THERAPEUTIC ACTION OF KETOGENIC ENTERAL NUTRITION IN OBESE AND OVERWEIGHT PATIENTS: A RETROSPECTIVE INTERVENTIONAL STUDY

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Abstract

Background: Ketogenic Enteral Nutrition (KEN™) is a modification of Blackburn's protein-sparing modified fast, using a hypocaloric, ketogenic liquid diet. The study is about Ketogenic enteral nutrition (KEN) in overweight and obese patients receiving short treatment of the nutritional solution as 24-hour infusion. It is a retrospective analysis that examines safety, weight loss and body composition changes after three sequential 10-days cycles of KEN therapy. **Methods:** Anthropometric and bio-impedance data from 629 patients who underwent KEN were collected before and after completing a ten-day cycle. The study focuses on the change in outcomes from the first cycle to the second cycle and from the first cycle to the third cycle. The following outcomes were explored: weight, waist circumference, BMI, fat mass, lean mass, dry lean mass, phase angle, wellness marker, water mass as a percentage of total body weight. Statistical tests were used to test for significant differences between paired cycle 1 and cycle 2 outcomes and also between paired cycle 1 and cycle 3 outcomes. For normally distributed outcomes, the paired t-test was used. Whereas for skewed outcomes, the Wilcoxon signed-ranks test was used. Scatter plots were used to plot percentage of excess weight loss against phase angle. The Pearson's correlation coefficient was calculated. Regression analysis for the outcome percent change in weight from cycle 1 to cycle 2 for phase angle and basal metabolic rate (BMR)/ Weight ratio as predictors was carried out. **Results**: The results suggested significant changes for all analyzed parameters. There were significant decreases in weight, waist circumference, BMI, fat mass, lean mass, dry lean mass and phase angle. Quantitative changes in lean mass and dry lean mass were minor changes with respect to changes in fat mass. There was also a statistically significant increase in water mass as a % of total body weight and wellness marker from cycle 1 to cycle 3.

The Pearson's correlation coefficients r=0.18, p=0.004 and r=22, p=0.04 indicated changes in cycle 1 and cycle 3 in percentage of weight excess to be significantly, positively correlated to phase angle. The multivariate linear regression model showed that for a 1 unit increase in BMR / weight there was a 3.3 percent decrease in percent change in weight. KEN treatment was overall well tolerated. Long term results need to be explored in further controlled studies **. Conclusions** KEN treatment is safe, well tolerated and results in rapid fat loss without detriment to dry lean mass

Introduction

The global health burden of obesity continues to rise despite improved public awareness of the importance of a healthy diet and regular exercise (1-3). Current treatment options for weight reduction include dietary measures, pharmacotherapy, endoscopic techniques and bariatric surgery. These are limited on the one hand by efficacy and long-term sustainability and on the other hand by safety and accessibility to the general public (4). Bariatric surgery is a valid therapeutic option (5) however inherently invasive and it should not be the first port of call after the failure of simple dietary measures (6, 7). Many of the currently available dietary strategies have not been shown to produce selective fat loss without a significant change in dry lean mass (4).

Dietary interventions that can produce weight reduction of the order of 5-10% of total body weight have been shown to reduce obesity-related morbidity (8-12).

Ketogenic Enteral Nutrition (KEN™) is a protein-sparing modified fast that has been developed in order to achieve rapid, safe, selective fat loss (13-16). Research studies have challenged the notion that ketogenic diets are harmful and demonstrate no loss of aerobic performance in athletes as well as obese individuals (18,19).

Lessons learnt from these studies suggest providing electrolyte and fluid replacement to counteract the natriuretic and kaliuretic effects of a ketogenic diet, together with adequate protein (0.9-1.2g/kg ideal body weight) can be safely administered to patients for long periods of time without adverse effect (20). Previous randomized controlled trials have demonstrated early satiety and significant weight loss using a low-carbohydrate ketogenic diet over a six- to twelve-month period with long-term safety and with preservation of lean mass (21-23).

On the basis of these observations, we proposed a system involving the continuous infusion of a specially formulated nasogastric feed over a ten-day period with a minimum of ten-day interval between each cycle to avoid the effects of keto-adaptation. The continuous nature of the infusion, as well as the ketogenic effects produced, and in contrast with bolus feeding, helps to create and maintain a sense of satiety (24, 25).

Methods

Anthropometric and bio-impedance data from 629 patients who underwent KEN were collected before and after completing each ten-day cycle. The study focused retrospectively on the British cohort of patients undergoing a prospective multicenter pilot study on Ken diet from 2006 to 2017 and were not included in previously published results (14). In particular the study refers to measurements made in the first three cycles of treatment. Patients who were responding but incompletely treated were eligible to continue with further cycles. Exclusion criteria included pregnancy, type I diabetes mellitus, severe hepatic or renal insufficiency (GFR < 20ml/h), inherited metabolic disorders and age < 16 years. Weight, height, waist and hip circumference, as well as bio-impedance measurements were carried out

immediately before the beginning of a KEN cycle and ten days following the completion of a KEN cycle.

Basal metabolic rate-weight ratio was measured at baseline and after each cycle by indirect calorimetry with a coefficient of variation of <10% was used for accurate analysis.

Patients repeated the KEN treatment cycle as many times as was required to achieve their target weight based on bio-impedance data.

The study focuses retrospectively on the change in outcomes from the first cycle to the second cycle and from the first cycle to the third cycle.

Informed consent was obtained from all individual participants included in the study and this have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Ethical approval was obtained from the University of Rome La Sapienza Ethics Committee, patients were self-referred and stratified for age and gender.

The following outcomes were explored: waist circumference, BMI, fat mass, lean mass, dry lean mass, phase angle, wellness marker, water mass as a percentage of total body weight. The cycle 1, 2 and 3 outcomes were analyzed using descriptive statistics (either mean and standard deviation, or median and inter-quartile range depending on the data distribution) summarizing the outcome at each cycle. Statistical tests were used to test for significant differences between paired cycle 1 and cycle 2 outcomes and also between paired cycle 1 and cycle 3 outcomes. Where changes in outcomes between timepoints were found to be normally

distributed, the paired t-test was used, whereas where the changes in outcomes had skeweddistributions, the Wilcoxon signed-rank test was used.

Linear regression was used to examine associations between changes in both phase angle and BMR/weight with percentage weight change Initially the simple relationship between variables was examined, and subsequently multiple linear regression was used to re-examine the relationships after adjusting for two pre-specified confounding variables.

A six-French polyurethane nasogastric tube (Pennine, UK) was placed by a trained nurse or physician. In addition, patients received a medication pack, which included, multivitamins and polyethylene glycol-based laxatives to ensure daily bowel movements. Patients were provided with Ketostix™ (Bayer, Switzerland) for daily urinalysis to assess for evidence of ketonuria. Patients were asked to provide a daily record of their weight, ketonuria, hunger assessment (subjective scale of 1 to 10), and bowel movements for the duration of the ten-day cycle. Ketonuria was used as indirect indicator of ketonemia and was collected for observational reasons only. At the end of the KEN cycle, patients attended the clinic for removal of their nasogastric tubes and repeat anthropometric and bio-impedance measurements. Patients were asked to adhere to a low-carbohydrate unsupervised diet and attended ten days later for further anthropometric and bio-impedance measurements. The K1000™ (Nutrimed 2000, Ancona, Italy) formula provides 65g daily protein (providing 1.2g/kg ideal body weight) in an electrolyte-rich solution. Carbohydrate and fat intake was completely restricted for the duration of the cycle.

Four-lead bio-impedance analysis measuring impedance at 5 and 50kHz, resistance at 50kHz, reactance and phase angle at 50kHz were carried out using the Bodystat™ 1500MDD analyzer (Bodystat, Isle of Man) (30-31).

Results

Results were available for the 50 days encompassing 3 treatment cycles in 629 patients. The results produced clinically relevant changes for all analyzed parameters (Tab.1 and 2).

PAUL: could you test collectively (by using ANOVA) differences in cyles 1, 2 and 3?

There were significant decreases in weight, waist circumference, BMI, fat mass, lean mass, dry lean mass and phase angle. Quantitative changes in lean mass and dry lean mass were negligible with respect to changes in fat mass. There was also a statistically significant increase in water mass as a percentage of total body weight and “wellness marker” from cycle 1 to cycle 3.

There was a significant negative association between change in BMR/weight from cycle 1 to cycle 2 and percentage change in weight during the same period. However, this association was no longer significant after adjusting for changes in waist circumference and fat mass.Change in fat phase angle from cycle 1 to cycle 2 was not associated with percentage weight change

PAUL: Diffence in study outcome in age,-sex or BMI in stratified groups ? Overweight vs obese (people with BMI >30)

When considering the change from cycle 1 to cycle 3, there was a significant association between change in BMR/weight and change in weight, which remained significant after adjusting for changes in phase angle, fat mass and waist

circumference. A one-unit increase in BMR/weight was associated with a 2.4% reduction in weight. There was no significant association between change in phase angle from cycle 1 to cycle 3 in the simple analysis. However, after adjustments greater change in phase angle was associated with a greater weight loss.

PAUL : Univariate linera regression analysis should also be performed for other counfonsing factors among all variables tested. Associated variables should then be included in adjustments models.

PAUL: Can cycle 3 be also be tested/included ?

Most patients' daily activities were not restricted, but many chose to spend their period of treatment away from the workplace. By the fifth day of treatment, 24% of patients reported a strong sense of asthenia, despite normal blood pressure levels. Twelve percent of patients reported a mild sense of hunger (score 2-4 / 10). Twenty-two percent of patients (n=138) were known to have type II diabetes mellitus receiving treatment for their condition, 92% (n=127) of these patients under KEN infusion were able to suspend their medication without adverse effect on their glucose homeostasis. No cases of clinically significant hypoglycemia were reported. Similarly, 80% of patients on anti-hypertensive medication also were able to suspend their medication during KEN infusion. Tube displacement and blockage occurred in 3% of cases but did not interrupt completion of the treatment. Patients with mild renal impairment or on anticoagulant therapy underwent close laboratory monitoring during treatment and completed KEN treatment successfully without adverse effects. One patient with renal salt wasting required supplemental sodium chloride to maintain electrolyte stability. Patients on Warfarin therapy were able to halve the dose for the duration of KEN treatment, whilst maintaining adequate anticoagulation.

Following KEN treatment, patients gained an average of 0.8kg after each of the ten-day intervals.

Discussion

This study was undertaken to investigate the hypothesis that KEN treatment results in selective fat loss and to assess patient safety and tolerability. Historical controls would suggest intensive dietetic intervention can achieve 1-2% weight reduction over a period of ten days. This modified fast provides a total of 205 – 270 calories and the 6kg net weight loss observed in ten days is of the same order of magnitude as observed following dietetic interventions in healthy and obese individuals over one year (32).

It might be assumed that such rapid weight loss was the consequence of relative dehydration, but the hallmark of successful KEN treatment is the phenomenon of selective fat loss without detriment to dry lean mass.

This effect might be due to the reduction in lipogenesis and increased lipolysis (33, 34).

Nair et al. reported that beta-hydroxybutyrate decreases leucine oxidation and promotes protein synthesis in human (35).

An other mechanism implicated in preservation of lean mass may be due to interaction of branched-chain amino acid leucine with the insulin signaling pathway to stimulate

downstream control of protein synthesis, resulting in maintenance of muscle mass during periods of restricted energy intake but high protein intake (36).

When water mass was expressed as a percentage of body weight in our patients, there was indeed an observed 1-2% increase after KEN therapy.

The study explored regression analysis of the outcomes percent change in weight from cycle 1 to cycle 2 for the predictors Phase angle and BMR / Weight. BMR/Weight showed a statistically significant correlation with percent change in weight in univariate analysis and multivariate analysis. Phase angle failed as predictor of weight loss in Ken in multivariate analysis. A proportion of 3:1 increase was reported for BMR/ Weight compared to percent change in weight in multivariate analysis. This stand to conclusions that metabolically active lean body tissue increased on a 1:3 basis against percent weight loss after each Ken cycle.

KEN treatment was well tolerated and the few mild to moderate adverse effects reported were all classified as reversible (Tab 4). Despite the placement of a fine-bore nasogastric feeding tube, KEN treatment may be considered a relatively non-invasive technique, when compared to weight management strategies such as endoscopic placement of intragastric balloons, endoscopic restrictive procedures and bariatric surgery. Tube-related complications, which included tube displacement and occlusion, were rare and did not lead to treatment failure.

It has been proposed that the mechanism of action of KEN treatment in inducing continuous satiety is two-fold: the continuous infusion of protein and electrolyte-rich solution into the small intestine producing continuous release of the satiety hormone Peptide YY, and the effects of ketogenic metabolism in suppressing hunger (33).

Effects of keton bodies (KBs) on appetite might be explained by the reduction in appetite control hormones, as ghrelin and leptin (16).

Preliminary data on mice suggest a third mechanism based on KEN-related delayed colonic transit and a subsequent increase in butyrate concentrations as a result of bacterial fermentation, as this may increase insulin sensitivity. Stimulation of sweet taste receptors on the tongue have also been shown to stimulate the release of insulin, counteracting the effects of ketogenesis (35).

We would like to highlight that ketosis is a physiological mechanism described by the biochemist Hans Krebs to differentiate it from the pathological keto acidosis seen in type 1 diabetes. In physiological ketosis ketonemia reaches maximum levels of 7/8 mmol/l (it does not go higher because the central nervous system is able to use KBs efficiently for energy in place of glucose) (16)

However, the majority of recent studies seem instead to amply demonstrate that the reduction of carbohydrates to levels that induce physiological ketosis can lead to significant benefits in blood lipid profile (16)

In summary, individuals with obesity, metabolic syndrome, insulin resistance and type 2 diabetes are likely to see symptomatic as well as objective biochemical improvements on very low- carbohydrate diet. Glucose control improves not only because there is less glucose coming in, but also because systemic insulin sensitivity improves as well.

Current studies are on-going to demonstrate the long-term sustainability of KEN treatment, which will clearly depend on the lifestyle changes adopted by patients after completing KEN therapy. Preliminary data suggest (14) 85% sustainability at

one year, i.e. patients regain a mean of 15% of their pre-treatment weight at one year following completion of the required number of KEN treatment cycles. A ten-fold reduction in all-cause mortality following KEN treatment has been observed (14). New strategies are being developed to assist patients in maintaining their rate of weight reduction between KEN treatment cycles (36,37).

KEN treatment is safe, well tolerated and results in rapid fat loss without detriment to dry lean mass. Controlled prospective research studies are warranted to compare KEN treatment with other more balanced dietary interventions.

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Author Contribution Statement: CP, RS, AF conceived the study; RS and VL collected data, PB analyzed data. CP, RS, AF, GC, VL, wrote the paper, CP had primary responsibility for final content. All authors red and approved the final manuscript.

Conflict of interests

C. Papadia: None Declared, P. Basset: None Declared, V. Lazarescu: None Declared, G. Cappello: None Declared, A Forbes: None Declared, R. Shidrawi: Director of Weight Management Systems Ltd, who are the sole representatives for KEN in the UK

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Table 1: Comparisons of changes in outcome from Cycle 1 to Cycle 2

Outcome	n	Cycle 1	Cycle 2	Change Cycle 1 to 2 (95% CI)	P-value
Weight	228	92.6 [80.6, 111.0]	89.3 [77.5, 107.4]	-3.7 [-4.2, -3.2]	<0.0001#
Waist circumference	227	104 [93, 115]	100 [90, 112]	-3 [-4, -3]	<0.0001#
BMI	226	33.6 [29.8, 37.8]	32.4 [28.9, 37.0]	-1.3 [-1.5, -1.1]	<0.0001#
Fat mass	226	38.4 [29.1, 46.3]	34.6 [27.5, 42.6]	-2.8 [-3.1, -2.4]	<0.0001#
Lean mass	223	52.9 [47.5, 65.2]	52.3 [46.9, 63.6]	-0.8 [-1.1, -0.5]	<0.0001#
Phase angle	223	5.91 ± 0.78	5.89 ± 0.90	-0.02 (-0.09, 0.05)	0.58*
Wellness marker	211	0.875 ± 0.022	0.876 ± 0.022	0.001 (-0.001, 0.003)	0.32*
Dry lean mass	225	15.1 ± 4.2	14.9 ± 4.1	-0.1 (-0.2, -0.1)	<0.0001*
Water mass as a % of total body weight	223	43.7 ± 5.2	44.6 ± 5.7	1.0 (0.7, 1.2)	<0.0001*

Statistics are: mean ± standard deviation plus mean change (95% confidence interval), or median [inter-quartile range] plus median change [95% confidence interval]

P-value from Wilcoxon Signed-ranks test; * P-value from Paired t-test; ~ descriptive statistics presented on the patients with both Cycle 1 and Cycle 2 outcomes available

Table 2: Comparisons of changes in outcome from Cycle 1 to Cycle 3

Outcome	n	Cycle 1	Cycle 3	Change Cycle 1 to 3 (95% CI)	P-value
Weight	126	95.9 [82.8, 115.7]	89.6 [78.4, 108.9]	-6.4 [-7.3, -5.6]	<0.0001#
Waist circumference	125	107 [97, 118]	101 [91, 112]	-6 [-8, -5]	<0.0001#
BMI	124	34.7 [31.2, 38.7]	32.8 [29.0, 36.2]	-2.4 [-2.8, -2.0]	<0.0001#
Fat mass	124	39.4 [32.1, 46.3]	34.3 [27.2, 41.8]	-4.9 [-5.8, -4.1]	<0.0001#
Lean mass	124	54.1 [47.5, 69.1]	52.4 [46.6, 67.8]	-1.3 [-1.6, -0.8]	<0.0001#
Phase angle	123	5.91 ± 0.87	5.77 ± 0.90	-0.13 (-0.21, -0.05)	0.002*
Wellness marker	117	0.875 ± 0.022	0.878 ± 0.022	0.003 (0.001, 0.006)	0.02*
Dry lean mass	124	15.1 ± 4.2	14.9 ± 4.1	-0.2 (-0.3, -0.1)	0.0002*
Water mass as a % of total body weight	124	43.1 ± 4.9	44.7 ± 5.9	1.6 (0.8, 2.4)	0.0001*

Statistics are: mean ± standard deviation plus mean change (95% confidence interval), or median [inter-quartile range] plus median change [95% confidence interval]

P-value from Wilcoxon Signed-ranks test; * P-value from Paired t-test; ~ descriptive statistics presented on the patients with both Cycle 1 and Cycle 2 outcomes available

Table 3: Linear regression analysis examining how changes in study meaures were associated with percent change in weight

Predictor	Unadjusted linear regression			Adjusted linear regression (*)		
	n	Regression coefficient	p-value	n	Regression coefficient	p-value

		(95% CI)			(95% CI)	
Cycle 1 to 2 Change in phase angle	223	0.12 (-0.77, 1.01)	0.79	222	0.22 (-0.39, 0.83)	0.47
Change in BMR / weight	222	-3.33 (-4.04, -2.61)	<0.0001	222	0.37 (-0.54, -1.29)	0.42
Cycle 1 to 3						
Change in phase angle	123	0.67 (-1.18, 2.53)	0.47	121	-1.34 (-2.27, -0.40)	0.006
Change in BMR / weight	124	-3.99 (-4.68, -3.29)	<0.0001	121	-2.38 (-3.28, -1.47)	<0.0001

(*) Adjusted for change in waist circumference, change in fat mass, in addition to change in phase angle, change in BMR/weight

Table 4 Complications/Side effects

Number of patients	Complications/Side effects
2	Diarrhoea
4	Panic attack
54	Asthenia
1	Paroxysmal Tachycardia
3	Difficult NG intubation
1	Hyponatremia (patient with diabetes insipidus)
10	Pharyngeal irritation
1	Hypertension
6	Tube dislocation without further complications