acids circulate in the blood in a completely dissociated form and are eliminated together with sodium and potassium ions (Siliprandi & Tettamanti 2011). This loss of cations implies a decrease of pH, which is normally balanced from the body apart when potassium and sodium intake are impaired (Phinney 2004) or in pathological overproduction of KBs during untreated diabetes type 1 which leads to diabetic ketoacidosis, characterized by a KBs level higher than 20 mmol/L with a decrease of pH. Biochemist Hans Krebs was the first who diversified physiological from pathological ketosis (Krebs 1966) (Table1). The wide range of possible KBs concentration in blood is similar to that of glucose and also in this case an extreme high level manifests itself in deleterious outcomes (Volek, Fernandez et al. 2008).

For skeletal and cardiac muscle, which normally oxidize fats, the use of KBs is a relative advantage, while for the central nervous system, in which fats don't have access due to the presence of the blood brain barrier (BBB), the availability of KBs is an important surrogate of glucose, which is the habitual substrate. During starvation, under a ketogenic diet or in new-born infants, the brain can utilize KBs as primary fuel instead of glucose (Laeger, Metges et al. 2010) in proportion to the degree of ketosis (Hartman, Gasior et al. 2007). βOHB is the most abundant circulating ketone body and its transport across the blood-brain barrier is mediated both by diffusion and by several monocarboxylic acid transporters as MCT1 and MCT2, the former being upregulated during a ketogenic diet (Newman, Verdin 2014).

This complementary action between the liver, which produces KBs in periods of shortage of carbs, and the CNS which use them, it's a very important event. The availability of KBs in extra hepatic tissues it's a way to spare glucose. In fact, the higher production of acetyl-CoA determines a higher production of citrate which, in the cytosol, inhibits the activity of glycolysis (Siliprandi & Tettamanti 2011).

Moreover, it was demonstrated that KBs are able to produce 25% more ATP than glucose or fatty acid (Volek, Fernandez et al. 2008), thanks to the high chemical potential of βOHB which leads to an increase of the ΔG_0 in the hydrolysis of ATP (Paoli, Canato et al. 2011).

Finally, glycaemia during the KD doesn't fall under physiological range, since glucose, which is formed through the gluconeogenesis process from glycerol released from the lysis of triglycerides, is sufficient for the maintenance of euglycaemia (Paoli, Canato et al. 2011).

Table 1. Blood values of several substances during a standard diet, a ketogenic diet and during the diabetic ketoacidosis (Paoli, Canato et al. 2011).

Blood values	**Standard diet**	**Ketogenic diet**	**Diabetic ketoacidosis**
Glucose (mg/dL)	80-120	65-80	**> 300**
Insulin (μU/L)	6-23	6.6-9.4	**≈ 0**
KBs (mmol/L)	0.1	7/8	**> 25**
pH	7.4	7.4	**< 7.3**

Ketogenic diets for weight loss: the inevitability of metabolism

"Everything should be made as simple as possible but not simpler"
Albert Einstein

In recent years grew a renewed scientific interest in understanding those metabolic mechanisms, previously suspected but never clarified (YUDKIN, CAREY 1960, Paoli, Canato et al. 2011), which could be the real reason behind the success of KDs in both in weight loss and in the control of the sense of hunger.

Considering weight loss, authors explain the better efficacy of KDs through a possible application of the second (instead of the first) low of thermodynamics, which predicts the flow of reactions in accordance to the increase of the entropy and of the dissipation of energy. Authors in favour of this mechanism hypothesize that the use of protein as source of energy in KDs is an "expensive" process for the organism and that could lead to a waste of calories. In a KD our body needs 60-65 g of glucose daily for red cells and neurons, which is obtained for the minor part from gluconeogenesis by glycerol and for most from gluconeogenesis by food or tissue proteins (Paoli, Canato et al. 2011, Landau, Wahren et al. 1996). About 100 g of protein can produce 57 g of glucose, so 110 g of proteins are needed to provide 60-65 g of glucose. Several authors have confirmed the role of energy loss for gluconeogenesis in KDs and the cost of this process has been estimated of about 400-600 Kcal/day.

Another aspect to be kept into consideration is the specific thermogenic response to food. This parameter calculates the energy expenditure to absorb and metabolise nutrients. For example, if we hypothesize a diet of 2000 Kcal/day with a Mediterranean percentage of carbohydrates, fat and proteins of 55:30:15 and we calculate its thermogenic effect (which correspond to an energy expenditure of 7%, 2.5% and 27% of total caloric intake brought by carbohydrates, fats and proteins respectively) calories actually available would be 1825.5. If we suppose to reduce the percentage of carbohydrates to 20% of total calories and to replace the removed calories with equal percentages of proteins and fats, we obtain another reduction of actually available calories of 80 Kcal. In this case, the actually available daily caloric intake would be of 1757 Kcal (so we "lost" 243 Kcal for thermogenesis). Finally, if we want to sharply reduce the total carbohydrates intake to about the 8% of total caloric intake, we would loose another 40 Kcal, reaching the final level of 1717.8 Kcal/day (Paoli, Canato et al. 2011).

To summarize, the hypothesized mechanisms for weight loss of KDs are, listed in order of available evidence, the following:

1. increased of the energy demanding processes of gluconeogenesis and the thermic effect of proteins;
2. increased fat oxidation and decreased lipogenesis through a strong action of KDs on hepatic gene expression (Volek, Fernandez et al. 2008);
3. greater metabolic efficiency in consuming fats highlighted by the reduction in the resting respiratory quotient (RQ). The RQ represents the ratio between produced CO_2 and consumed O_2 (CO_2/O_2): RQ of sugars is 1, while for a mixture of fatty acids is 0.7 (Paoli 2014).

Considering the reported food control effect, several mechanisms are possibly involved (Paoli, Bosco et al. 2015):

1. reduction in appetite due to the higher intake of proteins which show loss of appetite through several mechanisms (see page 14);

2. reduction of the orexigenic neuropeptide ghrelin (Hall, Bemis et al. 2015) and increase of the anorexigenic neuropeptide cholecystokinin (CCK) (Paoli, Bosco et al. 2015);
3. a direct effect on appetite suppression of the ketone bodies and of circulating free fatty acids (Paoli, Bosco et al. 2015).

Metabolic effects of ketogenic diets such as the increased fat oxidation and the decreased feeling of hunger give advantages to these diets compared to "balanced diets" proved in "real life" studies, which show a greater compliance and then a better weight and fat loss of KDs compared to "balanced diets" (Kris Gunnars 2014). Interestingly, these advantages seem to vanish in studies conducted in metabolic wards, where isocaloric high-carbohydrate diets with equal amount of proteins proved equal or better results of high-carb diets compared to KDs in terms of fat loss or energy expenditure (Hall, Chen et al. 2016, Hall, Bemis et al. 2015).

Pontential risks of ketogenic diets

If we treat KDs as high protein diets, which is not fully correct, risks related to this approach would be those of an excess of protein intake. The most feared negative effect of high protein diets is the impairment of renal function with an increase in glomerular pressure and hyperfiltration (Wyka, Malczyk et al. 2015). However, data in this regard are not uniform and some claim that a high protein intake is not harmful to renal function which can adapt to it (Skov, Haulrik et al. 2002, Martin, Armstrong et al. 2005), while others support the prolonged use of KDs as a therapy for diabetic nephropathy in mice (Poplawski, Mastaitis et al. 2011). Another common side effect of high protein diets is the so called "rabbit malaise", a typical Inuit's malady of early spring when lean rabbits were the only available food, which is characterised by headache and lassitude (Phinney 2004). Finally, fatigue and apparent cardiac dysfunction were experienced in 1976 following the popular Liquid Protein Diet (Phinney 2004). However, KDs, as we previously discussed, are only "relatively" high in protein and therefore these risks are inconsistent.

Regarding the feared risk of acidosis from hyperketonemia seen in untreated type-1 diabetes, such extreme ketone kevels (often above 20mmol/L) are caused by the absence of insulin and unregulated lypolisis and don't appear in fasting or during carbohydrate restriction when ketone levels are controlled (always lower than 12 mmol/L) (Volek, Fernandez et al. 2008) through regulatory mechanisms. Substrate availability for the ketogenic pathway is controlled both by the presence of insulin that impairs lipolysis and by KBs that act as feedback regulators. Moreover, βOHB itself stimulates beta-cell insulin release (Volek, Fernandez et al. 2008) while its production is limited through a feed-back regulation which prevents the excessive exhaustion of fat depots during prolonged starvation with action on GPR109A receptor, also known as niacin receptor (Laeger, Metges et al. 2010). Finally, the regulation of genetic expression of mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase into mRNA has been studied as a control point in the ketogenic pathway (Casals, Roca et al. 1992).

A lower bone density and a more intense onset of bone loss have also been related to the use of KDs. A study on bone mineral content of Alaskan Eskimos reported a normal bone growth among children, but a lower bone density and an earlier and more intense onset of bone loss after age 40 compared to citizens of the same age living in modernized and more sedentary USA. Whether it was the ketogenic state, a vitamin D deficiency and the decreased calcium absorption or the scarcity of alkalinizing fresh plant food that contributed to their earlier and faster bone loss is unclear (Mazess, Mather 1974). Another study tested the effect of 6 weeks of Atkins diet

and reported an increase of urine acidity and calcium excretion as well as a decrease of urinary citrate, an inhibitor of calcium stone formation. Researchers concluded that the Atkins diet *"delivers a marked acid load to the kidney, increases the risk for stone formation, decreases estimated calcium balance, and may increase the risk for bone loss"* (Reddy, Wang et al. 2002). Even if more studies are needed on long-term changes in bone mass or density, the intake of alkalinizing vegetables as well as a high fluid intake could be beneficial during KDs.

Regarding cardiovascular disease and diabetes, there have been doubts about the safety of use of KDs: however, concerns about diabetes mostly appear as long-term conjectures not supported by data (Feinman, Pogozelski et al. 2015), whether those regarding cardiovascular disease are about their long-term safety and better efficacy compared to "balanced" diets (Nordmann, Nordmann et al. 2006) and their risk related to the rise of blood cholesterol and triglycerides (Blackburn, Phillips et al. 2001). However, most recent works show a positive effect of KDs on this pathology (Volek, Phinney et al. 2009, Shai, Schwarzfuchs et al. 2008). Moreover, even if diabetes and cardiovascular disease have, until now, been treated separately, KDs show improvements in both conditions. According to the "Atherogenic Dyslipidaemia (AD)" theory, eating a high saturated fat diet causes increased LDL-cholesterol and triglycerides (TG), reduced HDL-cholesterol and arterial "clogging" and the suggestion to reduce saturated fat to prevent and to treat this condition is common (Volek, Fernandez et al. 2008). However, data show that there is no definitive evidence that dietary animal (saturated) fat causes heart disease (Siri-Tarino, Sun et al. 2010). Moreover, the centenarian Dr. Fred Kummerow has spent 60 years showing how "oxysterols" (oxidized cholesterol) and not "cholesterol" is the real cause the development of arterial plaque (Kummerow, Cook et al. 2001).

"Although the image of coronary arteries as kitchen pipes clogged with fat is simple, familiar, and evocative, it is also wrong" (Rothberg 2013)

Recent evidences show that increased synthesis of larger triglycerides-enriched VLDL and of small LDL particles and reduced HDL-C are caused by a high carbohydrate diet (Volek, Fernandez et al. 2008) and that the TG/HDL ratio has been discovered to be the best predictor of insulin resistance and LDL particle diameter (McLaughlin, Reaven et al. 2005). Diabetes and coronary heart disease could then be seen as a unique disease a high carbohydrate (high fructose) diet with a low intake of omega-3 and a high intake of omega-6 produces toxic changes as increased glucose, insulin, triglycerides, small LDL-C particles, uric acid, CRP, decreased HDL-C and fatty liver (Lee, Min et al. 2016, Raatz, Johnson et al. 2015, Zock, Blom et al. 2016, Lin, Chan et al. 2016). In order to treat both conditions, the single most important intervention in the management of coronary heart disease and diabetes would be to reduce the amount of carbohydrate in the diet, since it is the only intervention that addresses all risk factors. With this reduction, glucose and insulin levels decrease and dietary fats would be processed very differently from the body (Volek, Fernandez et al. 2008).

"The initial treatment for an abnormal carbohydrate/lipoprotein/inflammatory blood profile should be a high fat diet – not the use of statins which provide a marginal benefit in only 1 in 140 treated "healthy" subjects" (Abramson, Rosenberg et al. 2013)

KDs are then supported by strong evidences for their therapeutic action in both diabetes (with a reduction of blood insulin levels and improvement of systemic insulin sensitivity) and cardiovascular risk parameters (with a reduction of total cholesterol and TG and an increase of HDL-C) (Volek, Fernandez et al. 2008, Paoli, Rubini et al. 2013).

EXPERIMENTAL PART

1. LONG TERM SUCCESSFUL WEIGHT LOSS WITH A COMBINATION BIPHASIC KETOGENIC MEDITERRANEAN DIET AND MEDITERRANEAN DIET MAINTENANCE PROTOCOL

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.

Introduction

One of the major problems in weight control is the prevention of weight regain. Despite the majority of randomized controlled trials comparing *ad libitum* KDs with low-fat diets reported a greater weight loss over six months in the former (Nordmann, Nordmann et al. 2006, Shai, Schwarzfuchs et al. 2008, Schwarzfuchs, Golan et al. 2012), KDs are commonly criticized for the so called "yo-yo" effect or weight regain cycle (Jeffery 1996, Sumithran, Proietto 2013, Maclean, Bergouignan et al. 2011).

Recently Sumithran and colleagues have demonstrated that the increases in circulating ghrelin and in subjective appetite, which accompanied a hypocaloric diet, were reduced with a ketogenic approach (Sumithran, Prendergast et al. 2013). Thus, we hypothesized that certain aspects of the ketogenic diet such as muscle mass retention, RMR (resting metabolic rate) and orexigenic hormone stability combined with the acknowledged health benefits of traditional Mediterranean nutrition may favour long term weight loss. The aim of our study was to investigate the effect on weight and body composition of two short periods of a modified ketogenic diet, i.e., a very low carbohydrate ketogenic diet with phytoextracts (KEMEPHY) (Paoli, Cenci et al. 2010, Paoli 2011, Paoli 2012)interspersed between longer periods of maintenance nutrition, based on the traditional Mediterranean diet, over a total period of 12 months in obese/overweight healthy subjects.

Subjects and Methods

We designed this study as a retrospective analysis of the medical records of patients who began the weight loss intervention between 2006 and 2010 in selected medical centres. Patient charts were examined from the first clinical evaluation until after one year of dietary therapy supervised by a healthcare professional. The exclusion criteria from the baseline sample included: endocrine disease and cancer, which might induce weight variation, and severe mental illness. Inclusion criteria was BMI > 30, age between 25 and 65 years. Of 327 patients analysed, 89 obese subjects were selected that underwent a ketogenic Mediterranean diet with phytoextract (KEMEPHY), of these 81 fulfilled our inclusion criteria for this retrospective analysis: no use of antidepressant drugs, no diabetes and no change in quantity and quality of physical activity during the analysed time period. Of these 81 subjects, 68 completed the one year follow up protocol (84%). The general characteristics of the 68 remaining subjects analysed were: age 49.17 ± 10 years, height 167 ± 10 cm, weight 100.67 ± 16.54 kg, BMI 35.82 ± 4.11. All subjects were Caucasian (59 males, 12 females). During the first medical visit (t0) subjects

were educated on the KEMEPHY protocol and underwent anthropometric measurement, body composition and blood analysis. The year of treatment involved:

- An initial 20 days of very low carbohydrate ketogenic diet (K1)
- Followed by 20 days of a low carbohydrate non ketogenic diet (stabilization) (LC1)
- A first period of 4 months of normalcaloric Mediterranean diet (M1)
- A second 20 day very low carbohydrate ketogenic diet (K2)
- 20 Days of low carbohydrate non ketogenic diet (LC2)
- Final 6 months of normal caloric Mediterranean diet (M2)

During the KEMEPHY period the subjects followed a commercially available protocol called TISANOREICA® (Paoli, Cenci et al. 2010a, Paoli 2011, Paoli 2012). Two weight loss intervention periods were used as previously available clinical data (unpublished) suggested that this was required to achieve a 10% weight loss. Subjects were analysed at seven time points: before starting the diet (t0), after K1 (t1), after LC1 (t2), after M1 (t3), after K2 (t4), after LC2 (t5) and after M2 i.e., approximately one year after first visit (t6) (Figure 1.1). All subjects gave their informed consent to data use and the study was approved by the Ethical Commission of the Department of Biomedical Sciences of the University of Padova. Efforts to maximize retention in the protocol included e-mail and telephone reminders for appointments and a weekly phone call to verify compliance.

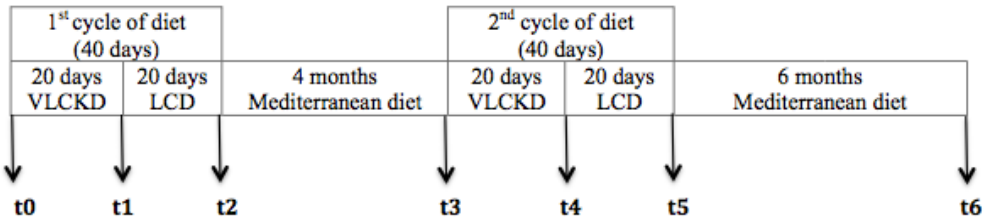


Figure 1.1. Experimental design: t0 = start (medical examination, anthropometric and body composition analysis, blood analysis); t1 = end of K1 (medical examination, anthropometric and body composition analysis); t2 = end of LC1 (medical examination, anthropometric and body composition analysis, blood analysis); t3 = start of K2 (medical examination, anthropometric and body composition analysis); t4 = end of K2 (medical examination, anthropometric and body composition analysis); t5 = end of LC2 (medical examination, anthropometric and body composition analysis); t6 = one year recall (medical examination, anthropometric and body composition analysis, blood analysis). VLCKD = very low carbohydrate ketogenic diet, LCD = low carbohydrate diet.

Diet Protocols

Each subjects received personal diet explanation by a qualified dietician during an individual visit. Dietary intake was measured with a validated 3-day food diary (Toeller, Buyken et al. 1997) and analysed by Dietnext® software (Caldogno, Vicenza, Italy). In the KEMEPHY protocol subjects almost totally exclude carbohydrates during the first three weeks. A detailed menu containing permitted and non-permitted foods was provided to each participant, along with the components of the ketogenic Mediterranean diet with phytoextracts. The diet consumed was primarily made of beef & veal, poultry, fish, raw and cooked green vegetables without restriction, cold cuts (dried beef, carpaccio and cured ham), eggs and seasoned cheese (e.g., parmesan). The drinks allowed were infusion tea, moka coffee and herbal extracts. The foods and drinks that subjects avoided included alcohol, bread, pasta, rice, milk, yogurt, soluble tea and barley coffee. In addition to facilitate the adhesion to the

nutritional regime, each subject was given a variety of specialty meals constituted principally of protein and fibers. These meals (TISANOREICA®, Asigliano Veneto, Vicenza, Italy) that are composed of a protein blend obtained from soya, peas, oats (equivalent to 18 g/portion) and virtually zero carbohydrate (but that mimic their taste) were included in the standard ration (Paoli 2011). During the KEMEPHY protocol, the subjects also consumed some specific herbal extracts useful to ameliorate the commonly reported symptoms of weakness and tiredness during the ketosis, improve glycaemic control and increase bile secretion helping digestion (choleretic effect) (Paoli, Cenci et al. 2010, Paoli 2011) (herbal blends are described in tables 1.4 and 1.5). During the ketogenic diet periods, subjects assumed 1 caplet in of a multivitamin-mineral supplement each morning (Gaby 2007, Zupec-Kania, Zupanc 2008, Phinney, Horton et al. 1980). Caplets (Multivitaminico Balestra e Mech, Gianluca MechSpA, Asigliano Veneto, Vicenza, Italy) contained Magnesium 19 mg, Calcium 16 mg, Phosphorus 8 mg, Zinc 4.5 mg, Iron 4.62 mg, Manganese 1 mg, Potassium 0.5 mg, Copper 0.4 mg, Chromium 28.55 µg, Selenium 4 µg, Niacin 10 mg, Beta carotene 1.8 mg, Folic Acid 66 µg, Biotin 30 µg, Vitamin C 19.8 mg, Vitamin E 3.3 mg, Pantothenic Acid 1.98 mg, Vitamin B6 0.66 mg, Vitamin B2 0.53 mg, Vitamin B1 0.426 mg, Vitamin D3 1.65 µg, Vitamin B12 0.33 µg.

During the months of the Mediterranean diet, subjects were instructed to follow a typical diet composed mainly of, whole grains (bread, pasta, whole wheat, rice), potatoes, meat, fish, eggs, poultry, vegetables, legumes, fruits, condiments (mainly olive oil), whole milk and wine. During the Mediterranean diet period the average macronutrients distribution was: 58% carbohydrate, 15% protein and 27% lipids (Kcal 1800 ± 108); the main sources of added fat were 30 to 50 g of olive oil per day (Shai, Schwarzfuchs et al. 2008). During the ketogenic period the prescribed daily intake of carbohydrate was about 30 g per day and the energy distribution of daily macronutrients was 12% carbohydrate, 36% protein and 52% lipids (Kcal 976 ± 118). During the low carbohydrate period the distribution was 25% carbohydrate, 31% protein and 44% lipids (Kcal 1111 ± 65) (Table 1.1).

Table 1.1. Characteristics of diets (data are expressed as mean and SD).

Macronutrients	Ketogenic phase	Lowcarbohydrate phase	Mediterranean phase
Kcal/day	976 ± 118	1111 ± 65	1800 ± 248
Protein (% total daily Kcal)	41 ± 2	27 ± 2	15 ± 2
Fat (% total daily Kcal)	46 ± 4	41 ± 2	27 ± 3
Carbohydrate (% total daily Kcal)	12 ± 2	33 ± 2	58 ± 4
Protein (g/day)	100 ± 11	74 ± 11	67.5 ± 9
Fat (g/day)	51 ± 9	50 ± 2	54 ± 6
Carbohydrate (g/day)	30 ± 0.2	91 ± 5	261 ± 18

Analysis

Dietary intake was measured by validated 3-day food diary (Toeller, Buyken et al. 1997b, Black, Skidmore et al. 2012) and analysed by Dietnext® (Caldogno, Vicenza, Italy) software. At each visit (timepoint t1–t6) subjects underwent anthropometric measurement, blood pressure measurements and body composition analysis, the latter

was assessed using bioelectrical impedance analysis (BIA Akern Bioresearch, Pontassieve, FI, Italy) which is a non-invasive and portable method for the estimation of fluid compartments, fat and fat-free mass in healthy subjects. Bioelectrical impedance analysis was chosen because it is a reliable method and its safety, convenience and non-invasive nature makes it useful procedure to be deployed in the routine monitoring of body composition also during the ketogenic diet (Piccoli, Brunani et al. 1998, Saunders, al-Zeibak et al. 1993). At t0, t3, and t6 fasting venous blood samples were collected at weeks 0 and 6 for total cholesterol (CHOL-T), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glucose, blood urea nitrogen (BUN), uricemia, eythrocyte sedimentation rate (ESR), creatinine, alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT). Blood was collected in EDTA treated vacutainer tubes. All measurements were made on a ROCHE/HITACHI 912, (Roche Diagnostics Ltd., Basel, Switzerland). Fasting total cholesterol was measured with an enzymatic colorimetric method. HDL-C and LDL-C were measured by an enzymatic colorimetric test in homogenous phase. Triglycerides with an enzymatic colorimetric TRINDER modified final point method. Plasma glucose was determined with the plasma glucose GOD-PAP enzymatic colorimetric method. Plasma urea nitrogen was measured using UV kinetic test. ALT and AST with IFCC enzymatic kinetic method. GGT with the SZASZ (kinetic photometric method), colorimetric method. Creatinine was measured using the JAFFE method with compensation, kinetic colorimetric test, and uric acid was determined using an enzymatic colorimetric method.

Statistical Analysis

The effect of the diet intervention was assessed using one way repeated-measures ANOVA. For body weight and fat percentage the measurements at t0, t1, t2, t3, t4, t5 and t6 were considered. For blood variables only t0, t3 and t6 time points were taken into account. When significant effects were found, post hoc analysis was performed using Tukey's test. An alpha level of $p < 0.05$ was used to denote a significant effect. Kolmogorov-Smirnov tests were used to assess the normality of the data. Mauchley's test of sphericity assessed the homogeneity of variance for the data. All statistical analyses were performed using the software package GraphPad Prism version 6.00 for Mac (San Diego, CA, USA). Values are represented as means and standard deviation (SD).

Results

There was a significant decrease in body weight after the first ketogenic period ($p < 0.0001$ t0 vs. t1); there was no significant difference between t1 and t2 nor between t2 and t3. At t4 body weight was significantly decreased compared to t3 and t1 ($p < 0.01$ and $p < 0.001$ respectively) after which it stabilized with no further significant changes at t5 and t6 (see Figure 1.2 A and Table 1.2). The same pattern was seen also for body fat percentage (see Figure 1.2 B and Table 1.2). Comparing bodyweight and body fat at t6 with t0 revealed that after one year there was an overall significant decrease in both parameters with no signs of weight regain over the course of the study. Systolic blood pressure showed a significant decrease comparing t0 vs. t2 (from 125 ± 10 to 117 ± 6 $p < 0.01$) whilst there was a decrease albeit not significant of diastolic blood pressure (from 86 ± 5 to 82 ± 8).

Table 1.2. Changes in body weight and body fat percentage during one year diet protocol. Values are expressed as mean and standard deviation. Significance was reported in Results section.

Anthropometric Data	t0	t1	t2	t3	t4	t5	t6
Body weight (kg)	100.7 ± 16.54	93.34 ± 15.04	90.33 ± 13.57	91.81 ± 12.58	86.64 ± 10.56	84.2 ± 10.04	84.59 ± 9.71
% Body fat	43.44 ± 6.34	36.93 ± 6.49	36.26 ± 6.46	37.15 ± 6.82	34.46 ± 6.3	33.50 ± 6.18	33.63 ± 7.6

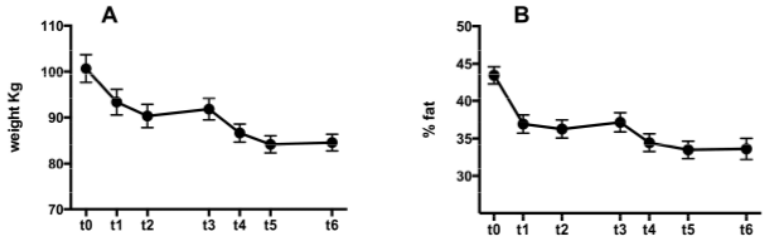


Figure 1.2. Changes in body weight (A) and fat percentage (B) from baseline to month 12. Error bars indicate standard error of the mean.

Blood measurements (Table 1.3) revealed a significant decrease in total cholesterol at t2 ($p < 0.0001$) vs. t0, followed by non-significant differences between t2 and t6 and a significant decrease comparing t6 with t0 ($p = 0.0003$). HDL-C showed a significant increase after the ketogenic and low-carbohydrate phases (K1 and LC1; at t2) but after 1 year there was overall no significant difference compared to starting (t6 vs. t0), the increase seen was short term. LDL-C also decreased at t2 and in contrast to HDL the decrease remained significant throughout the study ($p < 0.0001$ t2 vs. t0; $p = 0.004$ t6 vs. t0). TG declined significantly at t2 compared to t0 ($p = 0.0006$) and for t6 vs. t0 ($p = 0.01$) although there was no significant difference between t2 and t6. Finally blood glucose decreased significantly at t2 vs. t0 ($p < 0.0001$) after which it rose but at the end of the 12 months it was still significantly lower at t6 vs. t0 ($p = 0.0004$). No significant changes were observed in ALT, AST, GGT, Creatinine or BUN.

Table 1.3. Changes in blood biochemical and pressure parameters at baseline (t0), after first period of ketogenic diet and low carbohydrate diet (t2) and after one year from the start (t6). Values are expressed as mean and standard deviation.

Blood Parameters	t0	t2	t6	t0 vs t2	t0 vs t6	t2 vs t6
Chol-tot	193.2±37.87	171.9±31.94	179.8±32.42	$p < 0.0001$	$p = 0.0003$	n.s.
HDL-c	43.03±6.09	49.59±8	44.59±8	$p < 0.0001$	n.s.	$p < 0.001$
LDL-c	144.5±58.4	108.0±42.66	122.9±42.25	$p < 0.0001$	$p = 0.0004$	$p < 0.0001$
TG	112.7±61.02	88.62±40.65	95.45±39.99	$p = 0.0006$	$p = 0.0106$	n.s.
Glu	102.6±11.5	90.31±8.45	95.31±8.45	$p < 0.0001$	$p = 0.0004$	$p < 0.0001$
ALT	18.75±11.6	16.53±6.72	17.11±9.3	n.s.	n.s.	n.s.
AST	18±8.69	17.13±7.2	17.76±5.43	n.s.	n.s.	n.s.
GGT	20.68±16.16	16.1±5.3	17.8±6.8	$p = 0.012$	$p < 0.05$	n.s.
Creatinine	0.79±0.16	0.76±0.07	0.77±0.1	n.s.	n.s.	n.s.
BUN	15.87±3.83	16.1±85.29	15±3.87	n.s.	n.s.	n.s.
Uric acid	4.56±0.86	4.2±0.64	4.01±0.91	$p < 0.01$	$p < 0.05$	n.s.
SBP	125 ± 10	117±6	118±4	$p < 0.01$	$p < 0.01$	n.s.
DBP	86±5	82±8	82±5	n.s.	n.s.	n.s.

n.s. = not significant.

Discussion

There is no universally accepted definition of "successful weight loss maintenance" following a diet, but a reasonable candidate would be that proposed by Wing and Hill in 2001, which defines it as "individuals who have intentionally lost at least 10% of their body weight and kept off at least one year" (Wing, Hill 2001). The criterion of 10% is chosen for its well-documented effects in the improvements in risk factors for diabetes and cardiovascular disease, while the one year duration criterion was proposed in agreement with the USA Institute of Medicine (Thomas 1995). The data from our present study suggest that two brief periods of a "Mediterranean" variant on the VLCKD theme (which we call KEMEPHY) are able to induce significant weight and body fat loss that was maintained for at least one year. In particular, the weight loss reached at six months, after the second cycle of VLCKD, was maintained, without weight regain, over the subsequent six months of normocaloric Mediterranean nutrition.

The Mediterranean diet is associated with a longer life span (Trichopoulou, Kouris-Blazos et al. 1995), lower rates of coronary heart disease (Chahoud, Aude et al. 2004), hypercholesterolemia (Perona, Cabello-Moruno et al. 2006), hypertension, diabetes and obesity (Richard, Couture et al. 2013). However, it is difficult to isolate the "healthy" constituents of the Mediterranean diet, since it is not a single entity and varies between regions and countries. Since there is no "one size fits all" dietary recommendation, we have tried to merge the benefits of these two approaches: the long term "all-life" Mediterranean diet coupled with brief periods of a metabolism enhancing ketogenic diet.

Data from this study report that the majority of subjects maintained > 10% weight loss at 12 months, while 8 subjects didn't (Figure 1.3). These 8 subjects were included in the final statistical calculations, but the post dietary analysis showed that they were not compliant with the nutritional guidelines given for the Mediterranean diet period and they returned to their previous nutritional habits ("junk" food, high glycaemic index. etc). This is not in agreement with previous data, which show that low carb diets show lower compliance in the long term compared to low fat diets (Greenberg, Stampfer et al. 2009).

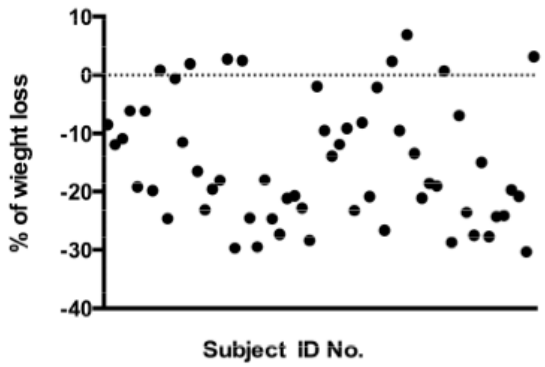


Figure 1.3. Changes in weight (% of change) of each subject (t6 compared to t0). Basal value is represented by the line zero. Each circle represents a single subject.

Many others factors beyond the return to previous nutritional habits can be involved in weight regain. These includes resting metabolic rate (RMR), insulin & leptin resistance and changes in the levels of several hormones involved in the homeostatic regulation of body weight (Sumithran, Prendergast et al. 2011). RMR could be affected by the loss of muscle mass due to an inadequate protein intake during dieting. The suggested daily protein consumption is around 15% in a classical hypocaloric Western diet of about 1200 Kcal/day so the actual

protein content will be approximately 45 g (180 Kcal; 4 Kcal/g). Hypothesizing a body weight of 70 kg this daily protein intake will be 0.64 g per kilogram of body weight, which is a possible cause of muscle loss and consequent reduction of resting energy expenditure. The VLCKD on the other hand appears not to influence (either positively or negatively) the basal energy expenditure (Paoli, Grimaldi et al. 2012). Regarding hormonal influences on weight regain a possible explanation involves a long-term increase in orexigenic signals. Sumithran and co-workers showed that a very low calorie diet causes a persistent elevation of the circulating mediators of appetite that encourages weight regain even one year after initial weight reduction (Sumithran, Prendergast et al. 2011). On the other hand it has been demonstrated that a ketogenic diet has only a minor effect on ghrelin levels and that the subjective ratings of appetite were lower when participants were in a state of physiological ketosis (Sumithran, Prendergast et al. 2013).

Conclusions

In summary the data from this study demonstrate that the majority of subjects showed significant weight loss (10%) as a result of a two-phase VLCKD and were compliant both during the six month weight loss phase and the six month normocaloric maintenance phase, with no weight regain. We can suggest that the proposed protocol was generally successful because of (a) the protein mass protective effects of a VLCKD and (b) the prescription of a traditional Mediterranean diet in the post weight-loss phase was especially important for achieving "weight loss success", i.e., continued weight loss for at least one year.

Table 1.4. Herbal extracts used during KEMEPHY diet.

Plant extracts	VLCKD	LCD	Composition
Extracts A, ml/day	20	20	Durvillea Antartica, Black Radish, Mint, Liquorice, Horsetail, Burdock Dandelion, Rhubarb, Gentian, Lemon Balm, Chinaroot, Juniper, Spear Grass, Elder, Focus, Anise, Parsley, Bearberry, Horehound
Extracts B, ml/day	20	20	Serenoa, Red clover, Chervil, Bean, Elder, Dandelion, Uncaria, Equisetum, Horehound, Rosemary
Extracts C, ml/day	50	50	Horsetail, Asparagus, Birch, Cypruss, Couch Grass, Corn, Dandelion, Grape, Fennel, Elder, Rosehip, Anise
Extracts D, ml/day	40	0	Eleuthero, eurycoma longifolia, ginseng, corn, miura puama, grape, guaranà, Arabic coffee, ginger

Table 1.5 Main actives ingredients of used phytoextracts, their reported effects and related references.

Extract	Main Active Ingredients	Reported beneficial effects	Refs
A	Mint, black radish, burdock	Indigestion Antioxidant Choleretic, increases bile secretion helping digestion	(Lugasi, Blazovics et al. 2005, Lou, Wang et al. 2010)
B	Serenoa Repens (saw palmetto)	Hormonal regulating effects	(Di Silverio, D'Eramo et al. 1992)
	White bean	Alpha-amylase inhibitory properties and has been reported to aid weight loss and glycaemic control	(Barrett, Udani 2011, Celleno, Tolaini et al. 2007)
C	Equisetum	Antioxidant Diuretic Glycaemic control	(Mimica-Dukic, Simin et al. 2008, Safiyeh, Fathallah FB, et al. 2007)
	Dandelion (Taraxacum officinale)	Diuretic	(Clare, Conroy et al. 2009)
D	Ginseng, Miura Puama, Guaranà	Ameliorate the commonly reported symptoms of weakness and tiredness during the initial VLCKD phase	(Pieralisi, Ripari et al. 1991, Piato, Detanico et al. 2010, Lima, Carnevali et al. 2005)

2. THE EFFECTS OF DIFFERENT HIGH-PROTEIN LOW-CARBOHYDRATES PROPRIETARY FOODS ON BLOOD SUGAR IN HEALTHY SUBJECTS

Lodi A, Karsten B, Bosco G, Gómez-López M, Brandão PP, Bianco A, Paoli A, *The Effects of Different High-Protein Low-Carbohydrates Proprietary Foods on Blood Sugar in Healthy Subjects.* J Med Food, 2016.

Introduction

Although it is recognized that the VLCKDs lead to greater weight losses than a low-calorie balanced diet at least in the short term (Bueno, de Melo et al. 2013), subjects with a sweet food preference may not adhere to this diet because of the lack of their preferred taste (Shai, Schwarzfuchs et al. 2008b, McVay, Voils et al. 2014).

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), whereas sweet taste is associated with energy and nourishment (Drewnowski, Krahn et al. 1992).

Populations experiencing an increase in obesity and CV diseases show common eating and drinking habits, notably a general decrease in intake of minimally processed foods and in an increase in the consumption of ultraprocessed ready-to-consume products. These foods, based on the accepted definition acknowledged by the Pan American Health Organization (PAHO WHO 2015) 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 ultraprocessed foods are savory and sweet snacks, ice cream, frozen and chilled ready meals, and soft drinks (PAHO WHO 2015) and they seem to be the cause of the extra daily diet calorie intake of both the young and the older populations (Monteiro, Moubarac et al. 2013). Ultraprocessed 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 flavorsome, and are relatively inexpensive.

However, when analyzing ultraprocessed products, less protein, potassium, and dietary fiber and more free sugar, total saturated and transunsaturated fats, and sodium are generally evident when compared with traditional foods (Monteiro, Levy et al. 2011, Moubarac, Batal et al. 2014). All these characteristics appear to be linked to the burden of obesity and metabolic syndrome (MetS) (Monteiro, Moubarac et al. 2013).

A potential solution to this scenario could be to review ultraprocessed, ready-to-consume products by reduction of their sugar and fat contents. A particular kind of these new ultraprocessed ready-to-consume products are proprietary foods that 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 also successfully used in more easy low-carbohydrate (CHO) diets as snacks or meal replacements (Paoli, Cenci et al. 2011). During ketosis, CHO intake must be under 30g/day (Paoli 2014, Paoli, Rubini et al. 2013d) and in previous studies (Paoli, Bianco et al. 2013, Paoli, Cenci et al. 2011) 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 behavior, because it is known that meal

replacement during the maintenance phase is useful to prevent weight gain (Vázquez, Montagna et al. 2009). During consumption of a VLCKD, it is mandatory to maintain a low level of glycaemia (about 80–90 mg/dL) to avoid insulin spikes (Paoli, Canato et al. 2011). This condition allows subjects to improve their fat oxidation as demonstrated by Paoli et al. (Paoli, Grimaldi et al. 2012) and by Tagliabue et al. (Tagliabue, Bertoli et al. 2012).

Another important aspect of a VLCKD is the influence of such dietary regimen on the perception of hunger (Gibson, Seimon et al. 2015). It has been suggested that ketone bodies reduce hunger through different and complex mechanisms (Paoli, Bosco et al. 2015); in contrast it is known that postprandial glucose and insulin spikes, typically produced after the intake of traditional ultraprocessed products that usually show a high glycaemic index (GI) (PAHO WHO 2015), elicit food craving and overeating, with a preference for high-GI CHOs(Lennerz, Alsop et al. 2013) a phenomenon defined as the CHO-craving effect (Ventura, Santander et al. 2014). Conversely, the consumption of nonprocessed foods low in simple sugars may ameliorate overeating and facilitate the maintenance of a healthy weight (Lennerz, Alsop et al. 2013).

The mentioned positive changes necessitate the need to analyze the effect of different high-protein low-CHO proprietary foods that are commonly used in diets, i.e. during VLCKD and low-CHO diets (LCD), on glycaemia compared with glucose.

Materials and Methods

Subjects were recruited through advertisement placed in two pharmacies located in the province of Vicenza. Exclusion criteria for this study were the presence of diabetes or prediabetes, being on a food diet, and females who were either pregnant or breast-feeding. After a preselection process of 32 participants, 14 females were eligible to participate in this 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 the exclusion from the study. 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 before the start of the study. Subjects were required to sign a participation consent form and they did not receive any monetary compensation.

Subjects were tested for individual glycaemic response curves elicited by the ingestion of 1000 kJ of glucose three times within a 3-week period (one test per week) and that of each of 10 high-protein low-CHO proprietary test foods once on separate days twice a week (Fig. 2.1). Tests were performed in the morning after 10–12 h 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.

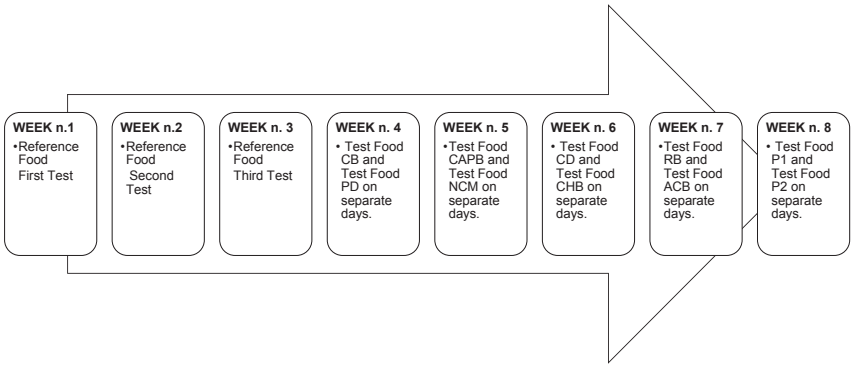


Figure 2.1. Experimental design

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*(Bijster 1993, Richter, Rassoul et al. 2007, Warnick, Boerma et al. 1993). Postprandial effect of sugar content on glycaemia is commonly defined through three methods: the GI, the glycaemic load (GL), and the glycaemic score (GS).

The GI method was developed to rank foods according to the extent to which they increase blood sugar concentrations (Holt, Miller et al. 1997) and it is a number that 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 h postprandial period is calculated. This value is consequently expressed as a percentage of the incremental AUC after the consumption of a reference food (commonly white bread or glucose) consumed by the same person on a different day (FAO 1998). The test food and the reference food must contain the same amount of available CHO (25 or 50g) and the individual has to perform the test under standardized conditions.

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

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 (Bao, Atkinson et al. 2011).

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

The GS method tests the glycaemic 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 glycaemia of a 1000 kJ portion of both test food and reference food (Bao, Atkinson et al. 2011).

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

Due to the very low-CHO content of the tested foods and because of the quantity of food required to reach the 25 g of available CHO for the calculation of GI being too large, this study utilized the GS method (Bao, Atkinson et al. 2011).

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 does not alter the incremental area under the glycaemic response curve (Brouns, Brouns et al. 2005)) and consumed within a period of 10 min. This 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 an LCD regimen (Paoli, Bianco et al. 2013, Paoli, Cenci et al. 2011, Paoli, Grimaldi et al. 2012b).

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], and 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 bread- sticks [RB; T-Smech], and the pizza dough [PD; Pizza Dough]) (Table 2.1). Glucose was used as reference food. This was dissolved in 220 mL of water and served as 1000 kJ portions (15.68kJ/g) (Plowman, Smith 2013) and had to be consumed within a 10 min period.

Table 2.1. Test Foods Characteristics

Test food name	*kJ/100 g*	*Protein/100 g, g*	*CHO/100 g (of which sugars/ of which polyalcohols), g*	*Fat/100 g (of which saturated), g*	*Fiber/100 g, g*	*Salt/100 g*
CB	1666.35 kJ/100 g	23.5	18 (0.1/14.5)	25.3 (11.2)	37.1	—
PD	1356 kJ/100 g	53.2	14.2 (1.6)	2.4 (0.9)	16.4	887 mg
CAPB	1498.87 kJ/100 g	34	10.9 (4/4.3)	14.4 (7.5)	28	1.13 g
NCM	1570 kJ/100 g	15.5	35.3 (0.1/30.1)	22.6 (5.5)	7.4	1.3 g
CD	1515.62 kJ/100 g	62.2	18.2 (0.4)	3.4	9.8	—
CHB	1775.2 kJ/100 g	6.1	51.6 (30.1/18.9)	28.2 (12)	7.6	0.02 g
RB	1900 kJ/100 g	49.10	8.96 (0.06)	21.06 (6.07)	16.12	0.80 g
ACB	1678 kJ/100 g	31.36	5.88 (0.06)	21.32 (12.42)	30.13	0.55 g
P1	962 kJ/100 g	44	5.9 (0.03)	3.3	34	720 mg
P2	1389 kJ/100 g	50	22.5 (0.7)	2.1 (1.6)	12.9	0.05 g

ACB, apple and cinnamon biscuits; CAPB, chocolate–almonds–pistachio bar; CB, chocolate biscuits; CD, chocolate drink; CHB, chocolate and hazelnut balls; CHO, carbohydrate; NCM, nuts and chocolate muffin; P1, pasta type 1; P2, pasta type 2; PD, pizza dough; RB, rosemary breadsticks.

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 an XY data table by selecting the AUC analysis. The effect of each test food on glycaemia compared with that of the reference food over time was assessed using a mixed model ANOVA (time · treatment). A post hoc Sidak's multiple comparison test was performed.

To select those test foods with a significant difference of blood sugar values compared with 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 stocked into a subcolumn. Tukey's multiple comparison test was chosen 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, and 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 glycaemia, and mean glycaemia in the different time points of the 10 test foods and the reference food among the subjects tested are listed in Table 2.2. Mean glycaemia after taking the reference food glucose resulted in 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 2.2 shows the comparison of mean blood sugar concentrations at the different time points between glucose and sweet test foods, whereas Figure 2.3 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 with that after the intake of the reference food glucose after 15, 30, 45, 60, and 90 min, although several test foods (CHB, CAPB, NCM, ACB, PD, and RB) were able to maintain this significance even after 120 min.

Figure 2.4 shows the comparison of blood sugar concentrations between sweet test foods and savory test foods. Comparison of mean blood sugar concentrations at the seven different time points highlighted a significant higher increase of glycaemia, particularly 15 and 30 min after taking the CHB and the two kinds of pasta P1 and P2 compared with the other test foods (Fig. 2.4).

In particular, the mean glycaemia increased significantly 15 and 30 min after the intake of CHB compared with the mean glycaemia after the ingestion of the CB, the CAPB, the RB, and the PD (Fig. 2.5).

After the intake of P2, the mean glycaemia increased significantly after 15 and 30 min. compared with the sweet test foods CB, CAPB, and NCM and with the savory test foods PD and SB (Fig. 2.6).

After the intake of P1, the mean glycaemia increased significantly after 15 and 30 min. compared with the sweet test foods CB and CAPB and with the savory test foods PD and RB (Fig. 2.7).

The statistical two-way ANOVA of the trend of blood sugar from before starting to eat up to 2 h after the intake of the reference food or of the test foods shows that, on average, the 40% of the total variation observed is because of the difference between the foods eaten (glucose or test foods). This result shows that, among all the ‘‘Sources of Variation’’ analysed (time, food, and 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 did not 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 (Fig. 2.8).

The average GS of each test food, calculated as the mean of GS values of each test food resulted from every subject, was always less than 25 compared with the GS reference value of glucose, which is 100 (Table 2.2). 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 is the sweet test food Cioco Mech and T-Smart, with a GS of 14.

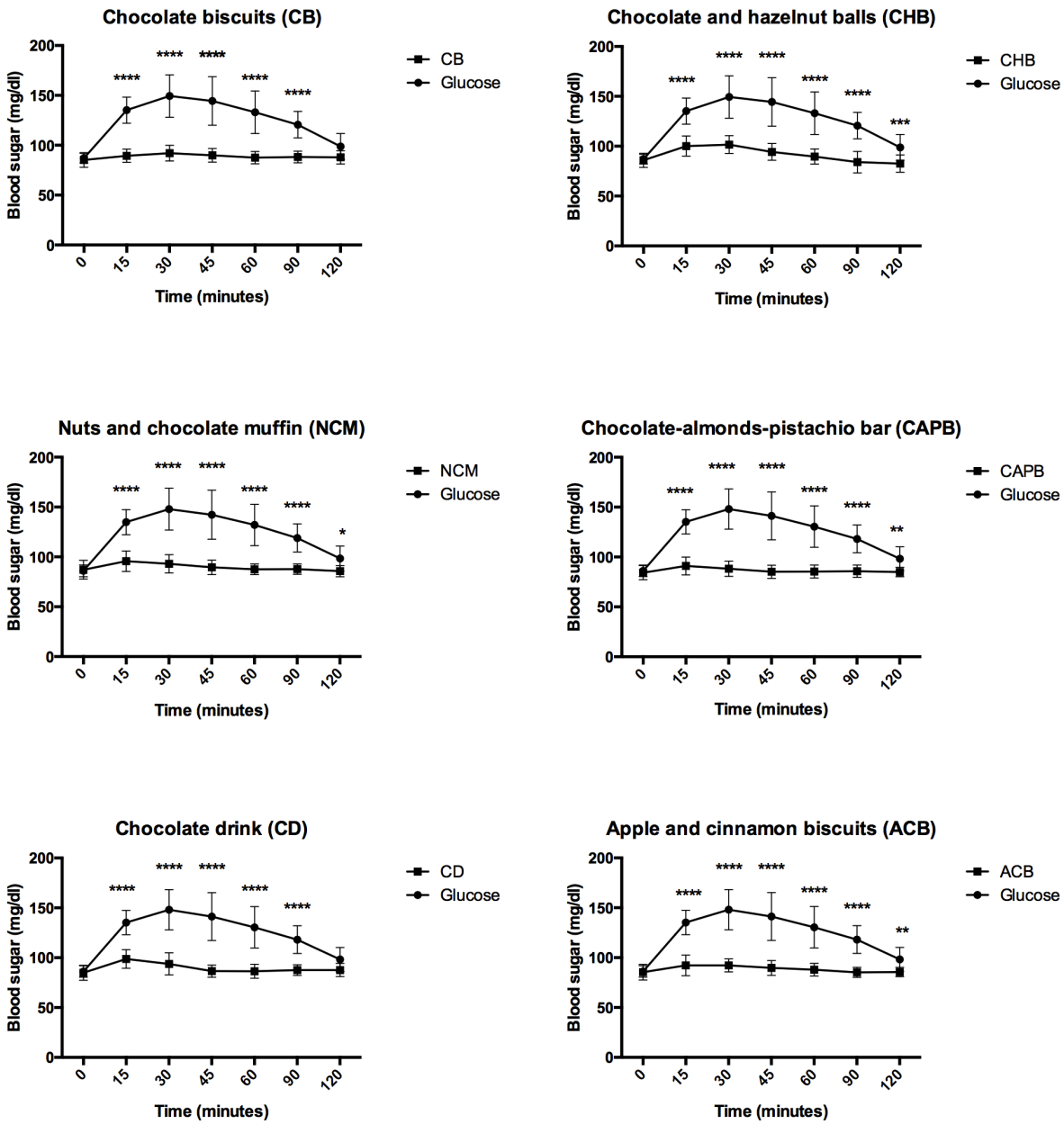


Figure 2.2. Comparison of mean blood sugar concentrations between glucose and sweet test foods (*P <0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001). Foods were tested among 14 healthy subjects. All tested foods and the reference food glucose were served as a 1000 kJ portion and consumed within 10 min.

Table 2.2. Mean GS, mean glycaemia and mean glycaemia in the different time points of the 10 test foods and of the reference food glucose.

Food tested	*Glucose*							*CB*							*CHB*						
Time (mins)	0	15	30	45	60	90	120	0	15	30	45	60	90	120	0	15	30	45	60	90	120
Mean glycemia (mg/dL) at different time points	86±6	135±12	148±20	141±24	130±20	118±14	98±12	85±7	90±6	89±6	92±8	90±7	88±6	88±7	86±7	100±10	102±9	94±8	90±8	84±11	83±8
Mean glycemia (mg/dL)				122±15							89±7							91±9			
Mean GS				100							14±9							23±15			

Food tested	*CD*							*ACB*						
Time (min)	0	15	30	45	60	90	120	0	15	30	45	60	90	120
Mean glycemia (mg/dL) at different time points	85±7	99±9	94±11	86±6	86±7	88±5	88±6	85±8	92±10	92±6	90±7	88±6	85±5	86±5
Mean glycemia (mg/dL)				89±7							88±7			
Mean GS				18±10							19±7			

Food tested	*NCM*							*CAPB*							*PD*						
Time (min)	0	15	30	45	60	90	120	0	15	30	45	60	90	120	0	15	30	45	60	90	120
Mean glycemia (mg/dL) at different time points	87±9	96±10	93±9	89±7	88±5	88±5	86±6	84±7	91±9	88±8	85±7	85±6	86±6	85±5	86±6	98±12	89±6	88±5	86±7	85±4	84±6
Mean glycemia (mg/dL)				89±7							86±7							88±6			
Mean GS				17±8							14±7							15±7			

Food tested	*RB*							*P2*							*P1*						
Time (min)	0	15	30	45	60	90	120	0	15	30	45	60	90	120	0	15	30	45	60	90	120
Mean glycemia (mg/dL) at different time points	82±7	90±7	91±8	90±7	89±8	88±6	86±5	88±8	106±13	100±13	89±10	85±7	87±4	88±6	87±8	101±10	99±10	94±5	94±8	93±9	90±6
Mean glycemia (mg/dL)				88±7							92±9							94±8			
Mean GS				18±8							19±7							21±11			

GS, glycemic score.

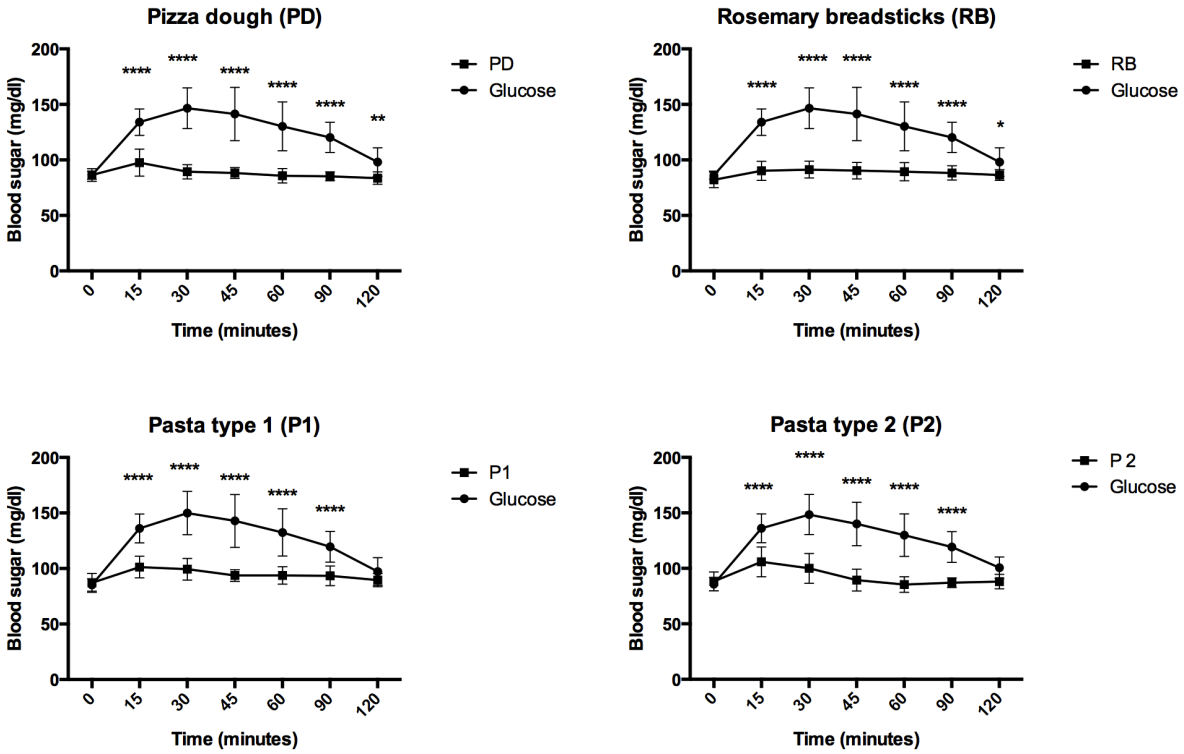


Figure 2.3. Comparison of blood sugar concentrations between glucose and savory test foods (*P<0.05; **P<0.01; ****P<0.0001). Foods were tested among 14 healthy subjects. All tested foods and the reference food glucose were served as a 1000 kJ portion and consumed within 10 min.

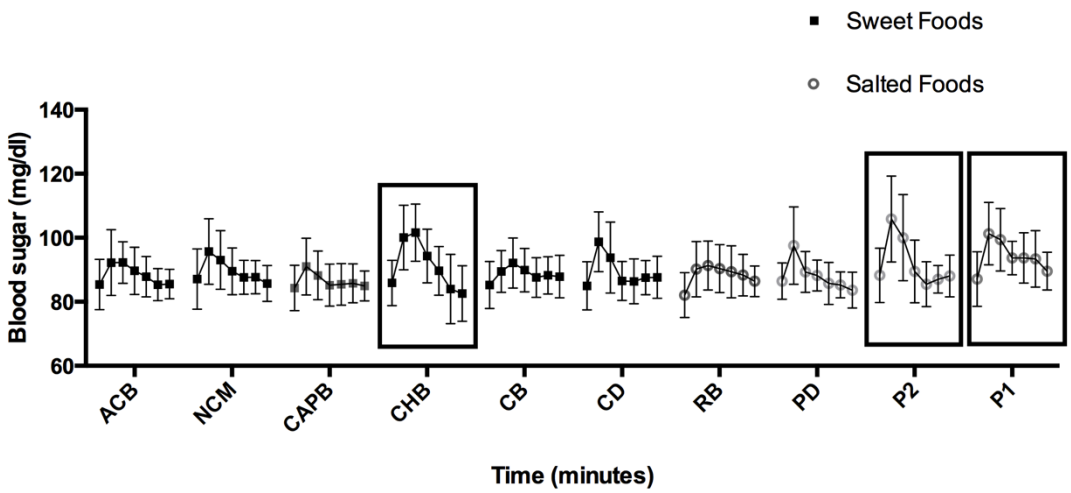


Figure 2.4 Comparison of blood sugar concentrations between sweet test foods and savory test foods. Among sweet test foods, the CHB show a higher increase in blood sugar than other test foods (significant differences are shown in Fig. 5). Among savory test foods, both pasta type 2 (P2) and pasta type 1 (P1) show a higher blood sugar trend (significant differences are shown in Figs. 6 and 7). CHB, chocolate and hazelnut balls.

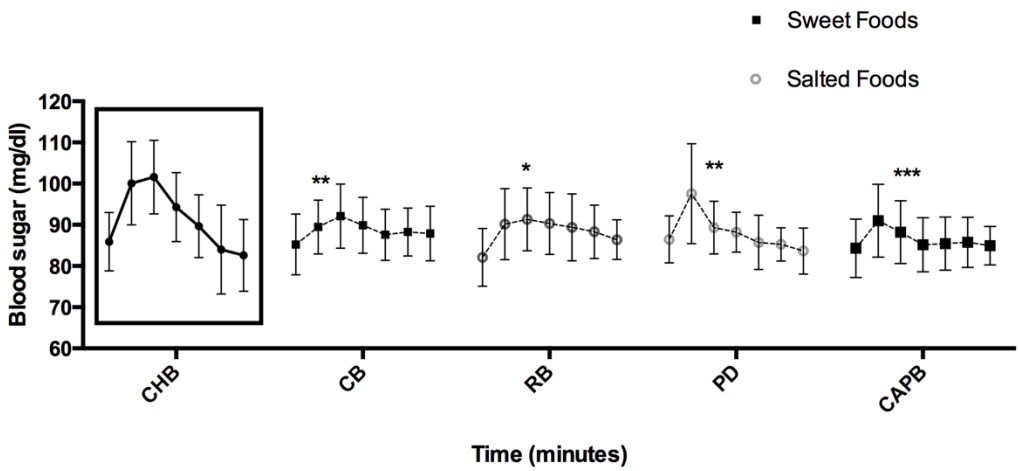


Figure 2.5. Comparison of mean blood sugar concentrations between the CHB and the other test foods. Results show significant differences after 15 or 30 min between CHB and CB, RB, PD, and CAPB. *P <0 .05; **P < 0.01; ***P < 0.001. CAPB, chocolate– almonds–pistachio bar; CB, chocolate biscuits; PD, pizza dough; RB, rosemary breadsticks.

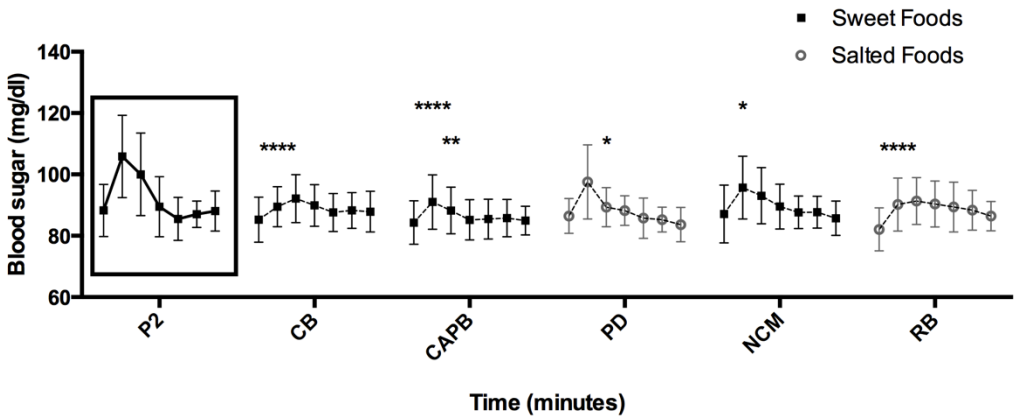


Figure 2.6. Comparison of mean blood sugar concentrations between pasta type 2 (P2) and the other test foods. Results show significant differences after 15 or 30min between P2 and CB, CAPB, PD, NCM, and RB. *P < 0.05; **P < 0.01; ****P < 0.0001. NCM, nuts and chocolate muffin.

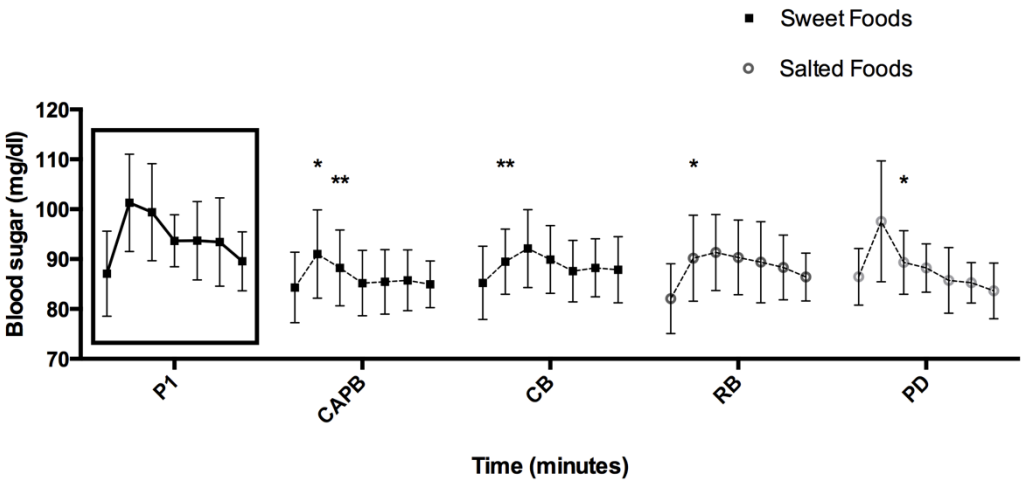


Figure 2.7. Comparison of mean blood sugar concentrations between the pasta type 1 (P1) and the other test foods. Results show significant differences after 15 or 30min be- tween P1 and CB, CAPB, PD, and RB. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

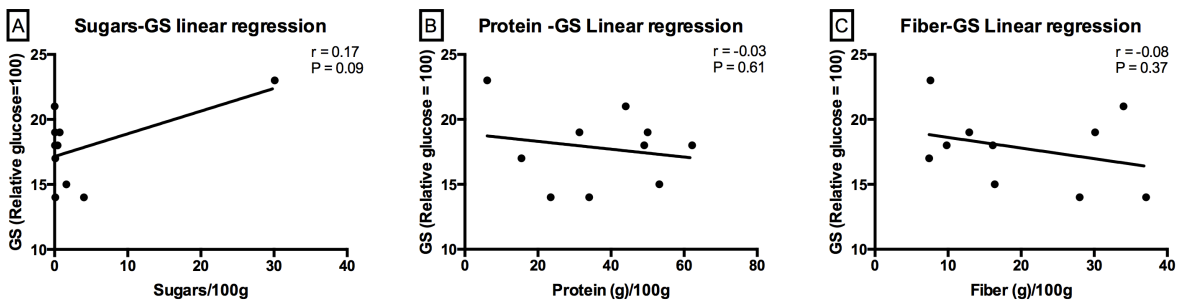


Figure 2.8. Bivariate correlations between observed glucose responses (GS) (relative to 1000 kJ glucose = 100), the available sugars, protein, and fiber contents of the 10 single test foods. Linear regression analysis was used to test the significance of the associations. Each point on the graph represents the mean result for each test meal (14 subjects). (A) Sugars—GS linear regression; (B) protein—GS linear regression; (C) fiber—GS linear regression. GS, glycaemic score.

Discussion

In this study, the GS of 10 proprietary foods high in proteins and fibers and low in sugars and saturated fats was tested. These proprietary foods claim to replicate the taste and aspect of high-CHO foods and are projected to be used as meals during VLCKD regimens. In our study, the products tested showed a significant lower blood sugar response and lower GS than an isoenergetic amount of glucose. Among he six sweet and four savory test foods, the CHB showed the highest GS (GS = 23). This result is consistent with the higher quantity of available sugars of CHB compared with the other test foods. The CB and the CAPB had the lowest GS of 14. This GS value is, according to the data available (Bao, Atkinson et al. 2011), similar to the GS value of a low-fat processed cheese. The macronutrient composition is important for glucose response, with CHO as the food component that acts directly on glycaemia, raising it and stimulating insulin secretion. However, even if CHO counting is still the basis for insulin dose adjustment in diabetes care management (Campbell, Walker et al. 2016), data show (Bao, Atkinson et al. 2011) that sugar content could be a stronger predictor of the observed glucose response than CHO. Other studies show that the structure of CHOs should also be kept under consideration: a disrupted structure, typical of processed whole grains, has a different effect on glycaemia compared with intact grains (Foster-Powell, Holt et al. 2002).

Even if there is a strong evidence supporting fibers' beneficial effect in reducing disease risk (Bao, Atkinson et al. 2011), only soluble fibers with gel-forming properties show a distinguishable effect for glycaemic control (Venn, Mann 2004, Gibb, McRorie et al. 2015).

This study does not show any significant relationship between GS and fiber content, but, differently from them, it does not show any correlations between GS and sugar content (Fig. 2.8). This conflicting result might be because of the very low quantity of available sugars in the test foods. Finally, protein content, despite being considered predictive for the GS (Bao, Atkinson et al. 2011), did not show such correlation in this study.

The low postprandial glycaemia produced by the proprietary foods tested is an important factor, because ultra-processed ready-to-consume products are commonly high in simple sugars that negatively affect a number of health parameters. Postprandial hyperglycaemia and compensatory hypoglycaemia are factors linked to the development of diabetes and CV diseases (Zeevi, Korem et al. 2015). Furthermore, the consumption of high-sugar snacks seems to be the main cause for the increase in intrahepatic triglyceride content (Koopman, Caan et al. 2014). Finally, the usual rapid and high glycaemic peak caused by ultra-processed products, together with their lack in fiber, proteins, and water, triggers an excessive consumption (Ventura, Santander et al. 2014, Blum, Thanos et al. 2014). Sugar is rapidly absorbed and produces a consequent high blood sugar spike that acts centrally, increasing the production and utilization of dopamine, which imitates the typical neuromodulation of addictive substances (Blum, Braverman et al. 2000). The abuse of high sugary ultraprocessed foods leads to the synthesis and the accumulation of fat and results in weight gain (Moubarac, Batal et al. 2014) which increases the risk of obesity and MetS. Bielemann et al. (Bielemann, Motta et al. 2015) recently demonstrated that ultraprocessed foods were responsible for 50% of the daily caloric intake among a cohort of 23-year-old participants in Brazil. Interestingly, the household availability of ultraprocessed ready-to-eat foods was associated with a low percentage of proteins and fibers intake.

Appetite control is related not only to glucose content and postprandial glycaemia but also to other factors (Farr, Chiang-shan et al. 2016) 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 reward is a goal that drives both appetite and

eating (Rolls 2016, Rogers, Brunstrom 2016). The larger the portion size, the more food is eaten, but eating is only indirectly related to energy balancing because it seems that we eat essentially for pleasure (Rogers, Brunstrom 2016). These new low-calorie proprietary foods could help to reduce energy intake, useful for a better weight maintenance or a more successful weight loss. Moreover, since high-energy-dense foods have the lowest satiating capacity even if they usually have a high palatability (Rogers, Brunstrom 2016), the high level of proteins and fibers and the high palatability, despite the low sugar content of these new ultraprocessed foods, are important features that contribute to both food reward and satiety (Tovar, del Carmen Caamaño et al. 2012).

A VLCKD that includes these proprietary foods that 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 (Mutch, Clement 2006, Paoli, Cenci et al. 2010).

Sweet foods are usually rich in refined CHO, have a high GI, and are related to an increased risk of overweight, obesity (Schwingshackl, Hoffmann 2013), and type 2 diabetes mellitus (T2DM) (Maki, Phillips 2015) . T2DM is increasing among young people (Reinehr 2013) and a dietary management is the most important factor to be considered to prevent the progression of impaired glucose tolerance to clinical DM. A dietary management is also important to minimize the glycaemic variability, which is the measure of blood sugar concentration changes over time (Tay, Thompson et al. 2015). An uncontrolled blood sugar concentration is the major risk factor in the development of T2DM complications such as retinopathy, neuropathy, nephropathy, and CV diseases (Brownlee, Hirsch 2006, Forbes, Cooper 2013, Bonora 2002, Pradeepa, Mohan 2011). 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 glycaemic fluctuations in T2DM management (Feinman, Pogozelski et al. 2015). 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 consumption of high-fiber, low-GI foods as CHO source (Tay, Thompson et al. 2015).

Dietary amino acids contribute to the de novo synthesis of glucose through gluconeogenesis and participate in the recycling of glucose carbon through the glucose–alanine cycle (Dashty 2013). However, dietary proteins have a minimal impact on glycaemia and insulin secretion compared with CHOs (Layman, Baum 2004), and a high-quality protein supplementation has been suggested during weight loss programs to preserve muscle mass, to improve glycaemic regulation, and to maintain euglycaemia (Verreijen, Verlaan et al. 2015, Pasiakos 2015). The 10 proprietary foods tested in this study are formulated with whey proteins. These, because of their high content of leucine, which promotes muscle mass synthesis and because of their fast digestion and delivery of amino acids in the circulation, are consequently considered the best type of proteins (Verreijen, Verlaan et al. 2015). Moreover, whey protein decreases appetite better than other types of proteins (Pal, Ellis 2010) and increases satiety through an increase of the release of CCK and GLP-1 and a reduction of ghrelin levels (Luhovyy, Akhavan et al. 2007).

The sweet proprietary foods tested in this study also contained low-calorie sweeteners. These are compounds able to stimulate, in the same way as sugar does, the sweet taste receptors (Brown, Rother 2012). Unlike sugar, low-calorie sweeteners do not release energy and hence they are used in weight loss programs even though perceived as controversial by the scientific community. This is due to low-calorie sweeteners producing possible adverse metabolic effects, such as increase of appetite, weight gain, and metabolic disorders (Yang 2010, Swithers, Martin et al. 2010, Ludwig 2009). 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 (Rogers, Hogenkamp et al. 2015). 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, because they avoid the high-calorie intake side effect of sugar (Rogers, Brunstrom 2016). These compounds could be useful in the prevention of overweightness and obesity in populations that are less sensitive to sweetness, predisposing them to consume more sugar to have the same ‘‘taste sensation’’ as people more sensitive to sweetness (Low, Lacy et al. 2014). Nowadays low-calorie sweeteners are important tools in DM management, in which dietary adherence is among the most difficult cornerstones (Anders, Schroeter 2015), 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 (Mulvaney, Schlundt et al. 2006).

Conclusions

The 10 proprietary foods tested showed a significant lower glycaemia than the standard food glucose and their GS resulted in always lower than 25. This low glycaemic response, together with their valuable ready-to-use format, makes these proprietary foods a valid tool during both weight management and weight loss programs, improving the adherence to KDs of individuals who tend to have a high preference for sweet foods. Moreover, these new ultraprocessed products high in protein and fibers and low in sugars could ameliorate 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.

3. WEIGHT LOSS AND COGNITIVE FUNCTIONS: THE EFFECTS OF GLYCAEMIA AND KETONEMIA VARIATION IN NON DIABETIC OVERWEIGHT YOUNG WOMEN

INTRODUCTION

The range of variation of both glucose and ketone bodies in the blood of non-diabetic individuals is wide. Glycaemia ranges from fasting concentration between 3.9 and 5.5 mmol/L (70 to 100 mg/dl) (Brent Wisse 2015) to higher values after eating, which rarely exceed 7.78 mmol/L (140 mg/dl) (Singh 2012). Ketone bodies (KBs) level ranges from 0.1 mmol/L during a standard carbohydrate-fed diet to 7/8 mmol/L during a ketogenic diet (Paoli, Canato et al. 2011) .

Both glucose and KBs are used as energy from the brain and have different effects on cognitive functions depending on their blood concentration.

Regarding blood glucose, acute hyperglycaemia typical of diabetic individuals is related with mild cognitive dysfunctions, producing a slowdown of all cognitive performance tests (Cox, Kovatchev et al. 2005); the same results were obtained testing hypoglycaemia (Graveling, Deary et al. 2013, Languren, Montiel et al. 2013) . However, recurrent hypoglycaemia was related with improved cognitive functions in both diabetic and healthy animals tested at euglycaemia (McNay, Sherwin 2004). In non-diabetic humans, a study reported that individuals with poorer glucose tolerance had faster working memory and lower depression compared to subjects with better glucose tolerance (Young, Benton 2014).

Considering KBs, their uptake in the brain is largely irreversible and doesn't saturate at physiological ranges of ketonemia, contrary to glucose which tend to decrease when glycaemia elevates (Bouteldja, Andersen et al. 2014).

When beta-hydroxybutyrate (βOHB) level is low (<0.5 mmol/L) it contributes to no more than 3% of brain energy requirements (Courchesne-Loyer, Croteau et al. 2016), whilst at level of 4.8 mmol/L KBs (AcAc plus βOHB) supply the 33% of total brain energy requirements(Courchesne-Loyer, Croteau et al. 2016). Studies comparing ketogenic diets with high carbohydrate diet reported either a similar effect on cognitive functions of both diets (Makris, Darcey et al. 2013) or an impairment during the ketogenic diet (Holloway, Cochlin et al. 2011, Edwards, Murray et al. 2011).

As there is no data of the effects of glycaemia and ketonemia variations on cognitive functions following different diets in overweight non-diabetic young women, the purpose of this study was then to compare the glycaemia and ketonemia before and after ten days of very low-carbohydrate ketogenic diet without any restriction of calories intake (KD) with a calorie-restricted ketogenic-mediterranean diet (KEMEPHY) and a calorie-restricted Mediterranean diet (MD) on working memory and executive functions in overweight young women.

SUBJECTS AND METHODS

Subjects

Subjects were recruited through leafleting in the university area.

Inclusion criteria for the diet-group were: overweight or obese young women, age between 20 and 35 years, 25<BMI>39.9 kg/m^2 (Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults. 1998)

Inclusion criteria for the non-diet group were: normal weight young women (18.5<BMI<25 kg/m^2), age between 20 and 35 years.

Exclusion criteria for both groups were: smokers, subjects under diet-treatment, subjects treated for diseases such as diabetes, cardiovascular diseases, depression, subjects doing sport more than 2 hours per week (Edwards, Murray et al. 2011)).

For the diet-group were recruited a total of 63 sedentary overweight and obese girls and 45 of them completed the study.

For the normal-weight group a total of 17 girls were recruited (21±1.8 kg) and 17 completed the study.

Study design

The study was driven from April 2015 to June 2016.

Subjects were contacted per mail and divided in groups according to the day of the beginning of their follicular phase in order to minimize hormonal effects on mood (Aitken, Baker et al. 2008).

Each group of subjects came for the basal measurements after an overnight fast to the Exercise Laboratory of the Physiology Department of the University of Padova. Subjects were instructed to avoid unusual exercise and big meals the night before the meeting.

Overweight and obese girls came for the basal measurements five days before the start of the dietary protocol . The following controls were set on the starting day of the diet (t1), on the third (t3), on the fifth (t5), on the seventh (t7) and on the last day (t10) (Figure 3.1).

On the basal control day, subjects signed the informal consent, were randomly divided in one of the three diet groups, received instruction about the diet protocol to follow and compiled a lifestyle questionnaire (Godwin, Streight et al. 2008). They were then weighed, their height was measured and they took a standard high carb breakfast (a high-carb 150 kcal muffin and one teaspoon of sugared instant tea dissolved in a glass of warm water). After breakfast, they completed the psychological tests. At t1, t3, t5, t7 and t10 ketone bodies levels and glycaemia were measured, as well as appetite levels which was scored through a visual analogue scale (VAS). On the last control day (t10) subjects repeated the body impedance analysis, the body weight measure and, after breakfast (each group had a different breakfast according to the prescribed diet), the psychological tests.

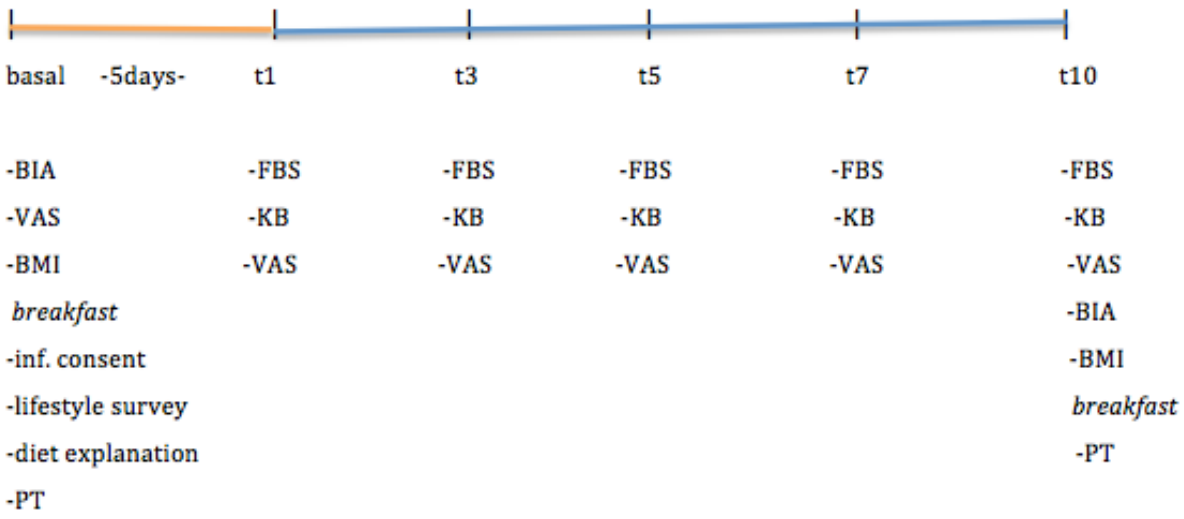


Figure 3.1: overweight subjects' study design. FBS:fasting blood sugar; KB: ketone bodies; VAS: visual analogue scale for appetite; BIA: bioelectrical impedance analysis; PT: psychological tests; inf. consent: sign of the informal consent.

Diet protocols

Diets tested were the Ketogenic Diet (KD), the Phytoketogenic Mediterranean Diet (KEMEPHY) and the Mediterranean Diet (MD) and were assigned randomly. A total of 13 girls followed the KEMEPHY diet, 16 followed the KD and 16 the MD.

The KD is a protocol in which all carbohydrate containing foods are excluded, whereas meat, eggs, fish, ham, green leafy vegetables, cruciferous, zucchini, cucumbers and eggplants can be assumed without any limit. This protocol allows the use of oil, lemon juice (2 tbs/day), spices and aromatic herbs with a limitation of the use of saturated fats like butter, margarine and lard. Coffee, tea and herbal tea could be sweetened with sweeteners.

The KEMEPHY(Paoli 2011) is a phytoketogenic mediterranean calorie-controlled diet in which the subjects were allowed to eat with no limits green leafy vegetables, cruciferous, zucchini, cucumbers and eggplants. The quantity of meat, eggs and fish was limited to once a day (120 g of meat or 200 g of fish or 1 egg). Moreover, subjects daily consumed four food supplements and liquid herbal extracts. Food supplements are high proteins (19 g/portion) / very low carbohydrate (3,5 g/portion) formulas simulating the aspect and taste of common carbohydrate rich foods added with dry phytoextracts. Among the dry phytoextracts, there are 50 mg of Griffonia simplicifolia seed extract (for a total of 200 mg/day), which are useful against anxiety, thanks to the content of 5-hydroxytryptophan, a direct precursor of serotonin (Carnevale, Di Viesti et al. 2011). Liquid herbal extracts were used for their depurative/ draining / toning activity, useful to reduce some commonly reported light side effects of ketogenic diets as constipation, headache and halitosis (Table 3.1).

The MD is a balanced calorie-controlled diet. The calorie intake was 1200 Kcal/day of which 15% were proteins, 60% carbohydrates and 25% fat. In this protocol was highlighted the use of the typical ingredients of the mediterranean tradition, such as extra virgin olive oil, vegetables, fruits, fish, lean meat and whole grain cereals.

Table 3.1: KEMEPHY DIET

KEMEPHY	
Daily Energy Kcal/day	882
Protein, g/day (%daily energy)	106 (48)
Carbohydrate, g/day (% daily energy)	20 (9)
Fat, g/day (% daily energy)	42 (42)

Glucose and ketones measurement

Levels of KB and glucose were assessed using Precision Xtra® Blood β-Ketone Test Strips and Precision Xtra® (Chu, Jiao 2015) (Abbott Laboratories, Illinois 60064-3500, USA) and On Call Plus Blood Glucose Test Strips and On Call Plus® (ACON Laboratoires, San Diego, CA92121, USA). Blood β-Ketone Test Strips measure blood βOHB level in fresh capillary whole blood from the fingertip between 0.0 and 8.0 mmol/l. The puncture was performed with the lancet Accu-Chek Softclix (Roche, Monza MB, Italy) on clean, dry and warm fingers.

Body composition analysis

Bioelectrical impedance analysis (BIA) is a reliable, non-invasive, safe and effective technique to measure body composition. Body composition was set through the four-electrode method using the instrument Akern STA-BIA (Akern s.r.l., Firenze, Italy) and its software BODYGRAM 3.0 (Savino, Cresi et al. 2004).

Psychological tests

Psychological tests consisted in a mood test, two cognitive tasks, one to investigate working memory and the second to stress executive functions, and in a VAS scale to test the appetite level.

The mood test is the Italian Version of the Depression Anxiety Stress Scales-21. It is a self report measure formed by 21 items that reliably measure depression (lack of incentive, diysphorya and low self-esteem) as well as anxiety (somatic and subjective symptoms), stress (irritability, impatience, tension and arousal) and general distress (related to anxiety and depression) (Bottesi, Ghisi et al. 2015).

The working memory test is an adapted version of the visuo-spatial n back (Cui, Bray et al. 2011, Haberecht, Menon et al. 2001). It consists in remembering the position of the letter "o" that, in each trial, could appear in different positions, since a 9-part grid divides the screen. In the control condition, the participant has to respond when the stimuli appears in the central part of the grid; in the low cognitive load when the letter is in the same position as the stimulus seen just before; in the high cognitive load when it matches the same position seen two positions before. The control condition is administrated between the other two. In total there are six blocks for the low cognitive load condition and six for the high one and the double amount for the control condition, for a total of 552 stimuli, 168 of which were targets and 384 were not-targets (Jaeggi, Buschkuehl et al. 2010). Each stimuli lasted 1500 ms, with a fixed inter-trial interval of 500 ms.

The executive function test is an adapted version of the inhibitory control task (Cona, Arcara et al. 2013, Amodio, Ridola et al. 2010). The stimulus are letters that appear in the centre of the screen. The task is formed by two main parts. In the first one the participant has to respond when the letter *x* or *y* appear on the screen; in the second part the participant has to answer only when *x* precedes *y* and then vice versa. In the first part 406

stimulus are present, 66 of them of which are "go-trials" and in the second one 1746 letters are present, 254 of which are "go-trials" and 47 are "no-go" (Cona, Arcara et al. 2013). Each stimuli lasted 500 ms, without interstimulus interval, in order to have a high time pressure task.
Each cognitive task is administered by E-prime software (Psychology Software Tools, Pittsburgh, PA) and before each part of the experiment a practice block was presented in order to make the participant familiar with the task. Both of the task needed 20-30 minutes to be completed.
Motivation to eat and appetite were investigated by Visual Analog Scale (Hill, Blundell 1982), a test formed by 6 scales. Each scale is 10 cm long and it is labelled with descriptions at both ends; moreover, every 10 mm, there are vertical lines labelled with numbers. Participants have to choose which part of the scale better describe how they feel. The scales investigate: appetite, fullness, desire to eat, how much would participant eat, urgency of eating and worries about food. Moreover, also the appetite (mean of all the responses) and gut based (hunger perceived) were taken into account (Stubbs, Hughes et al. 2000).

Tests were performed after breakfast. On the basal meeting, breakfast was given to participants and consisted in a high carbohydrate muffin and a hot tea. On the last day of the diet (t10) breakfast was brought from home by each participant according to the followed diet protocol.

STATISTICAL ANALYSIS

For the analysis of body weight, BMI, BIA parameters, glycaemia and KBs we used the software GraphPad PRISM version 6.0h. and we performed the RM two-way ANOVA analysis with matched values stacked into subcolums with Sidak's multiple comparisons test. In order to compare among eachother the measures of KB and glucose at each appointment, a Tukey's test was also performed. For the analysis of the correlations between glycaemia and ketonemia and psychological test parameters, Pearson correlation test was carried out by SPSS software (IBM SPSS, version 22, Armonk, NY).

RESULTS

Body weight and fat mass

Body weight decreased significantly ($p < 0.0001$) after the diet period in all three diet groups with no-significant difference between groups before and after diet. Mean body weight of KEMEPHY group was 79.7 ± 8.7 kg before and 76.1 ± 8.7 kg after diet with a mean loss of 3.5 ± 0.6 kg. Mean body weight of KD group was 75 ± 12.4 kg before and 71.9 ± 12.06 kg after diet with a mean loss of 3.1 ± 1.1 kg. Mean body weight of MD group was 77 ± 5.6 kg before and 75 ± 5.2 kg after diet with a mean loss of 2 ± 1 kg.
BMI decreased significantly ($p < 0.0001$) after the diet period in all three diet groups with no-significant difference between groups before and after diet. Mean BMI of KEMEPHY group was 28.4 ± 2.4 before and 27.1 ± 2.4 after diet with a mean loss of 1.3 ± 0.2. Mean BMI of KD group was 27 ± 1.9 before and 25.9 ± 1.9 after diet with a mean loss of 1.1 ± 0.4. Mean BMI of MD group was 27.8 ± 1.8 before and 27 ± 1.7 after diet with a mean loss of 0.7 ± 0.3.

The weight of fat mass decreases significantly ($p < 0.0001$) after the diet period in all three diet groups with no-significant difference between groups before and after diet. The mean weight of fat mass of KEMEPHY group was 31.7 ± 4.7 kg before and 29.7 ± 4.1 kg after diet with a mean weight loss of 2.1 ± 1.4 kg. The mean weight of fat mass of KD group was 28.8 ± 6.6 kg before and 27 ± 6.3 kg after diet with a mean weight loss of 1.8 ± 1.4 kg. The mean weight of fat mass of MD group was 30.1 ± 3.9 kg before and 28.3 ± 4.5 kg after diet with a mean weight loss of 1.8 ± 1.4 kg.

Glucose and ketones

Glucose levels decreased significantly after KEMEPHY ($p < 0.01$) (mean glycaemia pre-diet: 95 mg/dl; post-diet: 85.7 mg/dl with a mean reduction of 9 mg/dl) and KD ($p < 0.0001$) (mean glycaemia pre-diet: 92.8 mg/dl; post-diet: 80.6 mg/dl with a mean reduction of 12.2 mg/dl) with no-significant difference between diets. MD didn't show any significant difference in glucose level between pre and post-diet (mean glycaemia pre-diet: 94 mg/dl; post-diet: 92 mg/dl with a mean reduction of 2 mg/dl).

Levels of βOHB rose significantly in both ketogenic regimens with no-significant difference between KEMEPHY and KD. Levels increased more in KD ($p < 0.0001$) (mean pre-diet levels: 0.2 ± 0.1 mmol/l; mean post-diet levels: 1.7 ± 0.8 mmol/l with a mean increase of 1.5 ± 0.8 mmol/l) compared to KEMEPHY ($p < 0.001$) (mean pre-diet levels: 0.1 mmol/l; mean post-diet levels: 2.1 ± 1.6 mmol/l with a mean increase of 2.1 ± 1.6 mmol/l).

KBs increased significantly in KD group after 5 days of diet (t1 vs t5, $p < 0.0001$) whereas in KEMEPHY group KBs increased significantly after 7 days (t1 vs t7, $p < 0.0001$) (Figure 3.2).

Glucose levels decreased significantly in KD group after 3 days (t1 vs t3, $p < 0.05$) but mostly after 5 days (t1 vs t5, $p < 0.0001$), whereas in KEMEPHY KBs increased signficantly after 5 days (t1 vs t5, $p < 0.05$) (Figure 3.3).

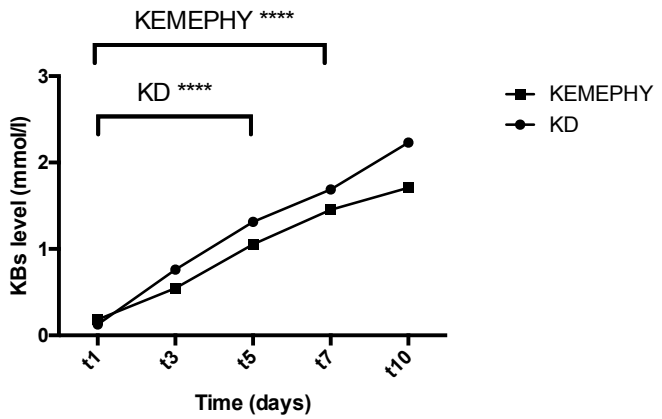


Figure 3.2. Strongest signficant increase of KBs level from t1 in both ketogenic diet groups (KD and KEMEPHY).

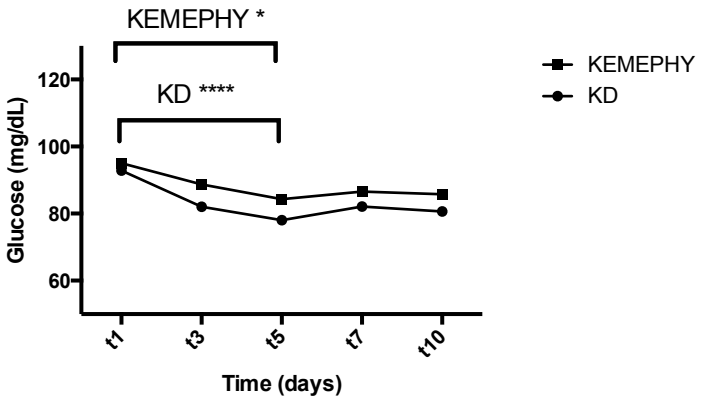


Figure 3.3. Strongest significant decrease of glucose level from t1 in both ketogenic diet groups (KD and KEMEPHY).

Psychological tests, glucose level and ketone bodies

Mood

No correlation was found between both glycaemia and ketonemia and depression, anxiety, stress or general distress, both in pre and post diet measurement.

Cognitive functions

- Working memory test (visuo-spatial n back): no correlation was found between glycaemia and working memory results, both before and after the diet period. Ketone levels, as well, were not correlated with working memory results, both in baseline ans post-diet measurement
- Executive function test (inhibitory control task):
    - Glycaemia: pre-diet levels were positively correlated with reaction time in the go-trials ($r(43) = 0.358$, $p = 0.018$). The same correlation was not found in the post-diet measuement, both considering all the participant together ($r(44) = 0.132$, $p = 0.392$) as well as dividing them by the type of diet followed: MD group r(15) = -0.025, $p = 0.930$ and ketogenic group (KEMEPHY and KD combined): $r(29) = 0.179$, $p = 0.354$.
    - Ketonemia: post-diet measurement showed a negative correlation between ketone levels and the accuracy of the no-go trials ($r(29) = -0.455$, $p = 0.027$), while in the baseline no correlation was found ($r(28) = -0.007$, $p = 0.974$).

VAS scale (appetite)

Glycaemia showed different correlation with VAS scale items. In the baseline measurement it was negatively correlated with how much would participants eat ($r(44) = -0.303$, $p = 0.045$). Differently, in the post test analysis, the glycaemia showed a negative correlation with the feeling of fullness ($r(44) = -0.455$, $p = 0.002$).

DISCUSSION

Normal level of glycaemia are difficult to be defined (WHO 2006), so that the WHO defines "normoglycaemia" as the level below that of intermediate hyperglycaemia or prediabetes (110-125 mg/dL) (Makaroff 2016, WHO 2006). Overweight and obesity are both risk factors for the development of diabetes (Sung, Jeong et al. 2012), but subjects of this study, despite being overweight, had a mean glycaemia below prediabetes levels (94±9 mg/dL), maybe thanks to their young age. However, following a high carb breakfast these subjcets reported a slower reaction time in the go-trial of the executive function test. Given that in the post-diet control (mean glycaemia: 86 ±9 mg/dL) this correlation was not found, we can speculate that a high glycaemia, which is typical after the consumption of ultra-processed products high in sugar and refined grains (Lodi, Karsten et al. 2016) such as the high carb muffin given to participants in the pre-diet control of this study, couldn't be the optimal choice as breakfast in the morning in order to achieve a good cognitive performance. In fact, data from this study show that glycaemia in the pre-diet control correlates positively with reaction time in the go-trials of the executive function test, which is a task that stresses functions which are frequently used during the day such as at work (the need to stay focused, the need to remember appointments) (Baddeley 2004, Wagner 1999), while studying (studying maths, problem solving, reading) (L.S. Siegel 1994, Raghubar, Barnes et al. 2010, Hambrick, Engle 2003) and during social life (remembering names and facts) (Logie, Law et al. 2010) .

Considering KBs, our study found a negative correlation between post-diet ketone levels and accuracy of the no-go trials of the executive function test. This detrimental effects could be related with the short diet period and then the relatively low levels of ketonemia achieved (mean ketonemia: 2 ± 1.3 mmol/L) and could be reversed, as hypothesized previously (Holloway, Cochlin et al. 2011), by higher levels of KBs.

Finally, our results showed that ketone levels (plasma βOHB) gradually increased during the first seven days, whereas the reduction of glucose levels mostly appeared on the third day, which is not consistent with previous findings which reported a gradual increase of KBs during the first four days and a deeper glucose level reduction on the fourth (Courchesne-Loyer, Croteau et al. 2016) .

CONCLUSIONS

Healthy young overweight subjects with fasting glycaemia below prediabetes level were negatively affected by a high-carb breakfast during an executive function test. Moreover, the effect of mild KBs levels (2 ± 1.3 mmol/L) negatively affected accuracy of the no-go trials of the executive function test.

BIBLIOGRAPHY

ABRAMSON, J.D., ROSENBERG, H.G., JEWELL, N. and WRIGHT, J.M., 2013. Should people at low risk of cardiovascular disease take a statin? *BMJ (Clinical research ed.),* **347**, pp. f6123.

AITKEN, R.J., BAKER, M.A., DONCEL, G.F., MATZUK, M.M., MAUCK, C.K. and HARPER, M.J.K., 2008. As the world grows: contraception in the 21st century. *The Journal of clinical investigation,* **118**(4), pp. 1330-1343.

AMODIO, P., RIDOLA, L., SCHIFF, S., MONTAGNESE, S., PASQUALE, C., NARDELLI, S., PENTASSUGLIO, I., TREZZA, M., MARZANO, C., FLAIBAN, C., ANGELI, P., CONA, G., BISIACCHI, P., GATTA, A. and RIGGIO, O., 2010. Improving the inhibitory control task to detect minimal hepatic encephalopathy. *Gastroenterology,* **139**(2), pp. 510-8, 518.e1-2.

ANDERS, S. and SCHROETER, C., 2015. Diabetes, diet-health behavior, and obesity. *Frontiers in endocrinology,* **6**, pp. 33.

ARENA, R., GUAZZI, M., LIANOV, L., WHITSEL, L., BERRA, K., LAVIE, C.J., KAMINSKY, L., WILLIAMS, M., HIVERT, M.F., FRANKLIN, N.C., MYERS, J., DENGEL, D., LLOYD-JONES, D.M., PINTO, F.J., COSENTINO, F., HALLE, M., GIELEN, S., DENDALE, P., NIEBAUER, J., PELLICCIA, A., GIANNUZZI, P., CORRA, U., PIEPOLI, M.F., GUTHRIE, G. and SHURNEY, D., 2015. Healthy Lifestyle Interventions to Combat Noncommunicable Disease-A Novel Nonhierarchical Connectivity Model for Key Stakeholders: A Policy Statement From the American Heart Association, European Society of Cardiology, European Association for Cardiovascular Prevention and Rehabilitation, and American College of Preventive Medicine. *Mayo Clinic proceedings,* **90**(8), pp. 1082-1103.

ATKINS, R.C., 1972. *Dr. Atkins' Diet Revolution. The HIgh Calorie Way to Stay Thin Forever.* New York, NY, USA.: D. McKay Co.

BADDELEY, A., 2004. Working memory. *Cognitive psychology: Key readings,* , pp. 355-361.

BANTING, W., 1863. *Letter on Corpulence, Addressed to the Public.* 1 edn. London: Harrison and Sons, St. Martin's Lane.

BAO, J., ATKINSON, F., PETOCZ, P., WILLETT, W.C. and BRAND-MILLER, J.C., 2011. Prediction of postprandial glycaemia and insulinemia in lean, young, healthy adults: glycaemic load compared with carbohydrate content alone. *The American Journal of Clinical Nutrition,* **93**(5), pp. 984-996.

BARRETT, M.L. and UDANI, J.K., 2011. A proprietary alpha-amylase inhibitor from white bean (Phaseolus vulgaris): a review of clinical studies on weight loss and glycaemic control. *Nutr J,* **10**, pp. 24.

BIELEMANN, R.M., MOTTA, J.V.S., MINTEN, G.C., HORTA, B.L. and GIGANTE, D.P., 2015. Consumption of ultra-processed foods and their impact on the diet of young adults. *Revista de saude publica,* **49**, pp. 1-10.

BIJSTER, P., 1993. 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,* **31**(3), pp. 173-178.

BLACK, K.E., SKIDMORE, P.M. and BROWN, R.C., 2012. Energy intakes of ultraendurance cyclists during competition, an observational study. *International Journal of Sport Nutrition andExercise Metabolism,* **22**(1), pp. 19.

BLACKBURN, G.L., PHILLIPS, J.C. and MORREALE, S., 2001. Physician's guide to popular low-carbohydrate weight-loss diets. *Cleveland Clinic journal of medicine,* **68**(9), pp. 761, 765-6, 768-9, 773-4.

BLUM, K., BRAVERMAN, E.R., HOLDER, J.M., LUBAR, J.F., MONASTRA, V.J., MILLER, D., LUBAR, J.O., CHEN, T.J. and COMINGS, D.E., 2000. The reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive and compulsive behaviors. *Journal of psychoactive drugs,* **32**(sup1), pp. 1-112.

BLUM, K., THANOS, P.K. and GOLD, M.S., 2014. Dopamine and glucose, obesity, and reward deficiency syndrome. *Frontiers in psychology,* **5**, pp. 919.

BONORA, E., 2002. Postprandial peaks as a risk factor for cardiovascular disease: epidemiological perspectives. *International journal of clinical practice.Supplement,* **(129)**(129), pp. 5-11.

BOTTESI, G., GHISI, M., ALTOÈ, G., CONFORTI, E., MELLI, G. and SICA, C., 2015. The Italian version of the Depression Anxiety Stress Scales-21: Factor structure and psychometric properties on community and clinical samples. *Comprehensive psychiatry,* **60**, pp. 170-181.

BOUTELDJA, N., ANDERSEN, L.T., MØLLER, N. and GORMSEN, L.C., 2014. Using positron emission tomography to study human ketone body metabolism: A review. *Metabolism,* **63**(11), pp. 1375-1384.

BRENT WISSE, M., 24/7/2015, 2015-last update, Blood sugar test - blood [Homepage of MedlinePlus], [Online]. Available: https://medlineplus.gov/ency/article/003482.htm [22/11/2016, 2016].

BROUNS, F., BROUNS, F., BJORCK, I., FRAYN, K., GIBBS, A., LANG, V., SLAMA, G. and WOLEVER, T., 2005. Glycaemic index methodology. *Nutrition research reviews,* **18**(1), pp. 145.

BROWN, R.J. and ROTHER, K.I., 2012. Non-nutritive sweeteners and their role in the gastrointestinal tract. *The Journal of Clinical Endocrinology & Metabolism,* **97**(8), pp. 2597-2605.

BROWNLEE, M. and HIRSCH, I.B., 2006. Glycaemic variability: a hemoglobin A1c–independent risk factor for diabetic complications. *Jama,* **295**(14), pp. 1707-1708.

BUENO, N.B., DE MELO, I.S., DE OLIVEIRA, S.L. and DA ROCHA ATAIDE, T., 2013. Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. *The British journal of nutrition,* **110**(7), pp. 1178-1187.

CAMPBELL, M.D., WALKER, M., KING, D., GONZALEZ, J.T., ALLERTON, D., STEVENSON, E.J., SHAW, J.A. and WEST, D.J., 2016. Carbohydrate Counting at Meal Time Followed by a Small Secondary Postprandial Bolus Injection at 3 Hours Prevents Late Hyperglycaemia, Without Hypoglycaemia, After a High-Carbohydrate, High-Fat Meal in Type 1 Diabetes. *Diabetes care,* **39**(9), pp. e141-2.

CARNEVALE, G., DI VIESTI, V., ZAVATTI, M. and ZANOLI, P., 2011. Anxiolytic-like effect of Griffonia simplicifolia Baill. seed extract in rats. *Phytomedicine,* **18**(10), pp. 848-851.

CASALS, N., ROCA, N., GUERRERO, M., GIL-GÓMEZ, G., AYTÉ, J., CIUDAD, C.J. and HEGARDT, F.G., 1992. Regulation of the expression of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene. Its role in the control of ketogenesis. *Biochemical Journal,* **283**(1), pp. 261-264.

CDC CENTERS FOR DISEASE CONTROL AND PREVENTION, August 28, 2014, 2014-last update, Diseases & Conditions A-Z Index [Homepage of Office of the Associate Director for Communication, Division of Public Affairs], [Online]. Available: http://www.cdc.gov/DiseasesConditions/az/a.html2016].

CELLENO, L., TOLAINI, M.V., D'AMORE, A., PERRICONE, N.V. and PREUSS, H.G., 2007. A Dietary supplement containing standardized Phaseolus vulgaris extract influences body composition of overweight men and women. *Int J Med Sci,* **4**, pp. 45-52.

CHAHOUD, G., AUDE, Y.W. and MEHTA, J.L., 2004. Dietary recommendations in the prevention and treatment of coronary heart disease: do we have the ideal diet yet? *The American Journal of Cardiology,* **94**(10), pp. 1260-1267.

CHIN, S.H., KAHATHUDUWA, C.N. and BINKS, M., 2016. Physical activity and obesity: what we know and what we need to know. *Obesity reviews : an official journal of the International Association for the Study of Obesity,* .

CHRISTENSEN, E.H. and HANSEN, O., 1939. I1. Zur Methodik der Respiratorischen Quotientâ€ Bestimmungen in Ruhe und bei Arbeit2. *Skandinavisches Archiv FÃ¼r Physiologie,* **81**(1), pp. 137-151.

CHU, C.B. and JIAO, L.D., 2015. (R)-3-oxobutyl 3-hydroxybutanoate (OBHB) induces hyperketonemiain Alzheimer's disease. *International journal of clinical and experimental medicine,* **8**(5), pp. 7684-7688.

CLARE, B.A., CONROY, R.S. and SPELMAN, K., 2009. The diuretic effect in human subjects of an extract of Taraxacum officinale folium over a single day. *J Altern Complement Med,* **15**, pp. 929-934.

CLARK, M.J. and SLAVIN, J.L., 2013. The effect of fiber on satiety and food intake: a systematic review. *Journal of the American College of Nutrition,* **32**(3), pp. 200-211.

Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults. 1998. *The American Journal of Clinical Nutrition,* **68**(4), pp. 899-917.

CONA, G., ARCARA, G., AMODIO, P., SCHIFF, S. and BISIACCHI, P.S., 2013. Does executive control really play a crucial role in explaining age-related cognitive and neural differences? *Neuropsychology,* **27**(3), pp. 378-389.

CONSULTATION, W.H.O., 2000. Obesity: preventing and managing the global epidemic. *World Health Organization technical report series,* (894),.

COURCHESNE-LOYER, A., CROTEAU, E., CASTELLANO, C.A., ST-PIERRE, V., HENNEBELLE, M. and CUNNANE, S.C., 2016. Inverse relationship between brain glucose and ketone metabolism in adults during short-term moderate dietary ketosis: A dual tracer quantitative positron emission tomography study. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism,* .

COX, D.J., KOVATCHEV, B.P., GONDER-FREDERICK, L.A., SUMMERS, K.H., MCCALL, A., GRIMM, K.J. and CLARKE, W.L., 2005. Relationships between hyperglycaemia and cognitive performance among adults with type 1 and type 2 diabetes. *Diabetes care,* **28**(1), pp. 71-77.

CUI, X., BRAY, S., BRYANT, D.M., GLOVER, G.H. and REISS, A.L., 2011. A quantitative comparison of NIRS and fMRI across multiple cognitive tasks. *NeuroImage,* **54**(4), pp. 2808-2821.

DASHTY, M., 2013. A quick look at biochemistry: carbohydrate metabolism. *Clinical biochemistry,* **46**(15), pp. 1339-1352.

DAVIS, C., 2014. Evolutionary and neuropsychological perspectives on addictive behaviors and addictive substances: relevance to the "food addiction" construct. *Substance abuse and rehabilitation,* **5**, pp. 129-137.

DEURENBERG, P. and YAP, M., 1999. The assessment of obesity: methods for measuring body fat and global prevalence of obesity. *Baillie`re's Best Practice & Research Clinical Endocrinology & Metabolism,* **13**(1), pp. 1-11.

DI SILVERIO, F., D'ERAMO, G., LUBRANO, C., FLAMMIA, G.P., SCIARRA, A., PALMA, E., CAPONERA, M. and SCIARRA, F., 1992. Evidence that Serenoa repens extract displays an antiestrogenic activity in prostatic tissue of benign prostatic hypertrophy patients. *Eur Urol,* **21**, pp. 309-314.

DREWNOWSKI, A., KRAHN, D.D., DEMITRACK, M.A., NAIRN, K. and GOSNELL, B.A., 1992. Taste responses and preferences for sweet high-fat foods: evidence for opioid involvement. *Physiology & Behavior,* **51**(2), pp. 371-379.

EDWARDS, L.M., MURRAY, A.J., HOLLOWAY, C.J., CARTER, E.E., KEMP, G.J., CODREANU, I., BROOKER, H., TYLER, D.J., ROBBINS, P.A. and CLARKE, K., 2011. Short-term consumption of a high-fat diet impairs whole-body efficiency and cognitive function in sedentary men. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology,* **25**(3), pp. 1088-1096.

FAO, 1998. *Carbohydrates in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation.*

FARR, O.M., CHIANG-SHAN, R.L. and MANTZOROS, C.S., 2016. Central nervous system regulation of eating: Insights from human brain imaging. *Metabolism,* **65**(5), pp. 699-713.

FEINMAN, R.D., POGOZELSKI, W.K., ASTRUP, A., BERNSTEIN, R.K., FINE, E.J., WESTMAN, E.C., ACCURSO, A., FRASSETTO, L., GOWER, B.A. and MCFARLANE, S.I., 2015. Dietary carbohydrate restriction as the first approach in diabetes management: critical review and evidence base. *Nutrition,* **31**(1), pp. 1-13.

FORBES, J.M. and COOPER, M.E., 2013. Mechanisms of diabetic complications. *Physiological Reviews,* **93**(1), pp. 137-188.

FOSTER-POWELL, K., HOLT, S.H. and BRAND-MILLER, J.C., 2002. International table of glycaemic index and glycaemic load values: 2002. *The American Journal of Clinical Nutrition,* **76**(1), pp. 5-56.

GABY, A.R., 2007. Natural approaches to epilepsy. *Alternative medicine review,* **12**(1), pp. 9.

GIBB, R.D., MCRORIE, J.W.,Jr, RUSSELL, D.A., HASSELBLAD, V. and D'ALESSIO, D.A., 2015. Psyllium fiber improves glycaemic control proportional to loss of glycaemic control: a meta-analysis of data in euglycaemic subjects, patients at risk of type 2 diabetes mellitus, and patients being treated for type 2 diabetes mellitus. *The American Journal of Clinical Nutrition,* **102**(6), pp. 1604-1614.

GIBSON, A., SEIMON, R., LEE, C., AYRE, J., FRANKLIN, J., MARKOVIC, T., CATERSON, I. and SAINSBURY, A., 2015. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obesity Reviews,* **16**(1), pp. 64-76.

GODWIN, M., STREIGHT, S., DYACHUK, E., VAN, D.H., PLOEMACHER, J., SEGUIN, R. and CUTHBERTSON, S., 2008. Testing the Simple Lifestyle Indicator Questionnaire: Initial psychometric study. *Canadian Family Physician,* **54**(1), pp. 76-77.

GRAVELING, A.J., DEARY, I.J. and FRIER, B.M., 2013. Acute hypoglycaemia impairs executive cognitive function in adults with and without type 1 diabetes. *Diabetes care,* **36**(10), pp. 3240-3246.

GREENBERG, I., STAMPFER, M.J., SCHWARZFUCHS, D., SHAI, I. and DIRECT GROUP, 2009. Adherence and success in long-term weight loss diets: the dietary intervention randomized controlled trial (DIRECT). *Journal of the American College of Nutrition,* **28**(2), pp. 159-168.

HABERECHT, M.F., MENON, V., WARSOFSKY, I.S., WHITE, C.D., DYER-FRIEDMAN, J., GLOVER, G.H., NEELY, E.K. and REISS, A.L., 2001. Functional neuroanatomy of visuo-spatial working memory in Turner syndrome. *Human brain mapping,* **14**(2), pp. 96-107.

HALL, K.D., 2017. A review of the carbohydrate-insulin model of obesity. *European journal of clinical nutrition, .*

HALL, K.D., BEMIS, T., BRYCHTA, R., CHEN, K.Y., COURVILLE, A., CRAYNER, E.J., GOODWIN, S., GUO, J., HOWARD, L., KNUTH, N.D., MILLER, B.V.,3rd, PRADO, C.M., SIERVO, M., SKARULIS, M.C., WALTER, M., WALTER, P.J. and YANNAI, L., 2015. Calorie for Calorie, Dietary Fat Restriction Results in More Body Fat Loss than Carbohydrate Restriction in People with Obesity. *Cell metabolism,* **22**(3), pp. 427-436.

HALL, K.D., CHEN, K.Y., GUO, J., LAM, Y.Y., LEIBEL, R.L., MAYER, L.E., REITMAN, M.L., ROSENBAUM, M., SMITH, S.R., WALSH, B.T. and RAVUSSIN, E., 2016. Energy expenditure and body

composition changes after an isocaloric ketogenic diet in overweight and obese men. *The American Journal of Clinical Nutrition,* **104**(2), pp. 324-333.

HAMBRICK, D.Z. and ENGLE, R.W., 2003. The role of working memory in problem solving. *The psychology of problem solving,* , pp. 176-206.

HARTMAN, A.L., GASIOR, M., VINING, E.P.G. and ROGAWSKI, M.A., 2007. The Neuropharmacology of the Ketogenic Diet. *Pediatric neurology,* **36**(5), pp. 281-292.

HILL, A.J. and BLUNDELL, J.E., 1982. Nutrients and behaviour: research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. *Journal of psychiatric research,* **17**(2), pp. 203-212.

HOLLOWAY, C.J., COCHLIN, L.E., EMMANUEL, Y., MURRAY, A., CODREANU, I., EDWARDS, L.M., SZMIGIELSKI, C., TYLER, D.J., KNIGHT, N.S., SAXBY, B.K., LAMBERT, B., THOMPSON, C., NEUBAUER, S. and CLARKE, K., 2011. A high-fat diet impairs cardiac high-energy phosphate metabolism and cognitive function in healthy human subjects. *The American Journal of Clinical Nutrition,* **93**(4), pp. 748-755.

HOLT, S.H., MILLER, J.C. and PETOCZ, P., 1997. An insulin index of foods: the insulin demand generated by 1000-kJ portions of common foods. *The American Journal of Clinical Nutrition,* **66**(5), pp. 1264-1276.

JAEGGI, S.M., BUSCHKUEHL, M., PERRIG, W.J. and MEIER, B., 2010. The concurrent validity of the N-back task as a working memory measure. *Memory (Hove, England),* **18**(4), pp. 394-412.

JEFFERY, R.W., 1996. Does weight cycling present a health risk? *The American Journal of Clinical Nutrition,* **63**(3 Suppl), pp. 452S-455S.

KARK, R., JOHNSON, R. and LEWIS, J., 1945. Defects of pemmican as an emergency ration for infantry troops. *War Medicine,* **7**, pp. 345-352.

KLEINERT, S. and HORTON, R., 2015. Rethinking and reframing obesity. *The Lancet,* **385**(9985), pp. 2326-2328.

KOOPMAN, K.E., CAAN, M.W., NEDERVEEN, A.J., PELS, A., ACKERMANS, M.T., FLIERS, E., FLEUR, S.E. and SERLIE, M.J., 2014. Hypercaloric diets with increased meal frequency, but not meal size, increase intrahepatic triglycerides: a randomized controlled trial. *Hepatology,* **60**(2), pp. 545-553.

KOSSOFF, E.H., CERVENKA, M.C., HENRY, B.J., HANEY, C.A. and TURNER, Z., 2013. A decade of the modified Atkins diet (2003–2013): Results, insights, and future directions. *Epilepsy & Behavior,* **29**(3), pp. 437-442.

KREBS, H.A., 1966. The regulation of the release of ketone bodies by the liver. *Adv Enzym Regul,* **4**, pp. 339-354.

KRIS GUNNARS, B., 2014-last update, 23 Studies on Low-Carb and Low-Fat Diets - Time to Retire The Fad. Available: https://authoritynutrition.com/23-studies-on-low-carb-and-low-fat-diets/2016].

KUMMEROW, F.A., COOK, L.S., WASOWICZ, E. and JELEN, H., 2001. Changes in the phospholipid composition of the arterial cell can result in severe atherosclerotic lesions. *The Journal of nutritional biochemistry,* **12**(10), pp. 602-607.

L.S. SIEGEL, 1994. Working Memory and Reading: A Life-soan Perspective. *INTERNATIONAL JOURNAL OF BEHAVIORAL DEVELOPMENT,* **17**(1), pp. 109-124.

LAEGER, T., METGES, C.C. and KUHLA, B., 2010. Role of β-hydroxybutyric acid in the central regulation of energy balance. *Appetite,* **54**(3), pp. 450-455.

LANDAU, B.R., WAHREN, J., CHANDRAMOULI, V., SCHUMANN, W.C., EKBERG, K. and KALHAN, S.C., 1996. Contributions of gluconeogenesis to glucose production in the fasted state. *The Journal of clinical investigation,* **98**(2), pp. 378-385.

LANGUREN, G., MONTIEL, T., JULIO-AMILPAS, A. and MASSIEU, L., 2013. Neuronal damage and cognitive impairment associated with hypoglycaemia: An integrated view. *Neurochemistry international,* **63**(4), pp. 331-343.

LAYMAN, D.K. and BAUM, J.I., 2004. Dietary protein impact on glycaemic control during weight loss. *The Journal of nutrition,* **134**(4), pp. 968S-73S.

LEE, J.E., MIN, S.H., LEE, D.H., OH, T.J., KIM, K.M., MOON, J.H., CHOI, S.H., PARK, K.S., JANG, H.C. and LIM, S., 2016. Comprehensive assessment of lipoprotein subfraction profiles according to glucose metabolism status, and association with insulin resistance in subjects with early-stage impaired glucose metabolism. *International journal of cardiology,* **225**, pp. 327-331.

LEIDY, H.J., CLIFTON, P.M., ASTRUP, A., WYCHERLEY, T.P., WESTERTERP-PLANTENGA, M.S., LUSCOMBE-MARSH, N.D., WOODS, S.C. and MATTES, R.D., 2015. The role of protein in weight loss and maintenance. *The American Journal of Clinical Nutrition,* .

LENNERZ, B.S., ALSOP, D.C., HOLSEN, L.M., STERN, E., ROJAS, R., EBBELING, C.B., GOLDSTEIN, J.M. and LUDWIG, D.S., 2013. Effects of dietary glycaemic index on brain regions related to reward and craving in men. *The American Journal of Clinical Nutrition,* **98**(3), pp. 641-647.

LI, Z., SONG, R., NGUYEN, C., ZERLIN, A., KARP, H., NAOWAMONDHOL, K., THAMES, G., GAO, K., LI, L., TSENG, C.H., HENNING, S.M. and HEBER, D., 2010. Pistachio nuts reduce triglycerides and body weight by comparison to refined carbohydrate snack in obese subjects on a 12-week weight loss program. *Journal of the American College of Nutrition,* **29**(3), pp. 198-203.

LIMA, W.P., CARNEVALI, L.C., EDER R, COSTA ROSA, L., BACCHI, E.M. and SEELAENDER, M.C., 2005. Lipid metabolism in trained rats: effect of guarana (Paullinia cupana Mart.) supplementation. *Clin Nutr,* **24**, pp. 1019-1028.

LIN, W.T., CHAN, T.F., HUANG, H.L., LEE, C.Y., TSAI, S., WU, P.W., YANG, Y.C., WANG, T.N. and LEE, C.H., 2016. Fructose-Rich Beverage Intake and Central Adiposity, Uric Acid, and Pediatric Insulin Resistance. *The Journal of pediatrics,* **171**, pp. 90-6.e1.

LODI, A., KARSTEN, B., BOSCO, G., GOMEZ-LOPEZ, M., BRANDAO, P.P., BIANCO, A. and PAOLI, A., 2016. The Effects of Different High-Protein Low-Carbohydrates Proprietary Foods on Blood Sugar in Healthy Subjects. *Journal of medicinal food,* .

LOGIE, R., LAW, A., TRAWLEY, S. and NISSAN, J., 2010. Multitasking, working memory and remembering intentions. *Psychologica Belgica,* **50**(3-4),.

LOU, Z., WANG, H., LI, J., CHEN, S., ZHU, S., MA, C. and WANG, Z., 2010. Antioxidant activity and chemical composition of the fractions from burdock leaves. *J Food Sci,* **75**, pp. C413-C419.

LOW, Y.Q., LACY, K. and KEAST, R., 2014. The role of sweet taste in satiation and satiety. *Nutrients,* **6**(9), pp. 3431-3450.

LUDWIG, D.S., 2009. Artificially sweetened beverages: cause for concern. *JAMA,* **302**(22), pp. 2477-2478.

LUGASI, A., BLAZOVICS, A., HAGYMASI, K., KOCSIS, I. and KERY, A., 2005. Antioxidant effect of squeezed juice from black radish (Raphanus sativus L. var niger) in alimentary hyperlipidaemia in rats. *Phytother Res,* **19**, pp. 587-591.

LUHOVYY, B.L., AKHAVAN, T. and ANDERSON, G.H., 2007. Whey proteins in the regulation of food intake and satiety. *Journal of the American College of Nutrition,* **26**(6), pp. 704S-712S.

MACLEAN, P.S., BERGOUIGNAN, A., CORNIER, M.A. and JACKMAN, M.R., 2011. Biology's response to dieting: the impetus for weight regain. *American journal of physiology.Regulatory, integrative and comparative physiology,* **301**(3), pp. R581-600.

MAKAROFF, L.E., 2016. The need for international consensus on prediabetes. *The lancet.Diabetes & endocrinology, .*

MAKI, K.C. and PHILLIPS, A.K., 2015. Dietary substitutions for refined carbohydrate that show promise for reducing risk of type 2 diabetes in men and women. *The Journal of nutrition,* **145**(1), pp. 159S-163S.

MAKRIS, A., DARCEY, V.L., ROSENBAUM, D.L., KOMAROFF, E., VANDER VEUR, S.S., COLLINS, B.N., KLEIN, S., WYATT, H.R. and FOSTER, G.D., 2013. Similar effects on cognitive performance during high- and low-carbohydrate obesity treatment. *Nutrition & diabetes,* **3**, pp. e89.

MARK, S., DU TOIT, S., NOAKES, T.D., NORDLI, K., COETZEE, D., MAKIN, M., VAN DER SPUY, S., FREY, J. and WORTMAN, J., 2016. A successful lifestyle intervention model replicated in diverse clinical settings. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde,* **106**(8), pp. 763-766.

MARTIN, W.F., ARMSTRONG, L.E. and RODRIGUEZ, N.R., 2005. Dietary protein intake and renal function. *Nutrition & metabolism,* **2**, pp. 25.

MAZESS, R.B. and MATHER, W., 1974. Bone mineral content of North Alaskan Eskimos. *The American Journal of Clinical Nutrition,* **27**(9), pp. 916-925.

MCLAUGHLIN, T., REAVEN, G., ABBASI, F., LAMENDOLA, C., SAAD, M., WATERS, D., SIMON, J. and KRAUSS, R.M., 2005. Is There a Simple Way to Identify Insulin-Resistant Individuals at Increased Risk of Cardiovascular Disease? *The American Journal of Cardiology,* **96**(3), pp. 399-404.

MCNAY, E.C. and SHERWIN, R.S., 2004. Effect of recurrent hypoglycaemia on spatial cognition and cognitive metabolism in normal and diabetic rats. *Diabetes,* **53**(2), pp. 418-425.

MCVAY, M.A., VOILS, C.I., COFFMAN, C.J., GEISELMAN, P.J., KOLOTKIN, R.L., MAYER, S.B., SMITH, V.A., GAILLARD, L., TURNER, M.J. and YANCY, W.S., 2014. Factors associated with choice of a low-fat or low-carbohydrate diet during a behavioral weight loss intervention. *Appetite,* **83**, pp. 117-124.

MEIDENBAUER, J.J., MUKHERJEE, P. and SEYFRIED, T.N., 2015. The glucose ketone index calculator: a simple tool to monitor therapeutic efficacy for metabolic management of brain cancer. *Nutrition & metabolism,* **12**, pp. 12-015-0009-2. eCollection 2015.

MILLER, B.V., BERTINO, J.S., REED, R.G., BURRINGTON, C.M., DAVIDSON, L.K., GREEN, A., GARTUNG, A.M. and NAFZIGER, A.N., 2003. An evaluation of the atkins' diet. *Metabolic syndrome and related disorders,* **1**(4), pp. 299-309.

MIMICA-DUKIC, N., SIMIN, N., CVEJIC, J., JOVIN, E., ORCIC, D. and BOZIN, B., 2008. Phenolic compounds in field horsetail (Equisetum arvense L.) as natural antioxidants. *Molecules,* **13**, pp. 1455-1464.

MONTEIRO, C.A., MOUBARAC, J., CANNON, G., NG, S.W. and POPKIN, B., 2013. Ultra-processed products are becoming dominant in the global food system. *Obesity reviews,* **14**(S2), pp. 21-28.

MONTEIRO, C.A., LEVY, R.B., CLARO, R.M., DE CASTRO, INÊS RUGANI RIBEIRO and CANNON, G., 2011. Increasing consumption of ultra-processed foods and likely impact on human health: evidence from Brazil. *Public health nutrition,* **14**(01), pp. 5-13.

MOUBARAC, J., BATAL, M., MARTINS, A.P.B., CLARO, R., LEVY, R.B., CANNON, G. and MONTEIRO, C., 2014. Processed and ultra-processed food products: consumption trends in Canada from 1938 to 2011. *Canadian Journal of Dietetic Practice and Research,* **75**(1), pp. 15-21.

MULVANEY, S.A., SCHLUNDT, D.G., MUDASIRU, E., FLEMING, M., VANDER WOUDE, A.M., RUSSELL, W.E., ELASY, T.A. and ROTHMAN, R., 2006. Parent perceptions of caring for adolescents with type 2 diabetes. *Diabetes care,* **29**(5), pp. 993-997.

MUTCH, D.M. and CLEMENT, K., 2006. Unraveling the genetics of human obesity. *PLoS genetics,* **2**(12), pp. e188.

NEWMAN, J.C. and VERDIN, E., 2014. Ketone bodies as signaling metabolites. *Trends in endocrinology and metabolism: TEM,* **25**(1), pp. 42-52.

NG, M., FLEMING, T., ROBINSON, M., THOMSON, B., GRAETZ, N., MARGONO, C., MULLANY, E.C., BIRYUKOV, S., ABBAFATI, C., ABERA, S.F., ABRAHAM, J.P., ABU-RMEILEH, N.M., ACHOKI, T., ALBUHAIRAN, F.S., ALEMU, Z.A., ALFONSO, R., ALI, M.K., ALI, R., GUZMAN, N.A., AMMAR, W., ANWARI, P., BANERJEE, A., BARQUERA, S., BASU, S., BENNETT, D.A., BHUTTA, Z., BLORE, J., CABRAL, N., NONATO, I.C., CHANG, J.C., CHOWDHURY, R., COURVILLE, K.J., CRIQUI, M.H., CUNDIFF, D.K., DABHADKAR, K.C., DANDONA, L., DAVIS, A., DAYAMA, A., DHARMARATNE, S.D., DING, E.L., DURRANI, A.M., ESTEGHAMATI, A., FARZADFAR, F., FAY, D.F., FEIGIN, V.L., FLAXMAN, A., FOROUZANFAR, M.H., GOTO, A., GREEN, M.A., GUPTA, R., HAFEZI-NEJAD, N., HANKEY, G.J., HAREWOOD, H.C., HAVMOELLER, R., HAY, S., HERNANDEZ, L., HUSSEINI, A., IDRISOV, B.T., IKEDA, N., ISLAMI, F., JAHANGIR, E., JASSAL, S.K., JEE, S.H., JEFFREYS, M., JONAS, J.B., KABAGAMBE, E.K., KHALIFA, S.E., KENGNE, A.P., KHADER, Y.S., KHANG, Y.H., KIM, D., KIMOKOTI, R.W., KINGE, J.M., KOKUBO, Y., KOSEN, S., KWAN, G., LAI, T., LEINSALU, M., LI, Y., LIANG, X., LIU, S., LOGROSCINO, G., LOTUFO, P.A., LU, Y., MA, J., MAINOO, N.K., MENSAH, G.A., MERRIMAN, T.R., MOKDAD, A.H., MOSCHANDREAS, J., NAGHAVI, M., NAHEED, A., NAND, D., NARAYAN, K.M., NELSON, E.L., NEUHOUSER, M.L., NISAR, M.I., OHKUBO, T., OTI, S.O., PEDROZA, A., PRABHAKARAN, D., ROY, N., SAMPSON, U., SEO, H., SEPANLOU, S.G., SHIBUYA, K., SHIRI, R., SHIUE, I., SINGH, G.M., SINGH, J.A., SKIRBEKK, V., STAPELBERG, N.J., STURUA, L., SYKES, B.L., TOBIAS, M., TRAN, B.X., TRASANDE, L., TOYOSHIMA, H., VAN DE VIJVER, S., VASANKARI, T.J., VEERMAN, J.L., VELASQUEZ-MELENDEZ, G., VLASSOV, V.V., VOLLSET, S.E., VOS, T., WANG, C., WANG, X., WEIDERPASS, E., WERDECKER, A., WRIGHT, J.L., YANG, Y.C., YATSUYA, H., YOON, J., YOON, S.J., ZHAO, Y., ZHOU, M., ZHU, S., LOPEZ, A.D., MURRAY, C.J. and GAKIDOU, E., 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet (London, England),* **384**(9945), pp. 766-781.

NINA TEICHOLZ, 2014. *The Big Fat Surprise.* New York: Simon & Schuster Papaerbacks.

NOAKES, T.D. and WINDT, J., 2017. Evidence that supports the prescription of low-carbohydrate high-fat diets: a narrative review. *British journal of sports medicine,* **51**(2), pp. 133-139.

NORDMANN, A.J., NORDMANN, A., BRIEL, M., KELLER, U., YANCY, W.S., BREHM, B.J. and BUCHER, H.C., 2006. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Archives of Internal Medicine,* **166**(3), pp. 285-293.

PAHO WHO, 2015. *Ultra-processed food and drink products in Latin America: Trends, impact on obesity, policy implications.* Washington D.C.: PAHO WHO.

PAL, S. and ELLIS, V., 2010. The acute effects of four protein meals on insulin, glucose, appetite and energy intake in lean men. *British journal of nutrition,* **104**(08), pp. 1241-1248.

PAOLI, A., CANATO, M., TONIOLO, L., BARGOSSI, A., NERI, M., MEDIATI, M., ALESSO, D., SANNA, G., GRIMALDI, K. and FAZZARI, A., 2011a. La dieta chetogenica: un'opportunità terapeutica ignorata? *Clin Ter,* **162**(5), pp. e137-146.

PAOLI, A., GRIMALDI, K., BIANCO, A., LODI, A., CENCI, L. and PARMAGNANI, A., 2012a. Medium term effects of a ketogenic diet and a Mediterranean diet on resting energy expenditure and respiratory ratio, *BMC Proceedings* 2012a, BioMed Central, pp. 1.

PAOLI, A., 2014. Ketogenic diet for obesity: friend or foe? *International journal of environmental research and public health,* **11**(2), pp. 2092-2107.

PAOLI, A., 2012. Ketogenic diet does not affect strength performance in elite artistic gymnasts. *Journal of the International Society of Sports Nutrition,* **9**(1), pp. 34-34.

PAOLI, A., 2011. 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. *Nutrition journal,* **10**, pp. 112.

PAOLI, A., BIANCO, A., GRIMALDI, K.A., LODI, A. and BOSCO, G., 2013. Long term successful weight loss with a combination biphasic ketogenic Mediterranean diet and Mediterranean diet maintenance protocol. *Nutrients,* **5**(12), pp. 5205-5217.

PAOLI, A., CANATO, M., TONIOLO, L., BARGOSSI, A.M., NERI, M., MEDIATI, M., ALESSO, D., SANNA, G., GRIMALDI, K.A., FAZZARI, A.L. and BIANCO, A., 2011. The ketogenic diet: an underappreciated therapeutic option? *Clin Ter,* **162**, pp. e145-e153.

PAOLI, A., CENCI, L., FANCELLI, M., PARMAGNANI, A., FRATTER, A., CUCCHI, A. and BIANCO, A., 2010a. Ketogenic diet and phytoextracts Comparison of the efficacy of Mediterranean, zone and tisanoreica diet on some health risk factors. *Agro Food Ind Hi-Tech,* **21**, pp. 24-+.

PAOLI, A., CENCI, L. and GRIMALDI, K.A., 2011. 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,* **10**, pp. 112.

PAOLI, A., RUBINI, A., VOLEK, J.S. and GRIMALDI, K.A., 2013. Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *European journal of clinical nutrition,* **67**(8), pp. 789-796.

PAOLI, A., BOSCO, G., CAMPORESI, E.M. and MANGAR, D., 2015. Ketosis, ketogenic diet and food intake control: a complex relationship. *Frontiers in Psychology,* **6**, pp. 27.

PAOLI, A., GRIMALDI, K., D'AGOSTINO, D., CENCI, L., MORO, T., BIANCO, A. and PALMA, A., 2012b. Ketogenic diet does not affect strength performance in elite artistic gymnasts. *Journal of the International Society of Sports Nutrition,* **9**(1), pp. 34.

PASIAKOS, S.M., 2015. Metabolic advantages of higher protein diets and benefits of dairy foods on weight management, glycaemic regulation, and bone. *Journal of Food Science,* **80**(S1), pp. A2-A7.

PERONA, J.S., CABELLO-MORUNO, R. and RUIZ-GUTIERREZ, V., 2006. The role of virgin olive oil components in the modulation of endothelial function. *The Journal of nutritional biochemistry,* **17**(7), pp. 429-445.

PESTA, D.H. and SAMUEL, V.T., 2014. A high-protein diet for reducing body fat: mechanisms and possible caveats. *Nutrition & metabolism,* **11**(1), pp. 53-7075-11-53. eCollection 2014.

PHINNEY, S.D., 2004. Ketogenic diets and physical performance. *Nutr Metab (Lond),* **1**, pp. 2.

PHINNEY, S.D., HORTON, E.S., SIMS, E.A., HANSON, J.S., DANFORTH, E. and LAGRANGE, B.M., 1980. Capacity for moderate exercise in obese subjects after adaptation to a hypocaloric, ketogenic diet. *J Clin Invest,* **66**, pp. 1152-1161.

PIATO, A.L., DETANICO, B.C., LINCK, V.M., HERRMANN, A.P., NUNES, D.S. and ELISABETSKY, E., 2010. Anti-stress effects of the "tonic"Ptychopetalum olacoides (Marapuama) in mice. *Phytomedicine,* **17**, pp. 248-253.

PICCOLI, A., BRUNANI, A., SAVIA, G., PILLON, L., FAVARO, E., BERSELLI, M. and CAVAGNINI, F., 1998. Discriminating between body fat and fluid changes in the obese adult using bioimpedance vector analysis. *International journal of obesity,* **22**(2), pp. 97-104.

PIERALISI, G., RIPARI, P. and VECCHIET, L., 1991. Effects of a standardized ginseng extract combined with dimethylaminoethanol bitartrate, vitamins, minerals, and trace elements on physical performance during exercise. *Clin Ther,* **13**, pp. 373-382.

PLOWMAN, S.A. and SMITH, D.L., 2013. *Exercise physiology for health fitness and performance.* Lippincott Williams & Wilkins.

POPLAWSKI, M.M., MASTAITIS, J.W., ISODA, F., GROSJEAN, F., ZHENG, F. and MOBBS, C.V., 2011. Reversal of diabetic nephropathy by a ketogenic diet. *PloS one,* **6**(4), pp. e18604.

PRADEEPA, R. and MOHAN, V., 2011. Postprandial glycaemic excursions and cardiovascular risk. *Journal of the Indian Medical Association,* **109**(12), pp. 912-917.

PROF. TIM NOAKES, JONNO PROUDFOOT and SALLY-ANN CREED, 2015. *The Real Meal Revolution.* Great Britain: Robinson.

RAATZ, S.K., JOHNSON, L.K. and PICKLO, M.J., 2015. Consumption of Honey, Sucrose, and High-Fructose Corn Syrup Produces Similar Metabolic Effects in Glucose-Tolerant and -Intolerant Individuals. *The Journal of nutrition,* **145**(10), pp. 2265-2272.

RAGHUBAR, K.P., BARNES, M.A. and HECHT, S.A., 2010. Working memory and mathematics: A review of developmental, individual difference, and cognitive approaches. *Learning and individual differences,* **20**(2), pp. 110-122.

REAVEN, G., 2012. Insulin resistance and coronary heart disease in nondiabetic individuals. *Arteriosclerosis, Thrombosis, and Vascular Biology,* **32**(8), pp. 1754-1759.

REDDY, S.T., WANG, C., SAKHAEE, K., BRINKLEY, L. and PAK, C.Y.C., 2002. Effect of low-carbohydrate high-protein diets on acid-base balance, stone-forming propensity, and calcium metabolism. *American Journal of Kidney Diseases,* **40**(2), pp. 265-274.

REINEHR, T., 2013. Type 2 diabetes mellitus in children and adolescents. *World J Diabetes,* **4**(6), pp. 270-281.

RICHARD, C., COUTURE, P., DESROCHES, S. and LAMARCHE, B., 2013. Effect of the Mediterranean diet with and without weight loss on markers of inflammation in men with metabolic syndrome. *Obesity,* **21**(1), pp. 51-57.

RICHTER, V., RASSOUL, F., LUTTGE, F. and THIERY, J., 2007. Cardiovascular risk factor profile on a population basis: Results from the Lipid Study Leipzig (LSL). *Experimental and clinical cardiology,* **12**(1), pp. 51-53.

ROBERTO, C.A., SWINBURN, B., HAWKES, C., HUANG, T.T., COSTA, S.A., ASHE, M., ZWICKER, L., CAWLEY, J.H. and BROWNELL, K.D., 2015. Patchy progress on obesity prevention: emerging examples, entrenched barriers, and new thinking. *The Lancet,* **385**(9985), pp. 2400-2409.

ROGERS, P.J. and BRUNSTROM, J.M., 2016. Appetite and energy balancing. *Physiology & Behavior,* .

ROGERS, P., HOGENKAMP, P., DE GRAAF, C., HIGGS, S., LLUCH, A., NESS, A., PENFOLD, C., PERRY, R., PUTZ, P. and YEOMANS, M., 2015. 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. *International journal of obesity,* .

ROLLS, E.T., 2016. Reward systems in the brain and nutrition. *Annual Review of Nutrition,* (0),.

ROTHBERG, M.B., 2013. Coronary artery disease as clogged pipes: a misconceptual model. *Circulation.Cardiovascular quality and outcomes,* **6**(1), pp. 129-132.

SAFIYEH, S., FATHALLAH FB, VAHID, N., HOSSINE, N. and HABIB, S.S., 2007. Antidiabetic effect of Equisetum arvense L. (Equisetaceae) in streptozotocin-induced diabetes in male rats. *Pak J Biol Sci,* **10**, pp. 1661-1666.

SAUNDERS, N.H., AL-ZEIBAK, S., RYDE, S.J. and BIRKS, J.L., 1993. The composition of weight loss in dieting obese females by electrical methods. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity,* **17**(6), pp. 317-322.

SAVINO, F., CRESI, F., GRASSO, G., OGGERO, R. and SILVESTRO, L., 2004. The Biagram vector: a graphical relation between reactance and phase angle measured by bioelectrical analysis in infants. *Annals of Nutrition & Metabolism,* **48**(2), pp. 84-89.

SCHIAVO, L., SCALERA, G., PILONE, V., DE SENA, G., QUAGLIARIELLO, V., IANNELLI, A. and BARBARISI, A., 2016. A Comparative Study Examining the Impact of a Protein-Enriched Vs Normal Protein Postoperative Diet on Body Composition and Resting Metabolic Rate in Obese Patients after Sleeve Gastrectomy. *Obesity Surgery,* .

SCHWARZFUCHS, D., GOLAN, R. and SHAI, I., 2012. Four-year follow-up after two-year dietary interventions. *New England Journal of Medicine,* **367**(14), pp. 1373-1374.

SCHWINGSHACKL, L. and HOFFMANN, G., 2013. Long-term effects of low glycaemic index/load vs. high glycaemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. *Nutrition, Metabolism and Cardiovascular Diseases,* **23**(8), pp. 699-706.

SHAI, I., SCHWARZFUCHS, D., HENKIN, Y., SHAHAR, D.R., WITKOW, S., GREENBERG, I., GOLAN, R., FRASER, D., BOLOTIN, A., VARDI, H., TANGI-ROZENTAL, O., ZUK-RAMOT, R., SARUSI, B., BRICKNER, D., SCHWARTZ, Z., SHEINER, E., MARKO, R., KATORZA, E., THIERY, J., FIEDLER, G.M., BLUHER, M., STUMVOLL, M., STAMPFER, M.J. and DIETARY INTERVENTION RANDOMIZED, C.T., 2008. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *N Engl J Med,* **359**, pp. 229-241.

SILIPRANDI & TETTAMANTI, 2011. *Biochimica Medica.* Padova: Piccin.

SINGH, S.K., 2012. Post-prandial hyperglycaemia. *Indian journal of endocrinology and metabolism,* **16**(Suppl 2), pp. S245-7.

SIRI-TARINO, P.W., SUN, Q., HU, F.B. and KRAUSS, R.M., 2010. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *The American Journal of Clinical Nutrition,* **91**(3), pp. 535-546.

SKOV, A.R., HAULRIK, N., TOUBRO, S., MOLGAARD, C. and ASTRUP, A., 2002. Effect of protein intake on bone mineralization during weight loss: a 6-month trial. *Obesity research,* **10**(6), pp. 432-438.

SOCIETÀ ITALIANA DI NUTRIZIONE UMANA - SINU, 2014-last update, LARN - Livelli di assunzione di riferimento per la popolazione italiana: CARBOIDRATI E FIBRA ALIMENTARE. Available: http://www.sinu.it/html/pag/05-CARBOIDRATI-E-FIBRA-ALIMENTARE.asp2016].

SOENEN, S., BONOMI, A.G., LEMMENS, S.G.T., SCHOLTE, J., THIJSSEN, M.A.M.A., VAN BERKUM, F. and WESTERTERP-PLANTENGA, M.S., 2012. Relatively high-protein or 'low-carb' energy-restricted diets for body weight loss and body weight maintenance? *Physiology & Behavior,* **107**(3), pp. 374-380.

STACKPOLE, E.A., 1965. *The long Arctic search: The narrative of Lieutenant Frederick Schwatka, USA, 1878-1880, seeking the records of the lost Franklin expedition.* Marine Historical Association.

STUBBS, R.J., HUGHES, D.A., JOHNSTONE, A.M., ROWLEY, E., REID, C., ELIA, M., STRATTON, R., DELARGY, H., KING, N. and BLUNDELL, J.E., 2000. The use of visual analogue scales to assess motivation

to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *The British journal of nutrition,* **84**(4), pp. 405-415.

SUMITHRAN, P., PRENDERGAST, L.A., DELBRIDGE, E., PURCELL, K., SHULKES, A., KRIKETOS, A. and PROIETTO, J., 2011. Long-term persistence of hormonal adaptations to weight loss. *New England Journal of Medicine,* **365**(17), pp. 1597-1604.

SUMITHRAN, P., PRENDERGAST, L.A., DELBRIDGE, E., PURCELL, K., SHULKES, A., KRIKETOS, A. and PROIETTO, J., 2013. Ketosis and appetite-mediating nutrients and hormones after weight loss. *European journal of clinical nutrition,* **67**(7), pp. 759-764.

SUMITHRAN, P. and PROIETTO, J., 2013. The defence of body weight: a physiological basis for weight regain after weight loss. *Clinical science (London, England : 1979),* **124**(4), pp. 231-241.

SUNG, K.C., JEONG, W.S., WILD, S.H. and BYRNE, C.D., 2012. Combined influence of insulin resistance, overweight/obesity, and fatty liver as risk factors for type 2 diabetes. *Diabetes care,* **35**(4), pp. 717-722.

SWITHERS, S.E., MARTIN, A.A. and DAVIDSON, T.L., 2010. High-intensity sweeteners and energy balance. *Physiology & Behavior,* **100**(1), pp. 55-62.

TAGLIABUE, A., BERTOLI, S., TRENTANI, C., BORRELLI, P. and VEGGIOTTI, P., 2012. 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,* **31**(2), pp. 246-249.

TAY, J., THOMPSON, C.H. and BRINKWORTH, G.D., 2015. Glycaemic variability: assessing glycaemia differently and the implications for dietary management of diabetes. *Annual Review of Nutrition,* **35**, pp. 389-424.

THOMAS, P.R., 1995. *Weighing the options: criteria for evaluating weight-management programs.* National Academies Press.

TOBIAS, D.K., CHEN, M., MANSON, J.E., LUDWIG, D.S., WILLETT, W. and HU, F.B., 2015. Effect of low-fat diet interventions versus other diet interventions on long-term weight change in adults: a systematic review and meta-analysis. *The lancet.Diabetes & endocrinology,* **3**(12), pp. 968-979.

TOELLER, M., BUYKEN, A., HEITKAMP, G., MILNE, R., KLISCHAN, A. and GRIES, F.A., 1997. Repeatability of three-day dietary records in the EURODIAB IDDM Complications Study. *Eur J Clin Nutr,* **51**, pp. 74-80.

TOVAR, A.R., DEL CARMEN CAAMAÑO, M., GARCIA-PADILLA, S., GARCÍA, O.P., DUARTE, M.A. and ROSADO, J.L., 2012. 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. *Nutrition journal,* **11**(1), pp. 1.

TRICHOPOULOU, A., KOURIS-BLAZOS, A., WAHLQVIST, M.L., GNARDELLIS, C., LAGIOU, P., POLYCHRONOPOULOS, E., VASSILAKOU, T., LIPWORTH, L. and TRICHOPOULOS, D., 1995. Diet and overall survival in elderly people. *BMJ (Clinical research ed.),* **311**(7018), pp. 1457-1460.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, June 22, 2016, 2016-last update, What is Metabolic Syndrome?. Available: http://www.nhlbi.nih.gov/health/health-topics/topics/ms2016].

VÁZQUEZ, C., MONTAGNA, C., ALCARAZ, F., BALSA, J., ZAMARRÓN, I., ARRIETA, F. and BOTELLA-CARRETERO, J., 2009. Meal replacement with a low-calorie diet formula in weight loss maintenance after weight loss induction with diet alone. *European journal of clinical nutrition,* **63**(10), pp. 1226-1232.

VELDHORST, M.A., WESTERTERP, K.R., VAN VUGHT, A.J. and WESTERTERP-PLANTENGA, M.S., 2010. Presence or absence of carbohydrates and the proportion of fat in a high-protein diet affect appetite suppression but not energy expenditure in normal-weight human subjects fed in energy balance. *The British journal of nutrition,* **104**(9), pp. 1395-1405.

VENN, B. and MANN, J., 2004. Cereal grains, legumes and diabetes. *European journal of clinical nutrition,* **58**(11), pp. 1443-1461.

VENTURA, T., SANTANDER, J., TORRES, R. and CONTRERAS, A.M., 2014. Neurobiologic basis of craving for carbohydrates. *Nutrition,* **30**(3), pp. 252-256.

VENTURA, A.K. and MENNELLA, J.A., 2011. Innate and learned preferences for sweet taste during childhood. *Current opinion in clinical nutrition and metabolic care,* **14**(4), pp. 379-384.

VERREIJEN, A.M., VERLAAN, S., ENGBERINK, M.F., SWINKELS, S., DE VOGEL-VAN DEN BOSCH, J. and WEIJS, P.J., 2015. 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. *The American Journal of Clinical Nutrition,* **101**(2), pp. 279-286.

VOLEK, J.S., FERNANDEZ, M.L., FEINMAN, R.D. and PHINNEY, S.D., 2008. Dietary carbohydrate restriction induces a unique metabolic state positively affecting atherogenic dyslipidemia, fatty acid partitioning, and metabolic syndrome. *Progress in lipid research,* **47**(5), pp. 307-318.

VOLEK, J.S., PHINNEY, S.D., FORSYTHE, C.E., QUANN, E.E., WOOD, R.J., PUGLISI, M.J., KRAEMER, W.J., BIBUS, D.M., FERNANDEZ, M.L. and FEINMAN, R.D., 2009. Carbohydrate restriction has a more favorable impact on the metabolic syndrome than a low fat diet. *Lipids,* **44**(4), pp. 297-309.

WAGNER, A.D., 1999. Working memory contributions to human learning and remembering. *Neuron,* **22**(1), pp. 19-22.

WARNICK, G.R., BOERMA, G.J., ASSMANN, G., ENDLER, A.T., GERIQUE, G., GOTTO, A.M., GRAZIANI, M.S., LIPPI, U., PATSCH, W. and RIESEN, W.F., 1993. Multicenter evaluation of Reflotron direct dry-chemistry assay of high-density lipoprotein cholesterol in venous and fingerstick specimens. *Clinical chemistry,* **39**(2), pp. 271-277.

WHELESS, J.W., 2008. History of the ketogenic diet. *Epilepsia,* **49 Suppl 8**, pp. 3-5.

WHO, 2015 – last update. Fact Sheet on Noncommunicable diseases. Available: http://www.who.int/mediacentre/factsheets/fs355/en/.

WHO, 2016-last update, Obesity. Available: http://www.who.int/topics/obesity/en/.

WHO, June, 2016, 2016b-last update, Obesity and overweight. Available: http://www.who.int/mediacentre/factsheets/fs311/en/2016].

WHO, 2006. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. Report of a WHO/IDF consultation. Available: http://www.who.int/diabetes/publications/diagnosis_diabetes2006/en/

WHO REGIONAL OFFICE FOR EUROPE, A healthy lifestyle. Available: http://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle2016].

WIEGMANN, T.B., ZLOMKE, A.M., MACDOUGALL, M.L. and KIPP, D.E., 1990. Controlled changes in chronic dietary protein intake do not change glomerular filtration rate. *American Journal of Kidney Diseases : The Official Journal of the National Kidney Foundation,* **15**(2), pp. 147-154.

WIEN, M.A., SABATE, J.M., IKLE, D.N., COLE, S.E. and KANDEEL, F.R., 2003. Almonds vs complex carbohydrates in a weight reduction program. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity,* **27**(11), pp. 1365-1372.

WIGGAM, M.I., O'KANE, M.J., HARPER, R., ATKINSON, A.B., HADDEN, D.R., TRIMBLE, E.R. and BELL, P.M., 1997. Treatment of diabetic ketoacidosis using normalization of blood 3-hydroxybutyrate concentration as the endpoint of emergency management. A randomized controlled study. *Diabetes care,* **20**(9), pp. 1347-1352.

WIJNHOVEN, T.M., VAN RAAIJ, J.M., SPINELLI, A., STARC, G., HASSAPIDOU, M., SPIROSKI, I., RUTTER, H., MARTOS, E., RITO, A.I., HOVENGEN, R., PEREZ-FARINOS, N., PETRAUSKIENE, A., ELDIN, N., BRAECKEVELT, L., PUDULE, I., KUNESOVA, M. and BREDA, J., 2014. WHO European Childhood Obesity Surveillance Initiative: body mass index and level of overweight among 6-9-year-old children from school year 2007/2008 to school year 2009/2010. *BMC public health,* **14**, pp. 806-2458-14-806.

WIKIPEDIA, 2012-last update, Ketogenesis. Available: https://en.wikipedia.org/wiki/Ketogenesis2016].

WIKIPEDIA, 2008-last update, Citric acid cycle. Available: https://en.wikipedia.org/wiki/Citric_acid_cycle2016].

WING, R.R. and HILL, J.O., 2001. Successful weight loss maintenance. *Annual Review of Nutrition,* **21**(1), pp. 323-341.

WORLD OBESITY FEDERATION, 2015. World Obesity (formerly IASO) history. Available: http://www.worldobesity.org/who-we-are/history/.

WYCHERLEY, T.P., MORAN, L.J., CLIFTON, P.M., NOAKES, M. and BRINKWORTH, G.D., 2012. Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials. *The American Journal of Clinical Nutrition,* **96**(6), pp. 1281-1298.

WYKA, J., MALCZYK, E., MISIARZ, M., ZOLOTENKA-SYNOWIEC, M., CALYNIUK, B. and BACZYNSKA, S., 2015. Assessment of food intakes for women adopting the high protein Dukan diet. *Roczniki Panstwowego Zakladu Higieny,* **66**(2), pp. 137-142.

YANG, Q., 2010. Gain weight by “going diet?” Artificial sweeteners and the neurobiology of sugar cravings. *Yale J Biol Med,* **83**(2), pp. 101-108.

YOUNG, H. and BENTON, D., 2014. The nature of the control of blood glucose in those with poorer glucose tolerance influences mood and cognition. *Metabolic brain disease,* **29**(3), pp. 721-728.

YUDKIN, J. and CAREY, M., 1960. The treatment of obesity by the "highfat" diet. The inevitability of calories. *Lancet (London, England),* **2**(7157), pp. 939-941.

ZEEVI, D., KOREM, T., ZMORA, N., ISRAELI, D., ROTHSCHILD, D., WEINBERGER, A., BEN-YACOV, O., LADOR, D., AVNIT-SAGI, T. and LOTAN-POMPAN, M., 2015. Personalized nutrition by prediction of glycaemic responses. *Cell,* **163**(5), pp. 1079-1094.

ZOCK, P.L., BLOM, W.A., NETTLETON, J.A. and HORNSTRA, G., 2016. Progressing Insights into the Role of Dietary Fats in the Prevention of Cardiovascular Disease. *Current cardiology reports,* **18**(11), pp. 111-016-0793-y.

ZUPEC-KANIA, B. and ZUPANC, M.L., 2008. Long-term management of the ketogenic diet: seizure monitoring, nutrition, and supplementation. *Epilepsia,* **49**, pp. 23-26.