Short Communication

Alkylglycerols reduce serum complement and plasma vascular endothelial growth factor in obese individuals

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Abstract

Alkylglycerols (AKGs), isolated or present in shark liver oil have anti-inflammatory properties. Complement 3 (C3) and 4 (C4) participate in lipid metabolism and in obesity, contributing to the metabolic syndrome and to the low-grade inflammation associated with obesity. In a randomized, controlled, crossover study, 26 non-diabetes obese individuals were assigned two preparations with low (LAC, 10 mg AKGs) and high (HAC, 20 mg AKGs) AKG content. Intervention periods were of 3 weeks preceded by 2-week washout periods in which shark liver oil was avoided. Cholesterol, C3, C4, and vascular endothelial growth factor (VEGF) decreased in a linear trend ($P<0.01$) from baseline (control) to LAC and HAC. Values after HAC were significantly lower ($P<0.05$) versus both baseline and after LAC. No adverse effects were observed or reported. Data from this pilot study open a promising field for the study of the beneficial effects of AKGs on cardiovascular risk factors in obese individuals.

Keywords Alkylglycerols, complement 3, complement 4, vascular endothelial growth factor, obesity, inflammation.

Introduction

Alkylglycerols (AKGs) the major component of shark liver oil (SLO), are glycerol ether lipids with structural characteristics of an ether linkage between fatty acid and α-position of the glycerol backbone (Brohult et al, 1970). AKGs are found in immune organs such as bone marrow and spleen, indicating their important role in human immune activity (Hallgren et al, 1974; Iannitti et al, 2010). AKGs mainly function has shown to be to stimulate the immune response in order to enhance defences against inflammation (Osmond et al 1963; Pugliese et al, 1998). In experimental models, AKGs have been applied for the treatment of radiation side effects, due to their ability to boost the immune system (Pugliese et al, 1998), and to treat leukemia and solid tumor as well (Langen et al, 1979). Adipose tissue macrophages play a key role in obesity induced inflammation and insulin resistance (Ortega Martinez de Victoria et al, 2009). A recent experimental study has shown that unsaturated AKGs can decrease the high-fat induced obesity and promote an improvement of insulin resistance in rats (Zangh M et al, 2013).

Few data exist on the *in vivo* effects of AKGs in humans. In the 1990s, AKGs were observed to be protective against mortality from uterine cervix cancer (Iannitti et al, 2010). In a cross-sectional study by telephone interview, AKGs administration has been reported to support the immune system by preventing symptoms in influenza patients (Ianitti et al, 2011). SLO is available as a dietary supplement in capsule and liquid form. Despite the health benefits above mentioned, to the best of our knowledge no randomized, controlled, clinical trial has been previously performed on the healthy effects of AKGs in humans. The aim of this work was to assess, in a randomized, controlled, crossover trial, the effects of AKGs on inflammatory markers in obese patients.

Methods

AKGs were prepared as previously described (Tenllado et al, 2011). Forty six obese outpatients, aged 23 to 59, from the Endocrinology Department of Hospital del Mar, Barcelona, Spain, were recruited prior to any type of treatment. Participants were community-dwelling with a body mass index (BMI) ≥ 30 kg/m^2 to ≤40 kg/m^2. Exclusion criteria were: intake of antioxidant supplements during the last two months, aspirin or any other drug with established antioxidant and inflammatory properties, BMI<30 or >40 kg/m^2, chronic diseases such as diabetes or cardiovascular, or any other condition that would impair compliance. All procedures performed in study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The local institutional Review Board approved the protocol and all participants included signed an informed consent.Trial is registered in Standard Randomized Controlled Trial (www.isrctn.org): NCT00123456**.**

A placebo-controlled, double-blind, cross-over, randomized, clinical supplementation trial was conducted with:1) high AKGs content (HAC: 20 mg AKG), and 2) low AKGs content (LAC: 10 mg AKG)**.** These oral AKG doses are lower than those which have shown do not cause adverse events, neither when administered to humans (100 mg three times a day) (Pugliese et al, 1998) or in oral safety studies in rats (1000 mg AKG-1 kg-1 of body weight) (Anadón et al, 2010).The randomization scheme was generated by using a web site (http://www.randomization.com). A latin square for the two treatments was used in the crossover clinical trial to randomize participants. HAC and LAC were sequentially administered over two 3-week periods preceded by two-week washout periods in which SLO or AKGs administration was avoided.

At the beginning of the study and after each intervention period the following variables were recorded: anthropometric measurements, a general questionnaire, a 3-day dietary record, and blood samples were collected at fasting. At the beginning and at the end of the study energy expenditure in physical activity (EEPA) was assessed by a modification of the Minnesota Leisure Time Physical Activity Questionnaire which has been validated by our group for its use in Spanish men and women (Elosua et al, 2000). Laboratory determinations for an individual were carried out in the same batch to avoid inter-assay imprecision. Serum glucose, total cholesterol, and triglycerides were performed by standardized enzymatic methods. Complement 3 (C3) and 4 (C4), and high sensibility C reactive protein (hs CRP) in serum were measured by immunoturbidimetry in a PENTRA-400 Analyzer (Horiba ABX, Montpellier, France). Plasma interleukine-8 (IL8), monocyte chemotactic protein-1(MCP-1), and vascular endothelial growth factor (VEGF) were measured by Luminex (x-MAP/x-MAG,) (Millipore, Billerica, Mass, USA). To ensure that AKGs consumption did not promote side effects, plasma aminotransferases, bilirrubin, γ-glutamil-transferase, urea, creatinine, and erythrocyte and leukocyte counts were measured at baseline and after each intervention.

A total sample size of 16 participants allows a 80% power to detect a significant difference between AKG groups of 0.03 g/L of C3 with consideration of a 2-sided type I error of 0.05. This sample size takes into account a 10 % drop-out rate. Calculations were made on the basis of previous data concerning healthy obese individuals and a standard deviation of C3, measured by immunoturbidimetry, of 0.04 (Weyer et al, 2002). Normality of continuous variables was assessed by normal probability plots. Non-normally distributed variables were log transformed and values expressed as antilogarithm. A general lineal model was performed to assess the effect of each

intervention compared to its baseline, and to assess the effect between interventions. Interactions with age, sex, and order, as well as with basal physical activity and triglycerides, variables in which differences between intervention orders were observed, were tested, and none found. A value of $P \leq 0.05$ was considered significant.

Results

From the 46 participants recruited, 26 met the eligibility criteria and were randomized. The high rate of ineligibility can be explained by the difficulty to found obese non-diabetic individuals among outpatients. Four participants dropped out due to difficulties for following the work timetable, three for collateral events, and one because he was unable to adhere to the treatment. Finally, 18 (7 men and 11 women) completed the study and entered in the analyses. Participants' baseline characteristics are shown in Table 1. No changes in energy and macro- or micronutrients of interest were observed in the dietary patterns throughout the study. No changes in daily energy expenditure in leisure-time physical activity were observed from the beginning to the end of the study. Routine biochemical and haematological analyses remained within the normal range throughout the study. None of the subjects reported adverse effects related to the interventions**.**

HAC intervention decreased cholesterol versus baseline and versus LAC intervention (mean differences =12.3mg/dL and 11.3 mg/dL, respectively)) ($P<0.05$). Changes in inflammatory markers are shown in Fig. 1. C3, C4, and VEGF decreased in a linear trend ($P<0.01$) from baseline to HAC the values after HAC being significantly lower ($P<0.05$)versus baseline (mean differences = 0.061 g/L, 0.025g/L, and 39ng/L, respectively) and versus LAC treatment (mean differences= 0.042 g/L, 0.018 g/L, and

27 ng/L, respectively). No changes were observed in other evaluated biomarkers. No adverse events were reported.

Discussion

Obesity is a chronic, low-grade inflammatory state. Adipose tissue is a key organ contributing to the inflammatory phenotype, and adipocytes are an important source of C3 (Gabrielsson et al, 2003). A three-fold increased risk of metabolic syndrome and its phenotypes, including abdominal obesity, impaired insulin sensitivity, reduced insulin resistance, and low HDL cholesterol concentrations, in individuals with C3 concentration above the median value has been previously described (Phillips et al, 2012). In our setting, C4 levels also decreased after HAC intervention both versus baseline and versus LAC intervention. Our results agree with those reported in patients with rheumatoid arthritis (RA) in whom SLO reduced complement level (Tchórzewski et al, 2002). The complement system plays a fundamental role in mediating the activity of RA (Montoro Alvarez et al, 2015). Elevated C3 predicts the development of cardiovascular disease, a leading cause of mortality in RA (Yang et al, 2015), and it is specifically related to the inflammatory processes involved (Engstrom et al, 2005). VEGF is a serum marker for RA (Selaas et al, 2015). VEGF expression in atherosclerotic plaques is associated with plaque angiogenesis and progression (Phillips et al, 2012). Plasma VEGF concentration is directly related to the severity of coronary artery disease (Kucukardali et al, 2008). The anti-angiogenic effect of AKGs observed in our study has been previously reported in mice models (Pedrono et al, 2004). In experimental models, C3 inhibition blocks ovarian tumour outgrowth and inhibits choroidal neovascularization by reducing VEGF expression (Rohrer et al, 2012). In

agreement with this, in our study besides a decrease in C3 we observed a decrease in VEGF values.

In summary, we describe for the first time a decrease in total cholesterol C3, C4, and VEGF after 20 mg daily doses of AKGs in obese patients. No adverse effects were observed. Our data open a promising field for the study of the beneficial effects of AKGs on both RA and CVD risk in obese individuals. Further research is warranted.

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Conflict of Interest

None declared

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Figure Legend

Fig.1. Inflammatory markers at baseline and after interventions. Values are expressed as Mean ± Standard Error. LAC, low alkylglycerol concentration; HAC, high alkylglycerol concentration. VEGF, vascular endothelial growth factor.* $P<0.05$ versus baseline, †$P<0.05$ versus LAC intervention.

line figure

Click here to download line figure Alkylglycerols Fig. 1.jpg

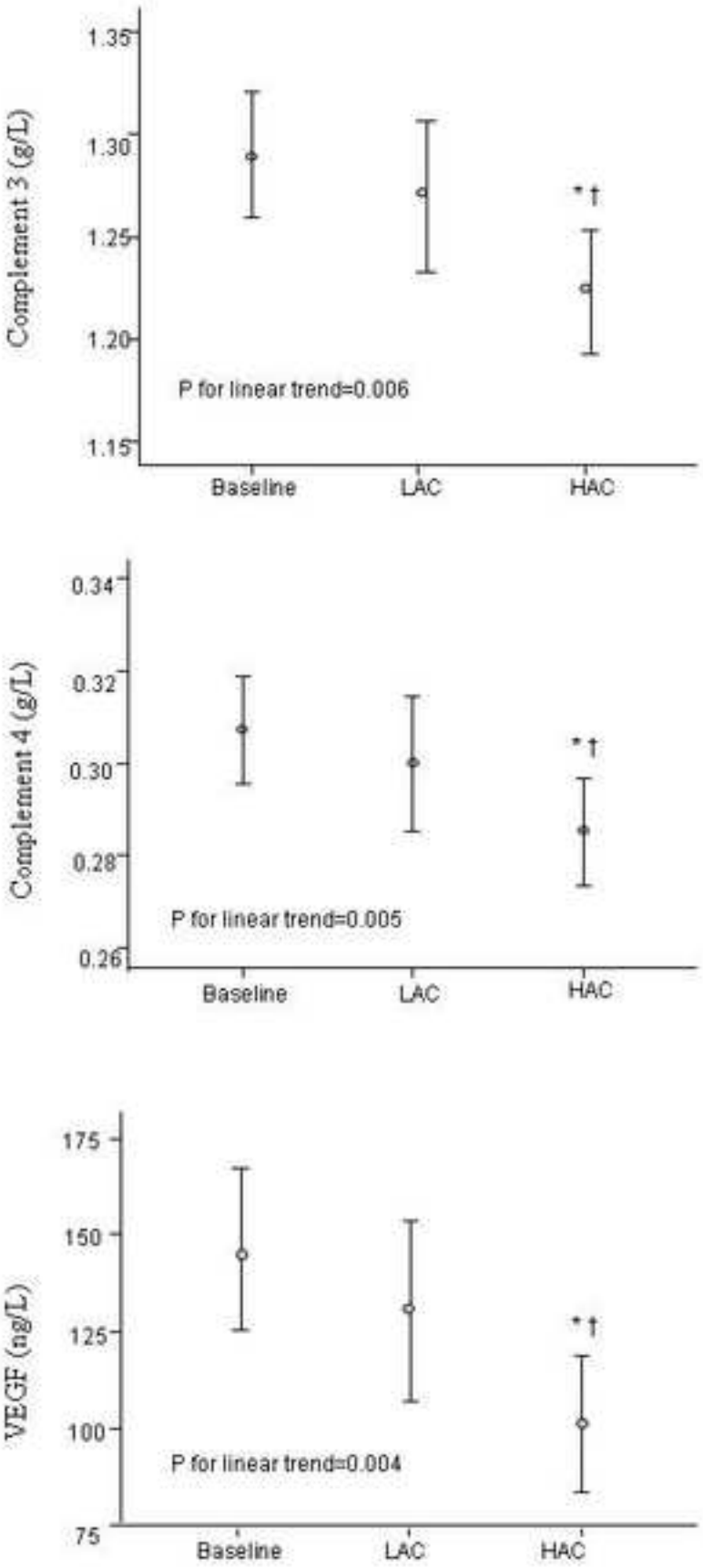

Table 1 Baseline characteristics of the participants by intervention order

	Order 1	Order 2
Gender (male/female)	3/10	3/10
Age, years	47.5 ± 9.7	44.1 ± 12.4
Body mass index, kg/m^2	38.7 ± 2.4	37.2 ± 3.1
Waist circumference, cm	117.1 ± 11.6	113.6 ± 7.8
Systolic blood pressure, mmHg	127.8 ± 11.3	120.3 ± 10.7
Diastolic blood pressure, mmHg	79.3 ± 7.4	77.9 ± 8.6
Smoking habits, %		
Current/Former/Never	46.1/23.1/30.8	43.3/25.0/31.7
Educational level, %		
Primary school	7.7	8.3
High school	38.5	41.6
University	53.8	5.0
Glucose, mmol/L	5.5 ± 0.75	5.1 ± 0.80
Total Cholesterol, mmol/L	5.3 ± 0.68	5.4 ± 1.17
Triglycerides, mmol/L	1.5 ± 0.61	0.99 ± 0.26*
EEPA, kcal/week	242 (189-307)	449 (262-836) †

Results are expressed as mean ± SD or median (25th to 75th percentile). Order 1, low- and high-alkyglycerol intervention; Order 2, high- and low-alkylglycerol intervention. EEPA, energy expenditure in physical activity * P<0.05, † P<0.05 versus Order 1.