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A novel nutritional supplement containing amino acids and chromium decreases postprandial glucose response in a randomized, double-blind, placebo-controlled study

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Abstract

High postprandial blood glucose levels are associated with increased mortality, cardiovascular events and development of diabetes in the general population. Interventions targeting postprandial glucose have been shown to prevent both cardiovascular events and diabetes. This study evaluates the efficacy and safety of a novel nutritional supplement targeting postprandial glucose excursions in non-diabetic adults. Sixty overweight healthy male and female participants were recruited at two centers and randomized in a double-blind, placebo-controlled, crossover design. The supplement, a water-based drink containing 2.6g of amino acids (L-Leucine, L-Threonine, L-Lysine Monohydrochloride, L-Isoleucine, L-Valine) and 250 mcg of chromium picolinate, was consumed with a standardized carbohydrate-rich meal. The primary endpoint was the incremental area under the curve (iAUC) for venous blood glucose from 0 to 120 minutes. Secondary endpoints included glucose iAUC $_{0–180\ minutes}$ and the maximum glucose concentration (C_{max}), for both venous and capillary blood glucose. In the intention-to-treat-analysis (n = 60) the supplement resulted in a decreased venous blood glucose $iAUC_{0\text{-}120min}$ compared to placebo, mean (SE) of 68.7 (6.6) versus 52.2 (6.8) respectively, a difference of -16.5 mmol/L•min (95% CI -3.1 to -30.0, $p = 0.017$). The C_{max} for venous blood glucose for the supplement and placebo were 6.45 (0.12) versus 6.10 (<0.12), respectively, a difference of -0.35 mmol/L (95% CI -0.17 to -0.53, $p<0.001$). In the per protocol-analysis (n = 48), the supplement resulted in a decreased C_{max} compared to placebo from 6.42 (0.14) to 6.12 (0.14), a difference of -0.29 mmol/L (95% CI -0.12 to -0.47, $p = 0.002$). No significant differences in capillary blood glucose were found, as measured by regular bed-side glucometers. The nutritional supplement drink containing amino acids and chromium improves the postprandial glucose homeostasis in overweight adults without diabetes. Future studies should clarify, whether regular consumption of the

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Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: EÖ, IB and RÖ are inventors of a patent family describing the supplement studied. EÖ and IB jointly own the right to the patent and Aventure AB/Double Good AB (RÖ) owns a license to use the patent. EÖ is an employee of Good Idea, Inc since August 2017. starScience GmbH (AS, PMH) have received funding for other studies by Aventure AB/Double Good AB. PH holds shares of Double Good AB. KA and LHL are employees of Aventure AB, the parent company of Double Good AB and Good Idea, Inc. The commercial affiliations of the authors do not alter our adherence to PLOS ONE policies on sharing data and materials.

supplement improves markers of disease or could play a role in a diet aiming at preventing the development of diabetes.

Introduction

High postprandial glucose levels after an oral glucose load are associated with the development of type 2 diabetes (T2D), cardiovascular disease (CVD) as well as increased mortality even in absence of a pre-diabetic condition such as impaired fasting glucose (IFG) [1–8]. A causal relationship between high postprandial glucose levels and T2D or CVD appears likely since interventions targeting postprandial glycemia have been shown to prevent these clinical conditions. This has explicitly been shown for pharmacologic interventions with acarbose [9–11] and is robustly supported by studies on low glycemic index (GI) diets [12–17] as well as experimental data describing possible underlying mechanisms [18–21].

Prevention of T2D and CVD are highly relevant and urgent public health issues since rates of obesity and overweight as well as the age of populations increase [1]. At the same time carbohydrate-rich foods with a high GI and a high glycemic load (GL), which became widespread throughout the 20th century paralleled by an increasing prevalence of obesity and T2D [22], are very popular and commonly available to the consumer [23]. These foods bear a high potential of causing pronounced glucose excursions and insulin spikes, which in turn might contribute to the development of T2D and CVD.

The aim of this study was to show efficacy and safety of a novel nutritional supplement drink (hereinafter called "the supplement")—a blend of five specific amino acids (5AA) and chromium picolinate (CrPic) in water, in a two center, double-blinded RCT. The 5AA were selected on basis of their rapid appearance in the blood stream after intake of whey protein [24] and chromium picolinate for its potential effect on insulin sensitivity [25]. The supplement has been developed to lower postprandial glycemia when consumed with a carbohydrate-rich meal and showed promising results in published [26] and unpublished pilot studies.

Methods

Study design

The randomized, double-blind, placebo-controlled, crossover study was performed by KGK Science Inc. at two centers (London ON, Canada and Orlando FL, US). Participants attended two single day periods separated by a 7-day washout period.

Ethics

This trial was registered at clinicaltrials.gov as NCT03152682. The registration was done with some delay with respect to patient recruitment, due to lack of clarity who was in charge of the submission. The authors confirm that all ongoing and related trials for this intervention are registered. The investigational product was reviewed by Health Canada and classified as a food. Issuance of a permit to conduct this clinical trial in Canada was therefore not required by Health Canada. Similarly, an Investigational New Drug permit was not required for conduct of the study in the USA. This study was approved for conduct at both the Canadian and the USA sites by the Institutional Review Board (IRB Services, Aurora, Ontario) on March 14, 2017. It was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and its subsequent amendments. Amendments to the protocol were

approved on June 14, 2017 and July 27, 2017. Written informed consent was obtained from all participants. The investigational products were produced in the USA and no permit for the import of the investigational product into Canada was required.

Participants

Individuals were recruited from Southwestern Ontario, Canada and Orlando, Florida, using the KGK Science Inc.'s internal participant database along with local electronic and physical advertisement devoid of gender or racial bias. Participants were required to be between 18 and 50 years of age, have a BMI between 25–29.9 kg/m^2 and be in a good physical and mental condition according to their own perception (on a five point scale from 'poor' to 'excellent'), medical history and laboratory results (full list of inclusion and exclusion criteria in S1 Table).

Test product and placebo

The supplement was a lightly carbonated drink containing a proprietary blend with molar ratios presented in [26] and a total of 2.6 g 5AA (L-Leucine, L-Threonine, L-Lysine Monohydrochloride, L-Isoleucine and L-Valine) as well as 250 mcg CrPic (Good Idea®, Good Idea, Inc., Larkspur, CA). Besides water and the active ingredients, the test product also contained citric acid, lemon natural flavor, sodium benzoate and potassium sorbate. The placebo product was identical to the test product with respect to all ingredients, but 5AA and CrPic. The investigational product and placebo were sealed in identically appearing bottles and matched in regard to color, taste and texture.

Intervention

After an overnight fast of 12h, the participants reported to the study facility in the morning. Upon confirmation of participant status and eligibility criteria, an intravenous cannula for repeated blood sampling was placed into the antecubital vein.

Participants started by consuming 175 ml of the supplement or placebo on an empty stomach (time 0), and 3 min later they began with the ingestion of a standardized breakfast meal, alternating between eating and drinking. Three portions of 50 ml each of the supplement or placebo were required to be consumed at min 3, 7 and 11 and the breakfast meal was to be completed by min 14. At the 14 min mark, the final 30 ml of test product or placebo were ingested. The total amount of supplement or placebo was 355 ml. The test meal consisted of white wheat bread (110 g without crust), butter (10 g) and ham (38 g). The calorie and macronutrient composition of the standardized breakfast meal at the London clinical site was: 377 kcal, 47.7g carbohydrates, 14.9 g proteins and 14.1 g fats. The corresponding figures for the Orlando site were: 350 kcal, 45.3 g carbohydrates, 13.1 g proteins and 13 g fats.

Blood samples were collected at 0, 15, 30, 45, 60, 90, 120 and 180 min for glucose (venous and capillary) and insulin (venous) analysis. Capillary blood glucose was measured by the CONTOUR® monitoring system (London site) and the OneTouch® Ultra 2 monitoring system (Orlando site). Quantitative determination of glucose in human serum was conducted via the enzymatic method with hexokinase utilizing the Roche Cobas e701 Analyzer (sensitivity limit 0.5 mmol/L, intra-assay coefficient of variation (CV) 2.7% at 3.1 mmol/L, and 1.4% at 19.8 mmol/L). Quantitative determination of insulin in human serum was conducted via electrochemiluminescence immunoassay utilizing the Roche Cobas e602 Analyzer (sensitivity limit 4 pmol/L, intra-assay CV 7.5% at 106 pmol/L, and 4.5% at 1 357 pmol/L).

Safety endpoints were analyzed in blood drawn at screening and both investigational visits (before starting the actual intervention and associated blood sampling) and included: blood count (hemoglobin, hematocrit, platelet count, red blood cell count, red cell indices, red cell

distribution width, white blood cell count, and differentials (neutrophils, lymphocytes, monocytes, eosinophils, basophils)), liver function (alanine transaminase, aspartate transaminase, bilirubin), and kidney function (creatinine, electrolytes (Na, K, Cl)). Besides the blood parameters, also blood pressure and heart rate were monitored. Urine pregnancy tests (Biostrip HCG, Innovatek Medical Inc.) were conducted at both sites for participants of childbearing age during the screening and baseline visits.

Outcome measures

The pre-defined primary outcome variable was 120-minute incremental area under the curve ($iAUC_{0\text{-}120min}$) for venous serum glucose. Time zero (0) indicates the time when the participant started eating the standardized meal and then repeated blood samples were taken until the last one at 120 min post meal start. The iAUC is the area under the curve, above the baseline levels. It corrects for variations in fasting plasma glucose levels within and between individuals and is thus optimal for comparing the effect of a food on blood glucose levels of individuals independently of their fasting plasma glucose. The iAUC is used, among others, for the calculation of the glycemic index [27]. Secondary outcome variables for venous sampling were: glucose $iAUC_{0\text{-}180min}$; serum insulin $iAUC_{0\text{-}120min}$ and $iAUC_{0\text{-}180min}$; peak glucose value in 120 minutes ($C_{max\ 0\text{-}120min}$) and 180 minutes ($C_{max\ 0\text{-}180min}$); time to peak glucose for 120 minutes ($T_{max\ 0\text{-}120min}$) and 180 minutes ($T_{max\ 0\text{-}180min}$); serum insulin $C_{max\ 0\text{-}120min}$ and $C_{max\ 0\text{-}180min}$. Secondary outcome variables for capillary measurements were glucose $iAUC_{0\text{-}120min}$ and $iAUC_{0\text{-}180min}$; $C_{max\ 0\text{-}120min}$ and $C_{max\ 0\text{-}180min}$; $T_{max\ 0\text{-}120min}$ and $T_{max\ 0\text{-}180min}$, as well as eating patterns as assessed by a 3-day food record for the weeks prior to days 0 and 8. The difference in each secondary outcome between supplement group and placebo group was tested separately by applying the method described in the Statistical analysis section.

Sample size calculation

A sample size of 30 participants per center (total of 60 participants) was calculated based on an expected iAUC of 66.09 mmol•min/L for the placebo and a pooled standard deviation of 61.50 mmol•min/L from a study by Lustig et al [28]. A mean difference of 36.8 mmol/L/min between the investigational product group and the placebo group is expected for the iAUC for each center, assuming a two-sided test with alpha equal to 5%, 80% power and a 20% drop-out rate from enrollment to final, post-baseline measurement.

Randomization

Randomization numbers were assigned by a blinded investigator per the order of a randomization list generated by www.randomization.com and allocated to their sequence in a decentralized manner in a 1:1 ratio, with a separate randomization list for each site. An un-blinded associate that was not involved in any data capture or study assessments labeled the investigational product. A randomization schedule was created and provided to the investigator indicating the order of randomization. Investigators, other site personnel and participants were blinded to the products.

Statistical analysis

All analyses were performed on the pooled data of the two centers. The following analytical populations were defined for the study: Safety population–all participants who received either product and on whom any post-randomization safety information was available; Intention-to-treat (ITT) population–all participants who received either product and on whom any post-

randomization efficacy information was available; Per Protocol (PP) population–all participants who consumed 100% of the investigational products, did not have any major protocol violations, and completed all study visits and procedures connected with measurement of the primary variable.

The trapezoid rule was used for calculating $iAUC_{0\text{-}120min}$ and $iAUC_{0\text{-}180min}$. The trapezoidal rule is a numerical integration method used to approximate the area under a curve. It is widely used to calculate the area under pharmacokinetic curves [29, 30]. Although simple, the trapezoidal rule is a reliable method for calculating iAUC, which has been shown to be more strongly correlated to glycemic response than total AUC [31, 32]. For the iAUC calculations, invalid data points in the ITT and PP analysis were handled by simple imputation methods–averaging values directly adjacent to the missing data point or using a participant's corresponding value from the other study period (pre-ingestion). It was possible to calculate the primary endpoint for 57 participants. Calculations for all other outcome variables were performed on the original data set without imputation. The between group changes from pre-ingestion were analyzed by a mixed model repeated measures analysis of variance. The model included subject as a random effect, with fixed effects of group, sequence, and visit. The p-values for each change was derived by a linear contrast statement of this model. P-values ≤ 0.05 were considered statistically significant. All statistical analysis was completed using the R Statistical Software Package Version 3.2.2 (R Core Team, 2015) and SAS version 9.3 (Cary, North Carolina) for Microsoft Windows.

Results

The participant recruitment began in March 2017 and the study was completed by August 2017. In order to recruit 60 men and women in equivalent numbers and according to predefined inclusion and exclusion criteria, a total of 110 candidates needed to be screened. The participant flow is outlined in Fig 1.

The ITT population included 60 participants (32 female, 28 male) from which 12 were excluded, with a resulting PP population of 48 participants (23 female, 25 male). Baseline characteristics are presented in Table 1. Out of the 12 excluded participants, seven were from the supplement → placebo sequence and five from the placebo → supplement sequence. The reasons for the exclusions were: starting antibiotic treatment (n = 1), delayed or missing blood samples (n = 3), non-adherence to the protocol (n = 2), fainting (n = 1) and wish to terminate the participation before second trial day (n = 5).

The glucose and insulin responses are presented in Fig 2 and Table 2.

In the ITT population, the supplement led to a 24% reduction in venous serum glucose $iAUC_{0\text{-}120\ min}$ ($p = 0.03$) as the primary outcome measure as well as a 5.4% reduction in $C_{max\ 0–120}$ ($p<0.01$) as compared to placebo. There were significant differences in serum glucose between placebo and the supplement at 30, 45, 60, 90 and 120 minutes (reduction by 4%, 3%, 5%, 6% and 4% respectively). No significant differences in the iAUCs for insulin, T_{max} or iAUC for capillary measurements were observed.

In the PP population a 4.5% reduction in $C_{max\ 0–120}$ ($p<0.01$) could be documented. There were significant reductions in serum glucose by the supplement compared with placebo at 30, 45 and 60 minutes (-4%, -4% and -5% respectively). No significant differences in the iAUCs for venous serum glucose or insulin as well as T_{max} or iAUC for capillary measurements, were observed.

An analysis of subgroups was performed in the PP population. Pronounced effects were observed in the middle-aged group (40–50 years old, n = 23) with a 30% reduction in venous glucose $iAUC_{0\text{-}120\ min}$ ($p = 0.006$) and a 5% reduction in C_{max} ($p = 0.004$) after the supplement

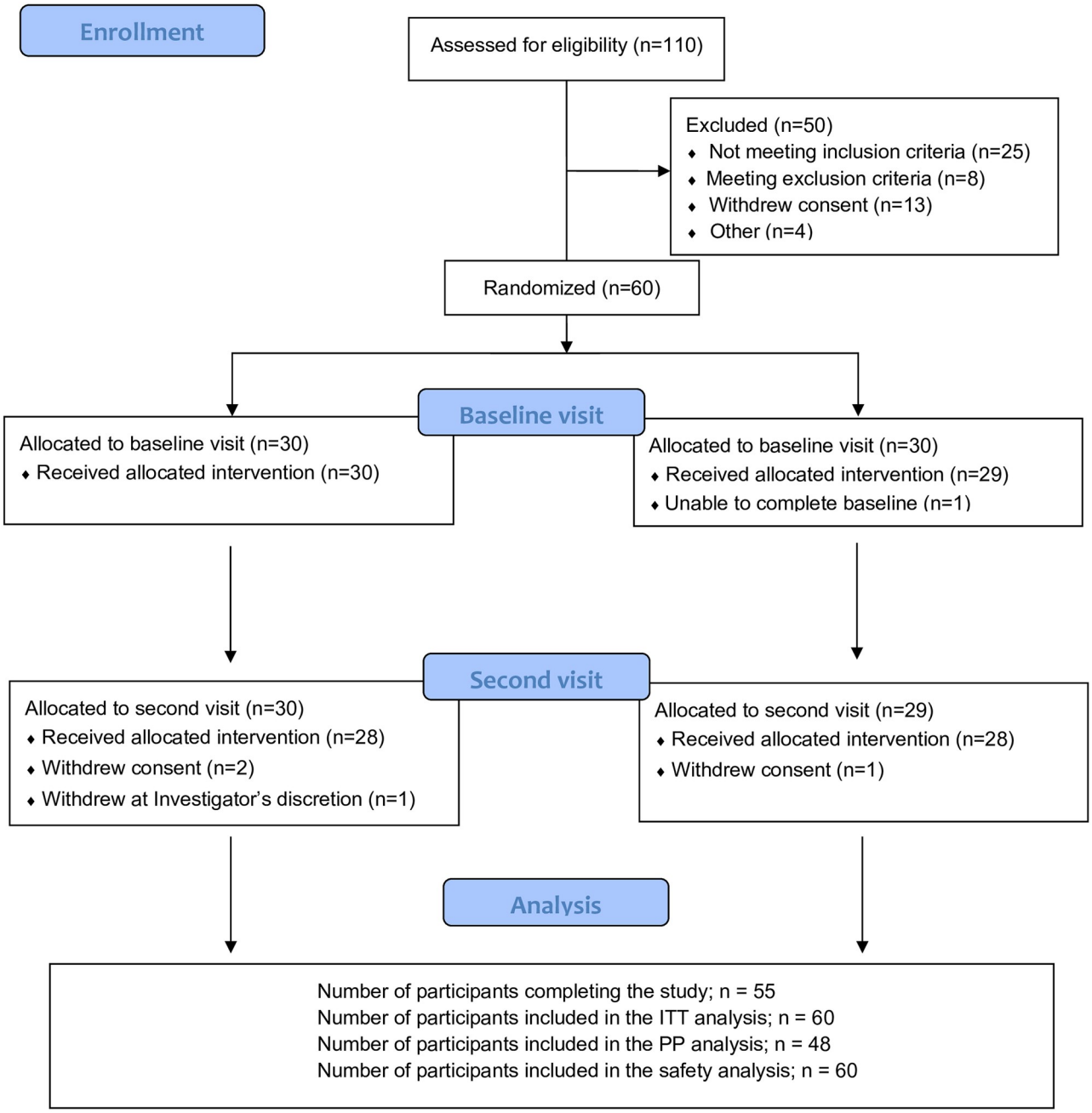

Fig 1. Participant flow.

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compared to placebo. The other subgroups (gender, race) did not show any significant changes in the primary endpoint.

There were no severe adverse events, as categorized by the Medical Dictionary for Regulatory Activities, version 17. Out of 19 mild to moderate adverse events (AE) recorded in 16 individuals experiencing these events, three events were categorized as possibly related to the supplement (one nervous system disorder and two gastrointestinal disorders) and one as

Table 1. Participant characteristics at screening[1].

	ITT population (n = 60)	PP population (n = 48)
Gender, *n* (%)		
Female	32 (53%)	23 (48%)
Male	28 (47%)	25 (52%)
Alcohol consumption status, *n* (%)		
None	21 (35%)	17 (35%)
Occasional	26 (43%)	21 (44%)
Weekly	13 (22%)	10 (21%)
Smoking status, *n* (%)		
Ex-smoker	5 (8%)	3 (6%)
No	55 (92%)	45 (94%)
Health questionnaire, *n* (%)		
Excellent	22 (37%)	18 (38%)
Very good	27 (45%)	21 (44%)
Good	11 (18%)	9 (19%)
Race, *n* (%)		
Western European white	28 (47%)	21 (44%)
Black or African American	15 (25%)	12 (25%)
Other	17 (28%)	15 (31%)
Age, yrs (SD)	34.6 (10.4)	35.5 (10.5)
Weight, kg (SD)	79.7 (10.2)	80.8 (10.7)
BMI, kg/m^2 (SD)	27.4 (1.59)	27.5 (1.59)
Fasting venous blood glucose, mmol/L (SD)	4.93 (0.42)	4.95 (0.42)
HbA1c, % (SD)	5.48 (0.33)	5.47 (0.31)
Systolic blood pressure, mmHg (SD)	119.8 (8.9)	117.7 (9.0)
Diastolic blood pressure, mmHg (SD)	76.2 (8.1)	75.8 (8.7)
Heart rate, bpm (SD)	69.0 (11.1)	69.2 (11.2)

[1] Values are *n* (%), or means (SD)

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possibly related to placebo (vascular disorder). All AEs were resolved by the end of the study without requiring medical treatment or hospitalization.

Discussion

In this controlled clinical trial, we show a significant reduction of the serum glucose iAUC in healthy overweight subjects after a mixed meal consumed together with the supplement, compared to placebo. There are different ways in which the supplement could beneficially influence the glucose metabolism and health in the target population of individuals that are not yet affected by cardiovascular disease or diabetes. From a GI point of view [33], the supplement was able to reduce the overall GI of the high glycemic test meal. Although there is still some controversy on the role of low GI food and meals [12, 34], most studies comparing diets with different GI´s favor the ones with a lower GI in regards to the development of T2D [12–17, 35, 36].

Elevated postprandial glucose has been identified as an important risk factor for developing T2D as well as CVD and even overall mortality in individuals without impaired glucose tolerance (IGT) or T2D. The factual endpoints studied in these epidemiological studies were the 60

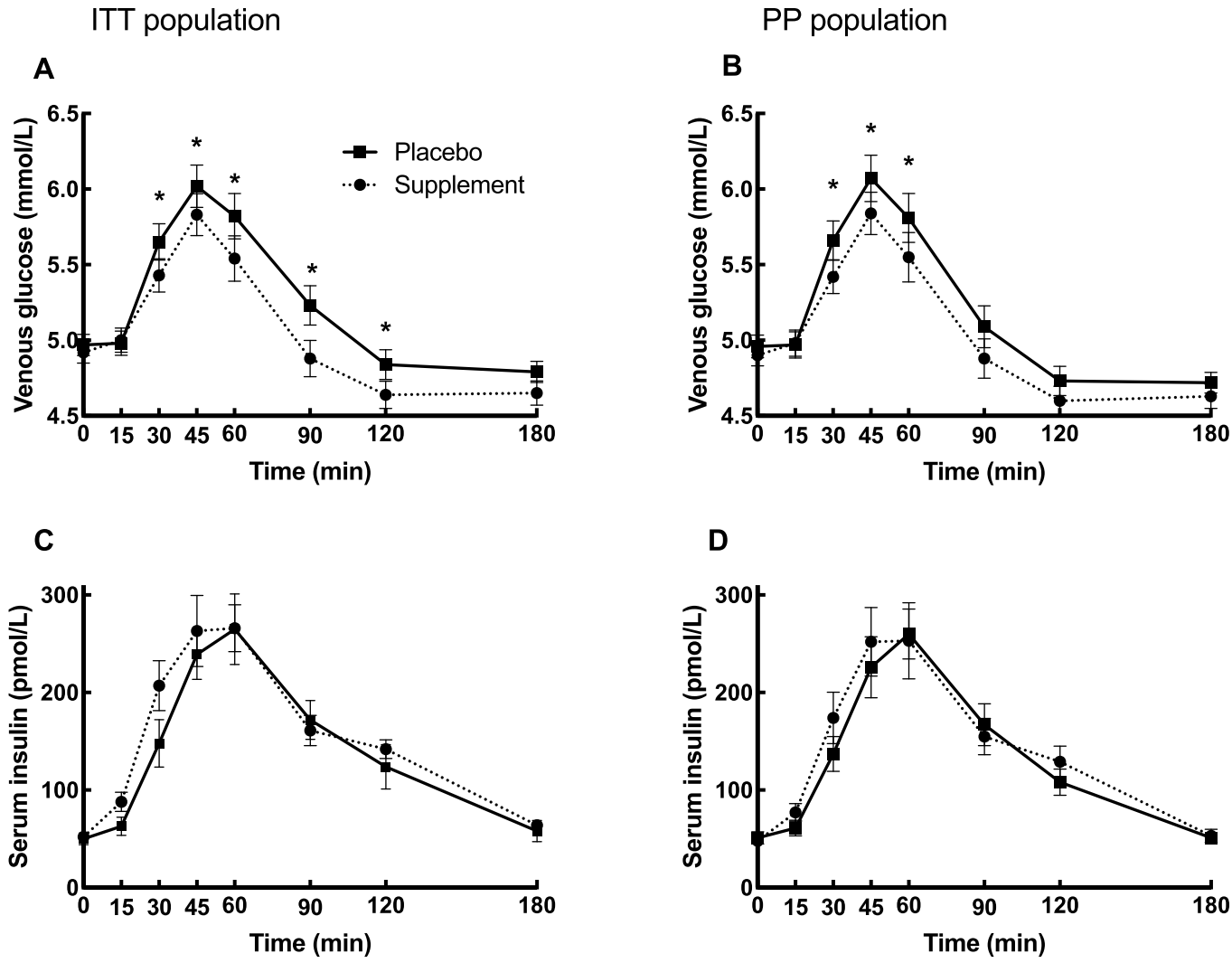

Fig 2. Postprandial glucose and insulin responses. Glucose and insulin responses for the ITT (n = 54–58, for different time points) and PP (n = 48) populations respectively after intake of a high carbohydrate sandwich meal with supplement or placebo. Data are expressed as means ± SE. Asterisc (*) indicates a significant difference between treatments ($p<0.05$) at the respective time point.

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and 120 minutes glucose values after a standardized glucose load varying between 50g and 100g of glucose [1–8]. This study showed significant differences for venous glucose at 30, 45, 60 and 90 minutes in favor of the supplement, thus showing a significant reduction of the surrogate risk factor 60-minute postprandial glucose. The use of a mixed meal as opposed to glucose solutions tests efficacy of the supplement closer to a real-life scenario.

There is ample evidence for a causal relationship between elevated postprandial glucose and the development of T2D and CVD. Experimental evidence on a cellular level shows that an induction of endothelial dysfunction by postprandial hyperglycemia is most likely a consequence of increased oxidative stress [18–21]. In human studies, postprandial glucose excursions have been shown to correlate with the extent of atherosclerosis [21]. Interventions targeting postprandial glycemia have been shown to prevent atherosclerosis but also the development of T2D [9–11] and possibly even cardiovascular events [9, 10].

Table 2. Glucose and insulin responses for ITT and PP populations[1].

	Supplement			Placebo			Group Difference		95% CI		
ITT population	*n*	Mean	SE	*n*	Mean	SE	Mean	SE	Upper	Lower	*p*-value
Serum glucose											
$iAUC_{0\text{-}120\ min}$, mmol/L•min	54	52·20	6·79	58	68·72	6·62	-16·54	6·71	-3·08	-30·01	0·017
$iAUC_{0\text{-}180\ min}$, mmol/L•min	54	62·44	8·42	58	79·07	8·19	-16·63	8·56	0·55	-33·81	0·058
$C_{max0\text{-}120\ min}$, mmol/L	54	6·10	0·12	58	6·45	0·12	-0·35	0·09	-0·17	-0·53	<0·001
$T_{max0\text{-}120\ min}$, min	54	51·04	2·49	58	52·29	2·85	-1·25	5·32	5·32	-7·82	0·704
Serum insulin											
$iAUC_{0\text{-}120\ min}$, pmol/L•min	54	15 803	1 589	58	14 523	1 556	1 279	1 334	3 958	-1 399	0·342
$iAUC_{0\text{-}180\ min}$, pmol/L•min	54	19 361	1 908	58	17 253	1 869	2 108	1 589	5 298	-1 083	0·191
$C_{max\ 0\text{–}120\ min}$, pmol/L	54	380·99	35·19	58	345·70	34·63	35·29	25·58	86·66	-16·07	0·174
$T_{max\ 0\text{–}120\ min}$, min	54	59·38	3·43	58	58·78	3·33	0·60	3·76	8·16	-6·95	0·873
	Supplement				**Placebo**		**Group Difference**		**CI 95%**		
PP population	*n*	Mean	SE	*n*	Mean	SE	Mean	SE	Upper	Lower	*p*-value
Serum glucose											
$iAUC_{0\text{-}120\ min}$, mmol/L•min	48	53·73	7·26	48	66·48	7·26	-12·75	6·95	1·24	-26·74	0·073
$iAUC_{0\text{-}180\ min}$, mmol/L•min	48	62·36	8·72	48	74·37	8·72	-12·01	8·91	5·93	-29·94	0·185
$C_{max\ 0\text{–}120\ min}$, mmol/L	48	6·12	0·14	48	6·42	0·14	-0·29	0·09	-0·12	-0·47	0·002
$T_{max\ 0\text{–}120\ min}$, min	48	49·30	2·98	48	49·61	2·98	-0·31	3·22	6·17	-6·79	0·923
Serum insulin											
$iAUC_{0\text{-}120\ min}$, pmol/L•min	48	14 833	1 607	48	13 546	1 607	1 268	1 416	4 119	-1 582	0·375
$iAUC_{0\text{-}180\ min}$, pmol/L•min	48	17 737	1 791	48	15 713	1 791	2 024	1 719	5 483	-1 436	0·245
$C_{max\ 0\text{–}120\ min}$, pmol/L	48	357·15	35·81	48	324·42	35·81	32·73	26·62	86·30	-20·85	0·225
$T_{max\ 0\text{–}120\ min}$, min	48	59·50	3·48	48	58·17	3·48	1·33	3·96	9·29	-6·64	0·739

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Participants in this study were mostly overweight and the glucose lowering effect of the supplement was pronounced in the older subgroup (40–50 yrs) showing a significant and impressive 30% reduction of $iAUC_{0\text{-}120\ min}$ glucose. Since overweight and increasing age are the main risk-factors for developing diabetes [1], efficacy of the supplement has been shown in a group, in which diabetes prevention is highly relevant.

The exact mechanisms leading to the observed effects of the supplement on postprandial glucose are not completely clear. Chromium is, however, known to play a role in glucose metabolism, possibly by potentiating insulin interaction with its receptor via binding of a low molecular chromium binding protein [37–39]. Yet, its role in improving glucose metabolism as a supplement is not well described in controlled clinical studies, most of which were performed in patients with T2D [40–43]. Milk and whey protein are known to stimulate insulin [44–50] and thus reduce the postprandial glucose response to a glucose solution [45, 47–51]. However, in the case of single amino acids, the insulinogenic effect is not attributable to all amino acids [52–55] and requires higher doses than the ones used here [52, 53, 56–58]. Hence, the iAUC for insulin was not significantly increased by the supplement compared to placebo and there was only an indication of a trend towards an earlier insulin response when looking at insulin iAUC´s. Studies looking at insulin and the development of T2D or IGT suggest that a pronounced first-phase insulin response compared to a late insulin increase could be beneficial [55, 59–61]. In addition it has been described, that proteins and amino acids have a distinct effect on gastric emptying, possibly delaying the glucose uptake [62–64]. A 'priming' of the stomach with the first sips of the supplement leading to a slowing of gastric emptying and a

better insulin homeostasis could contribute to the effects on postprandial glucose observed. Further research will have to substantiate these hypotheses.

The limitations of this study include missing values due to the high number of blood draws required for each participant and thus the need for imputation of some results when performing the iAUC calculation in the ITT analysis. Another limitation is the lack of data on menstrual cycle for the female participants, since it is known that blood glucose regulation differs between the follicular and luteal phases [65]. Overall, there were no significant effects when looking at the capillary blood measurements. This can be explained by the use of patient glucometers, which are known for their imprecision [66, 67] and cannot be expected to produce reliable data for the calculation of differences in glucose iAUC. This is surely a systematic problem for the analysis of this secondary end point. However, in a previous study of an early version of the supplement, professional glucometers used for capillary blood glucose measurements showed significant reductions of postprandial glucose, in line with that shown for venous glucose in this study [26]. Another limitation of this study was the variability in brands of bread used for the standardized test meal between the Canadian and American sites. This resulted in a slight difference in fiber content between the meals, with the Canadian brand having 2 grams more dietary fiber than the US brand. However, the macronutrient and caloric content of the test meals remained relatively similar between sites and individuals at each center received the same brand in the supplement and placebo test meal.

In conclusion, the supplement drink tested in this study efficiently reduced the postprandial glucose excursions after a meal with a high carbohydrate content without causing any adverse events. Future studies will have to evaluate whether regular consumption of the supplement improves markers of disease or might even prevent the development of diabetes. Effects on satiety and food cravings will also have to be studied. Definition of the mechanisms involved in the observed effects will help to develop supplements that could not only be used for the prevention of diabetes but may also be an option for dietary interventions in metabolic disease.

Supporting information

S1 Table. Inclusion and exclusion criteria.
(PDF)

S1 Checklist. Consort check-list.
(DOC)

S1 Data. Original data.
(XLSX)

S1 File.
(PDF)

Author Contributions

Conceptualization: Elin Östman, Azat Samigullin, Lovisa Heyman-Lindén, Kristina Andersson, Inger Björck, Rickard Öste, Per M. Humpert.

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Version 1.9

Clinical studies of Good Idea®

This document provides brief summaries of the studies underlying the concept of Good Idea®.
The final drink formula is based on a series of clinical studies investigating different doses of the active ingredients, *ie* five free amino acids (5AA; leucine, isoleucine, valine, lysine, threonine) and chromium (as Cr-picolinate, CrPic). The latter are blended in carbonated and lightly flavored water that are consumed in conjunction with carbohydrate containing meals. With few exceptions, the meal studies have been performed in normal to overweight participants, without diabetes.

For more details about the research and clinical studies, please contact
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Proof of concept studies of Good Idea®

1. Proof of concept North America (PLoS ONE 15(6): e0234237.)

Registered at clinicaltrials.gov as NCT03152682.
https://doi.org/10.1371/journal.pone.0234237

- **Study design:** Double-blind, randomized, placebo controlled, cross-over study
- **Study locations:** London, ON, Canada and Orlando, FL, USA
- **Subjects:** 60 overweight men (28) and women (32), without diabetes
  - Mean BMI: 27.4 kg/m^2 (24-30)
  - Mean age: 34.6 years (20-50)
- **Meal:** White bread, butter and ham. In total 46 g carbohydrates, 14 g protein and 14 g fat (total 372 kcal). Good Idea® or Placebo was consumed 3 min before (175 ml) and then during the meal (180 ml). The latter volume was divided in 4 servings à 50 ml at times 3 min (after first bite), 7 min and 11 min, as well as the remaining 30 ml at time 14 min.
- **Test product:** 355 ml (12 fl.oz) Good Idea® LemonLime (2.6 g 5AA + 250 µg CrPic) or Placebo (carbonated and lemon-lime flavored water without the 5AA+CrPic)
- **Main results:** Acute consumption of Good Idea® with a standardized meal elicited a significant 24% reduction in post-prandial blood glucose $iAUC_{0\text{-}120min}$, as well as a 5.4% decrease in C_{max} compared to placebo (ITT-population, n=60). There was no significant difference in overall insulin responses after Good Idea® compared to placebo.

2. Proof of concept Europe (completed in 2016, unpublished data)

Registered at clinicaltrials.gov as NCT03411395.

- **Study design:** Double-blind, randomized, placebo controlled, cross-over study
- **Study location:** Lund, Sweden
- **Subjects:** 25 normal weight, men (12) and women (13), without diabetes
  - Mean BMI: 21.9 kg/m^2 (19-25)
  - Mean age: 26.8 years (19-52)
- **Meal:** White bread, butter and ham. In total 50 g carbohydrates and 14 g protein.
  Good Idea® or Placebo was consumed before (110 ml) and during the meal (220 ml) in 6 servings à 55 ml at time - 3 min, -1.5 min, 0 min (start of meal), 3 min, 6 min and 9 min.
- **Test products:** Good Idea® LemonLime (2.6 g AA + 250 µg CrPic)
  GI_{half} (1.3 g AA + 250 µg CrPic)
  $GI_{quarter}$ (0.65 g AA + 250 µg CrPic)
- **Main results:** Acute consumption of Good Idea® with a standardized meal elicited a significant 12% reduction in the post-prandial blood glucose $iAUC_{0\text{-}180min}$, as well as a borderline decrease in C_{max} (-16%, p=0.056), compared to Placebo. No other differences were found for glucose responses between the different GI-doses and the placebo. There was no significant difference in $iAUC_{0\text{-}180min}$ for insulin after Good Idea® or any of the other GI-doses, compared to Placebo.

Pilot trials with Good Idea®

3. Pilot study of Good Idea served without a meal (completed in 2018, unpublished data)

- **Study design:** Single-blind, randomized, placebo controlled, cross-over study
- **Study location:** Lund, Sweden
- **Subjects:** 9 overweight, men and women, without diabetes
  - Mean BMI: 28.1 kg/m^2 (25.8-32.4)
  - Mean age: 34 years (23-47)
- **Meal:** No meal was served
- 175ml of Good Idea® or Placebo was consumed at 0 min and then 3 servings of 50 ml each were consumed after 3, 7 and 11 min and a final dose of 30 ml was consumed at 14 min.
- **Test product:** Good Idea® LemonLime (2.6 g AA + 250 µg CrPic)
- **Main results:** There was no overall difference in insulin concentrations between treatments over 120 minutes (p=0.362, RM 2-way Anova), but there was a statistically significant (p<0.001) interaction between the time x treatment effect. A post hoc test revealed that there was a small, but statistically significant increase of insulin after consuming Good Idea at 15 minutes (p=0.010) and at 30 minutes (p<0.001), compared to Placebo.
  For capillary glucose there was a statistically significant overall difference between the investigational product and placebo over 120 minutes (p=0.040) and a significant interaction between treatment and time (p=0.006). A post hoc statistical comparison revealed statistically significant lower glucose values after Good Idea at time points 45 and 120 minutes (p= 0.027 and p<0.001 respectively). Important to note is that all capillary glucose levels measured were well within the range of normal fasting glucose values for both Good Idea and Placebo.

4. Pilot trial with Good Idea and a commercial meal (completed in 2017, unpublished data)

- **Study design:** Single-blind, randomized, placebo controlled, cross-over study
- **Study location:** Lund, Sweden
- **Subjects:** 11 overweight men (4) and women (7) without diabetes
    - Mean BMI: 27.1 kg/m^2 (24.8-30.9)
    - Mean age: 28 years (19-52)
- **Meal:** Commercial thick crust pizza served with iceberg lettuce. In total 36 g carbohydrates and 10 g protein (total 243 kcal).
- Good Idea® or Placebo was consumed before (175ml at 0 min) and during the meal (175 ml) in 3 servings of 50 ml (at 3, 7 and 11 min) and one serving of 30 ml at 14 min.
- **Test product:** Good Idea® LemonLime (2.6 g AA + 250 µg CrPic)
- **Main results:** Acute consumption of Good Idea® with the standardized pizza meal elicited a 37% reduction in the post-prandial blood glucose $iAUC_{0\text{-}180min}$, as well as a 35% decrease in C_{max} compared to Placebo. None of the results were statistically significant but that was expected since this pilot trial was not powered to reach statistical differences. There was no significant difference in $iAUC_{0\text{-}180min}$ for insulin after Good Idea® compared to Placebo.

5. Pilot study in patients with prediabetes and type 2 diabetes

(Completed in 2016, data presented at German Diabetes congress, Berlin, Germany, 2016)

- **Study design:** Double-blind, randomized, controlled, cross-over pilot study
- **Study location:** Heidelberg, Germany
- **Subjects**: Patients with type 2 diabetes (13) and prediabetes (7)
    - Mean BMI: 34 kg/m^2
    - Mean age: 52 years
- **Meal:** White bread, butter & jam. In total 50 g carbohydrates. 100 ml of carbonated, flavored water without (Placebo) or with 1.75 g 5AA+125 µg CrPic was taken before the meal. Another 230 ml of the drink was consumed with the meal.
- **Main results:** There was no reduction of post-prandial plasma glucose either in patients with diabetes or pre-diabetes by the 5AA+CrPic drink *vs*. Placebo. The patients with diabetes showed an early increase of serum insulin, whereas the patients with prediabetes did not.

Studies to optimize recipe and timing of intake

Please note that the test products included in the studies below vary somewhat as to their contents of the 5AA and CrPic compared with Good Idea®.

6. Two doses study

(Data presented at American College of Nutrition conference, San Diego, CA, 2016)

- **Study design:** Single-blind, randomized, placebo controlled, cross-over study
- **Study location:** Aventure, Lund, Sweden
- **Subjects:** 19 normal and overweight men (9) and women (10), without diabetes
    - Mean BMI: 24.1 kg/m^2 (18.6-31.9)
    - Mean age: 26.2 years (21-43)
- **Meal:** White bread, butter and strawberry jam. In total 50 g carbohydrates and 7 g protein. Carbonated, flavored water without (Placebo) or with two different doses of 5AA+CrPic were consumed as half the bottle (165 ml) before the meal and the other half (165 ml) during the meal.
- **Test products:** 2.6 g AA+186 µg CrPic (5AACrPic_2.6)
  5.2 g AA+186 µg CrPic (5AACrPic_5.2)
- **Main results:** Glucose $iAUC_{0\text{-}180\ min}$ was significantly reduced by 31% for 5AACrPic_2.6 compared to Placebo, with only a non-significant trend of reduction (26%) for 5AACrPic_5.2. The overall insulin responses were not significantly increased by any dose of 5AA+CrPic compared to Placebo.

7. Timing study (unpublished data)

- **Study design:** Single-blind, randomized, placebo controlled, cross-over study
- **Study location:** Lund University, Sweden
- **Subjects**: 20 normal and overweight men (11) and women (9), without diabetes
    - Mean BMI 27.6 kg/m^2 (22.9-30.8)
    - Mean age 33.5 years (19-59)
- **Meal:** 125 g cod, 250 g mashed potatoes (44 g powder + 200 ml boiled water), 20 g melted butter, 50 g lingonberry jam, 50 g cucumber. In total 49 g carbohydrates and 34 g protein.
- **Test product:** Carbonated flavored water without (Placebo) or with 2.6 g 5AA+186 µg CrPic (5AACrPic)
- **Timing of product intake:**
    1. Placebo, ½ serving consumed before the meal (and the rest together with the meal).
    2. 5AACrPic, ½ serving consumed before the meal (and the rest together with the meal).
    3. Placebo, together with the meal (no drink before eating).
    4. 5AACrPic, together with the meal (no drink before eating).
- **Main results:** No differences in glucose responses (iAUC) were found between any products or intake regimes. C_{max} for glucose was significantly lower after taking 5AACrPic in the "pre-meal" regimen compared to the same product taken "during" the meal. No statistical differences were found in the overall insulin response ($iAUC_{0\text{-}120min}$). The insulin iAUC for 0-15 min was significantly higher after the 5AACrPic taken in the "pre-meal" regimen compared to all other test meals. For iAUC 0-30 min the insulin iAUC was significantly higher for 5AACrPic, both in the "during" and "pre-meal" regimens.

8. Breakfast study

(Data presented at German Diabetes congress, Berlin, Germany, 2016)

- **Study design:** Single-blind, randomized, controlled, cross-over
- **Study location:** Lund University, Sweden
- **Subjects:** 20 normal and overweight men (11) and women (9), without diabetes
    - Mean BMI: 24 kg/m^2 (20-31)
    - Mean age: 42 years (22-59)
- **Meal:** White wheat bread, butter and jam. In total 63 g carbohydrates, 9 g protein and 12 g fat (total 380 kcal)
- **Test product:** Carbonated, flavored water without (Placebo) or with 1.75 g 5AA + 125 µg CrPic. Subjects were instructed to take a couple of sips before starting the meal.
- **Main results:** When having 5AACrPic with the meal, a 41% reduction in blood glucose response was observed compared to Placebo, without a reduction in C_{max}. No statistical increase in the insulin response was observed.

9. Dose-response study (Data presented at IDF Congress, Vancouver, Canada, 2015)

- **Study design:** Single-blind, randomized, placebo controlled, cross-over trial
- **Study location:** Lund University, Sweden
- **Subjects:** 16 normal and overweight men (9) and women (7), without diabetes
    - Mean BMI: 27.5 kg/m^2 (23-32)
    - Mean age: 54 years (37-66)
- **Meal:** Mashed potato, oven cooked fish (cod), melted butter, cucumber, lingonsylt.
  In total 50 g carbohydrates, 27 g protein and 17 g fat (total 472 kcal).
  100 mL of the drink taken within 3 min before starting the meal and the rest during the meal.
- **Test products:** Carbonated and flavored water without (Placebo) or with:
    a) 6.9 g 5AA + 500 µg CrPic (5AACrPic-high)
    b) 3.5 g 5AA + 250 µg CrPic (5AACrPic-medium)
    c) 1.75 g 5AA + 125 µg CrPic (5AACrPic-low)
- **Main results:** All three doses of 5AACrPic reduced the blood glucose response significantly by 21-28%, compared to Placebo. C_{max} of glucose was reduced by 15-20%. By reducing the dose of the 5AACrPic mix, less insulin was required to obtain the reduction in postprandial glycemia compared with the highest dose. The overall insulin response was not significantly increased for 5AACrPic-medium and 5AACrPic-low, compared to Placebo. GLP-1 tended to increase for all 5AACrPic containing meals (+39% (high), +55% (med), +65% (low)) but did not reach significance.

10. Juice study (unpublished data)

- **Study design:** Single-blind, randomized, controlled, cross-over trial
- **Study location:** Lund University, Sweden
- **Subjects**: 13 normal and overweight men (6) and women (7), without diabetes
    - Mean BMI: 27.8 kg/m^2 (24-30)
    - Mean age: 54 years (40-60)
- **Meal:** White bread, butter & orange jam – Totally 78 g available starch (50 g from the bread), 9 g protein and 10 g fat (total 448 kcal)
- **Test product:** Water (110 ml), orange juice concentrate (48 g), aroma, malic acid and without (Placebo) or with 6.9 g 5AA+500 µg CrPic
- **Main results:** The 5AA+CrPic juice lowered blood glucose iAUC 0-120 min significantly by 22% and C_{max} by 25%. The postprandial insulin responses were significantly higher for the 5AA+CrPic juice compared to Placebo.

11. Single or combined active ingredients

(Data published in *Functional Foods in Health and Disease*, Feb 2017, https://www.ffhdj.com/index.php/ffhd/article/view/304)

- **Study design:** Single-blind, randomized, controlled, cross-over trial
- **Study location:** Lund University, Sweden
- **Subjects:** 19 overweight men (11) and women (8), without diabetes
    - Mean BMI: 27.3 kg/m^2
    - Mean age: 51 years
- **Meal:** White bread, butter & orange jam. In total 64 g carbohydrates (50 g from the bread), 9 g protein and 12 g fat (total 380 kcal)
- **Test product:** Carbonated, flavored water without (placebo) or with 6.9 g 5AA alone or in combination with 500 µg CrPic, taken together with the meal.
- **Main results:** 5AA+CrPic significantly lowered C_{max} (called iPeak) by 27% compared to Placebo, and tended to reduce glucose response $iAUC_{0\text{-}120\ min}$ by 20% (non-significant). Early insulin increase did not reach significance after 5AA+CrPic, although the overall iAUC for insulin was significantly higher (about 30%) for 5AA+CrPic compared with Placebo. Compared with the 5AA drink, the 5AA+CrPic reduced the insulin release by about 50%, indicating an improved insulin economy by combining 5AA with CrPic.

Research Article Open Access

A drink containing amino acids and chromium picolinate improves postprandial glycemia at breakfast in healthy, overweight subjects

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ABSTRACT

Background: Chromium (Cr) and certain amino acids (AA) have been individually shown to improve postprandial glycemia.

Method: The present randomized, controlled, cross-over trial in 19 healthy, overweight subjects (age 51±1y and BMI 27.3±0.3 kg/m^2; mean±SEM) evaluated a combination of leucine, isoleucine, valine, lysine, and threonine (5AA) with Cr. Postprandial glycemia and insulinemia were measured following a bread meal, served with carbonated water (Ref) or carbonated water containing 5AA, Cr-picolinate (CrPic) or a combination (5AA+CrPic).

Results: The 5AA+CrPic and 5AA respectively lowered the incremental glucose peak ($P< 0.001$) by almost 30% compared to Ref. No significant differences in incremental insulin peaks were found. However, during the first 15 minutes 5AA induced a higher insulin response (+112%; $p<0.01$) compared to Ref. Interestingly, 5AA+CrPic reduced the initial AA-induced insulin increase by more than 50%, indicating improved insulin economy.

Conclusions: These observations suggest that a drink containing both 5AA and CrPic attenuate postprandial glycemia in healthy "at risk" subjects.

Keywords: Amino acids, chromium picolinate, postprandial glycemia, insulin economy, drink

INTRODUCTION

Low glycemic diets are associated with reduced inflammatory tonus (CRP, adiponectin) [1], lowered oxidative stress (NFκβ) [2] and reduced risk of type 2 diabetes mellitus (T2DM), and heart disease [3]. Ingestion of whey proteins has been reported to positively affect glucose metabolism and increase satiety [4]. One suggested mechanism is the insulinogenic properties

of whey protein, and particularly the hypoglycemic effect, which has been related to five specific amino acids (5AA; leucine, isoleucine, valine, threonine, and lysine) that rise rapidly in blood upon whey ingestion [5]. Accordingly, the efficiency of the 5AA to reduce the postprandial glycemic response has been repeatedly proven in healthy subjects [5, 6]. Furthermore, the specific mix of 5AA has been shown to improve hepatic steatosis and glucose tolerance in mice [7]. In another setting, a mix of AA containing leucine, isoleucine, valine, cysteine, and methionine improved postprandial glucose responses in healthy overweight subjects [8]. When it comes to the role of AA in diabetes, Solerte et al, has shown that supplementation of the diet with all essential AA, significantly improved glucose regulation (lowered HbA1c) in poorly controlled T2DM subjects [9].

Another dietary factor of interest in relation to glycemic regulation is chromium [10]. However, most of the studies made on Cr supplementation have been performed in T2DM subjects, where improved effects on glycemic control, improved lipid profiles, attenuated weight gain, and reduced central fat distribution have been reported [11-14]. In hyperglycemic subjects, dietary supplementation with Cr decreased glucose and insulin responses to an oral glucose challenge [15]. Moreover, in subjects at high risk of developing T2DM, increased insulin sensitivity was reported in the absence of effects on body composition after 8 months intake of Cr-picolinate (CrPic) [16]. However, there are also trials in non-diabetic or T2DM subjects which have failed to show metabolic improvements associated with Cr [17-20].

We hypothesized that a combination of 5AA and Cr in a drink could synergistically improve postprandial glycemia when co-ingested with a composite and challenging high glycemic sandwich breakfast in healthy, overweight subjects. Three carbonated test drinks were studied containing either the 5AA, CrPic or a combination of both (5AA+CrPic). Carbonated water without the addition of either ingredient was used as reference. The test and reference drinks, respectively, were provided with a sandwich meal consisting of white wheat bread, butter, and marmalade, and postprandial blood glucose and insulin were registered for 3 hours. Additionally, subjective ratings of appetite sensations were registered.

METHODS

Study design

The study was a randomized, controlled, cross-over, single blind human trial. Nineteen subjects were included (11M:8F). The volunteers were non-smoking, aged 51±1y (mean±SEM), considered to be healthy, and had body mass indices in the overweight range (BMI 27.3±0.3 kg/m^2, mean±SEM). Five persons were receiving either of the following medication: one blood-thinning (Trombyl), two hypotensive (Enalapril and Tenormin, respectively), one vitamin B supplementation (Behepan), and one allergen extract from grass pollen (Grasax). These medications were not considered to affect the parameters tested in this study and were therefore allowed. However, the volunteers were asked to not take the medication in the morning before the test. All subjects participated as volunteers, gave their written informed consent, and were aware of the possibility of withdrawing from the study without explanation at any time. The study was approved by the Regional Ethical Board at Lund University (Dnr 2010/499).

The test and reference breakfast meals were served in random order over four separate occasions after an overnight fast, approximately one week apart. The subjects were instructed to eat the same type of dinner in the evening before each test day and a late evening snack consisting of white wheat bread with spread and a drink. Furthermore, they were asked to not

perform any extensive exercise, drink alcohol, or eat fiber-rich food on the day before each visit. All meals were well tolerated and subjects finished them within 10-15 minutes as requested.

Reference and test drinks

Five AA (L-leucine, L-isoleucine, L-threonine, L-valine, and L-lysine) and CrPic were added either alone or in combination to the three test drinks. Both CrPic and the 5AA were kindly provided by Einar Willumsen (Brondby, Denmark). Reference and test drinks were named as follows: water with grapefruit aroma and no other ingredients (Ref), water with grapefruit aroma and 5AA (5AA), water with grapefruit aroma and CrPic (CrPic), and water with grapefruit aroma and both 5AA and CrPic (5AA+CrPic). CrPic was added in the amount of 500 µg, which corresponds to 62 µg of Cr. The molar ratio of AA was 1.1:1.1:1.7:2.1:2.2 for Val:Ile:Lys:Thr:Leu. Carbon dioxide and aroma was added to improve palatability. No sugar was added to the drinks. More details on the drinks are presented in Table 1. The carbonated drinks were prepared the day before each test (except for Ref that was provided by the flavoring company in one batch).

Table 1. Ingredients in the test and reference drinks.

	Water (g)	Amino acids[1] (g)	CrPic (µg)	Aroma (g)	Total weight[3] (g)
Ref	312.0	-	-	X[2]	312.0
CrPic	308.0	-	500	0.19	312.0
5AA	305.1	6.9	-	0.19	312.0
5AA+CrPic	301.1	6.9	500	0.19	312.0

CrPic, Chromium picolinate; [1]Molar ratio of the amino acids; 1.1:1.1:1.7:2.1:2.2 for Val:Ile:Lys:Thr:Leu, respectively; [2]Aroma added by flavoring company in unknown amount; [3]Total weight without aroma taken in account.

Breakfast meal

A standardized, composite breakfast meal was served along with each of the reference or test drinks. The breakfast meal consisted of white wheat bread (WWB) (Dollarfranska, Lockarp, Sweden) corresponding to 50 g available starch [21] with 24 g orange marmalade (Onos, Orkla Foods, Sweden) and 10 g butter (Bregott, Arla Foods, Sweden). All breakfast meals contained the same amount of carbohydrates and fat, whereas protein varied depending on whether or not AA were included in the accompanying drink. The nutritional composition of the sandwich meal was 64.0 g carbohydrates, 9.1 g protein, and 11.7 g fat with an energy content of 380.1 kcal. The AA in the drinks (5AA and 5AA+CrPic) corresponded to 6.9 g protein equaling 27.6 kcal. Hence, the meals with the AA-containing drinks contained 407.8 kcal and a total of 16.0 g protein.

Blood analysis

Capillary blood samples were taken at time 0 (fasting) and at 15, 30, 45, 60, 90, 120, and 180 minutes after breakfast to determine glucose and insulin. Blood glucose was evaluated directly using a B-glucose analyzer (HemoCue Glucose 201$^+$ Analyser, HemoCue AB, Angelholm, Sweden). Serum was separated by centrifuging samples for 5 min (3000 rpm,

Eppendorf mini spin, F-45-12-11) and frozen at -18°C until analysis. Insulin was measured using an enzyme immunoassay kit (Mercodia AB, Uppsala, Sweden) on an integrated immunoassay analyzer (CODA Open Microplate System; Bio-Rad Laboratories, Hercules, CA, USA).

Subjective appetite rating

During each session, the test subjects were asked to rank their subjective appetite at 0, 15, 30, 45, 60, 90, 120, and 180 minutes on a 100 mm visual analogue scale (VAS) ranging from "not at all" to "extremely". Feeling of satiety, feeling of hunger, and desire to eat were evaluated.

Calculations and statistical analysis

Nineteen subjects participated in the study, with all but one completing all test meals. Of the nineteen participants, one subject was excluded from final analysis as he was indicated as a statistical outlier with Grubbs' test for 5 time points (Minitab Statistical Software, version 16, State College, PA). As a result, data from 18 subjects was further analyzed. One person's data is missing for 5AA due to illness (n=17). One person missed VAS scoring for the subjective appetite ratings; therefore, one set of appetite scores are missing for the 5AA+CrPic drink (n=17).

All results are expressed as mean ± SEM. The incremental area under the curve (iAUC) for glucose and insulin was calculated according to the trapezoidal method, excluding areas below fasting level. Total areas under the curve (tAUC) were calculated for feeling of satiety, feeling of hunger, and desire to eat respectively, using GraphPad Prism (ver 5, GraphPad Software, San Diego, CA, USA). Incremental peaks (iPeaks) were calculated per individual for glucose and insulin respectively, as the maximum elevation from baseline. The glucose profile (GP) was calculated by dividing the duration (the time the curve remained above baseline) by the iPeak [22].

All statistical calculations were performed in Minitab Statistical Software (versions 14 and 16, State College, PA, USA). To evaluate significances for iAUCs of glucose and insulin, in addition to iPeaks, GI, II, and GP, analysis of variance (ANOVA) was used followed using Tukey's pair wise comparisons test. For appetite ratings, analysis of variance was conducted with covariate (ANCOVA) using total area values and the 0-values as covariate. In the cases where the residuals were not normally distributed, Box Cox transformation was applied before analysis with ANOVA and ANCOVA.

Time × treatment interactions were analyzed for glucose and insulin responses using a mixed model (PROC MIXED in SAS, release 8, SAS Institute Inc., Cary, NC) with repeated measures and an autoregressive covariance structure. The subjects were the random variable and the fasting values the covariate.

RESULTS

Postprandial glycemia

The glycemic responses are represented in **Figure 1** and **Table 2**. A main effect for product (P=0.0346) and a product × time interaction was found (P=0.0020). 5AA+CrPic and 5AA respectively resulted in significantly lower glucose response (iAUC) compared to Ref during the first hour. The iPeak (Δ mmol/L) was significantly lower for 5AA and 5AA+CrPic compared to both Ref and CrPic respectively. GP was significantly higher after 5AA+CrPic

and 5AA compared to Ref. There was no significant difference in glycemia between CrPic and Ref at any time.

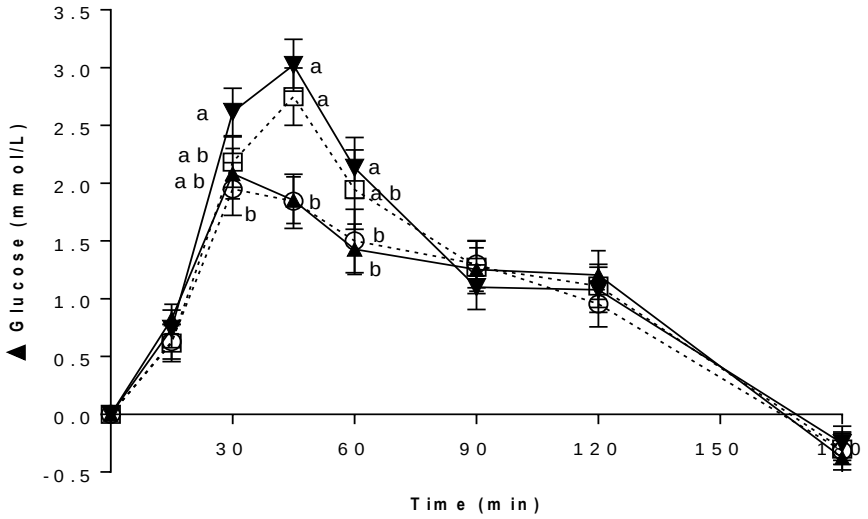

Fig. 1 Glycemic responses following intake of a breakfast with four different test drinks: reference (Ref) (▼), chromium picolinate (CrPic) (□), mix of amino acids leucine, isoleucine, valine, lysine and threonine (5AA) (▲) and 5AA+CrPic (○). Values expressed as mean ± SEM; n = 18 (except for 5AA, n = 17). Incremental glucose values at each time point, not sharing the same letter are significantly different.

Table 2. Glucose and insulin responses after intake of a standardized breakfast with different test and reference drinks.

	Ref	CrPic	5AA	5AA+CrPic
Glucose				
iAUC[1] *0-60 min* (Δ %)[2]	111.7±8.0*a* (-)	98.1±9.5*ab* (-12)	82.1±7.4*b* (-26)	78.1±8.4*b* (-30)
iAUC[1] *0-120 min* (Δ %)[2]	192.8±17.3 (-)	182.2±20.7 (-5)	159.5±16.2 (-17)	155.0±18.1 (-20)
iAUC[1] *0-180 min* (Δ %)[2]	223.9±22.7 (-)	212.6±25.0 (-5)	195.5±21.9 (-13)	183.5±22.5 (-18)
iPeak, mmol/L (Δ %)[2]	3.3±0.2*a* (-)	3.0±0.2*a* (-10)	2.3±0.2*b* (-29)	2.4±0.2*b* (-27)
GP[3], min·(mM)$^{-1}$ (Δ %)[2]	52±3*a* (-)	60±5*ab* (+15)	74±5*b* (+42)	75±7*b* (+44)
Insulin				
iAUC[1] *0-15 min* (Δ %)[2]	0.41±0.12*a* (-)	0.40±0.07*a* (-3)	0.87±0.16*b* (+112)	0.61±0.14*ab* (+49)
iAUC[1] *0-60 min* (Δ %)[2]	7.52±0.66*a* (-)	7.17±0.84*a* (-5)	10.9±1.11*b* (+45)	9.31±1.06*ab* (+24)
iAUC[1] *0-120 min* (Δ %)[2]	14.2±1.2*a* (-)	14.1±1.4*a* (-1)	20.6±2.3*b* (+45)	19.0±2.3*b* (+34)
iAUC[1] *0-180 min* (Δ %)[2]	16.6±1.7*a* (-)	16.6±1.9*a* (-)	23.9±3.1*b* (+44)	21.8±2.7*b* (+31)
iPeak, nmol/L (Δ %)[2]	0.24±0.02*ab* (-)	0.24±0.02*b* (+3)	0.30±0.03*a* (+22)	0.29±0.03*ab* (+17)

Ref, Reference; CrPic, Chromium picolinate; 5AA, 5 amino acids (Val:Ile:Lys:Thr:Leu); iAUC, incremental area under the curve; iPeak, incremental peak; GP, glycemic profile; [1]Expressed as min•mmol/L; [2]Percentage change from Ref.; [3]Residuals not normally distributed after Box Cox transformation. Values are expressed as mean ± SEM; n = 18 (except for 5AA, n = 17). Values on the same row not sharing the same letter are significantly different ($p<0.05$).

Postprandial insulinemia

The insulin responses are represented in **Figure 2** and **Table 2**. A main effect was found for the product (p=0.0035), as well as a product × time interaction (p<0.0001). During the first 15 minutes after start of breakfast, the 5AA induced a significant increase in insulin compared to Ref. The insulin increase during the same time for 5AA+CrPic was about half of the 5AA response, and did not differ significantly from Ref or CrPic. The overall insulin responses (iAUC 0-180 min) were significantly higher for both 5AA and 5AA+CrPic compared to Ref and CrPic. The iPeak (Δ mmol/L) was significantly higher after 5AA compared with CrPic but not different from Ref or 5AA+CrPic.

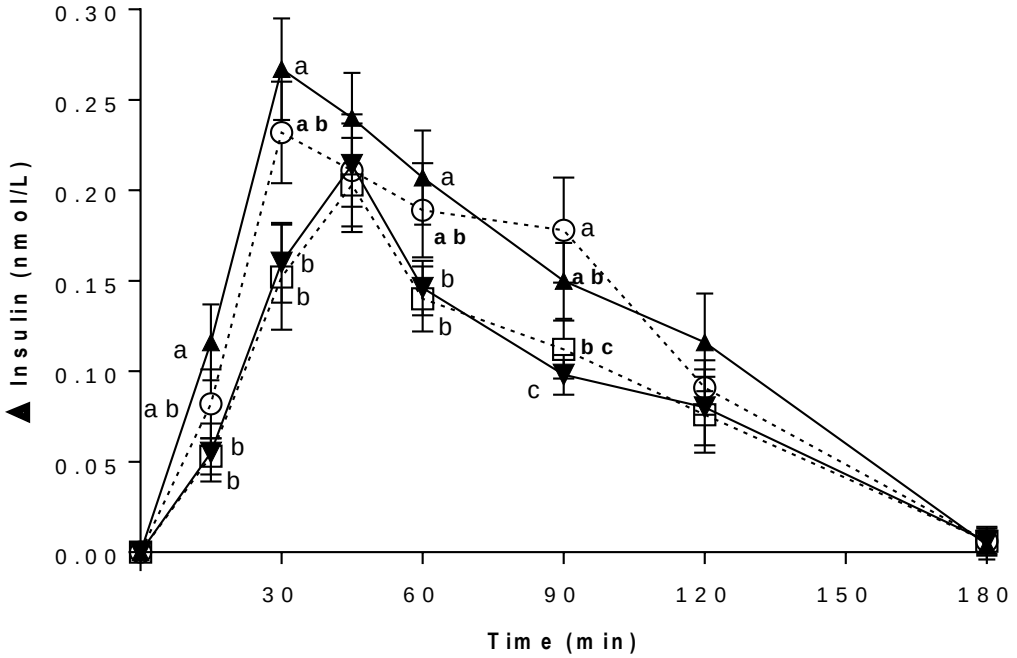

Fig. 2 Insulinemic responses following intake of a breakfast with four different test drinks: reference (Ref) (▼), chromium picolinate (CrPic) (□), mix of amino acids leucine, isoleucine, valine, lysine and threonine (5AA) (▲) and 5AA+CrPic (○). Values expressed as mean ± SEM; n = 18 (except for 5AA, n = 17). Incremental insulin values at each time point, not sharing the same letter are significantly different.

Subjective appetite ratings

Among the subjective appetite ratings there was one significant difference observed for *feeling of satiety* (tAUC 0-180), with a lower rating for CrPic compared to 5AA (results not shown).

DISCUSSION

This study demonstrates that both AA-containing test drinks (5AA and 5AA+CrPic respectively) caused similar improvements in glycemia after breakfast, as measured by lowered iAUC 0-60 min, reduced iPeak and increased GP, compared to the Ref drink. However, a difference between 5AA+CrPic and 5AA was found in the insulin response. Whereas a significant early increase in postprandial plasma insulin was discovered following the 5AA meal (+112%, iAUC 0-15 min) compared to Ref, the corresponding insulin increase after the meal with 5AA+CrPic (+49%) was about half and not different from Ref. The

finding that the specific AA have an insulin stimulating and glucose reducing effect is in line with previous results [5, 6]. However, there seems to be less insulin needed to obtain the same glucose lowering effect if CrPic is added to the 5AA-drink. Therefore, the drink with 5AA+CrPic appeared to improve insulin economy.

The fact that the peak of the mean glucose responses occur at 30 min for 5AA and 5AA+CrPic and at 45 min for Ref and CrPic indicate that the early insulin release caused by 5AA is effective in counteracting the glucose rise after intake of a challenging breakfast. In fact, the improved first phase insulin responses have been reported as important for overall glucose regulation and decreased risk of T2DM [23]. Furthermore, in a previous study the early insulin release (0-30 min) to a carbohydrate challenge supplemented with whey protein and 5AA was correlated to the beneficial impact of glycemia, as manifested by lowered glucose responses and increased glycemic profile in healthy, normal weight subjects [6]. In the present study, no such correlations were found (results not presented); additionally, it should be noted that in the study by Gunnerud, et al. [6] the 5AA was taken as a pre-meal drink, while in the present study the 5AA were ingested together with the breakfast meal.

The present results suggest that the addition of CrPic to the AA-containing drink blunted the insulin responses elicited by the 5AA. To our knowledge, such an acute insulin saving effect of Cr-supplementation has not been reported before. In previous acute and longer term studies of Cr-supplementation, beneficial effects on glycemic control have been reported as reduced acute glucose responses and glycemic peaks, as well as reduced fasting glucose and improved insulin sensitivity [12, 24]. Furthermore, Cr has been hypothesized to be insulin sensitizing both in cell [25] and animal [26] studies. Acute effects of CrPic have been reported for doses ranging from 200-1000 µg [12, 15, 24]. In this study, the dose was set to 500 µg CrPic and no significant glucose lowering effect was found. However, results regarding the efficacy of dietary supplementation with Cr on indicators of metabolism are inconclusive [27]. One reason for this could be that the acute effect of Cr may differ in between subjects [24]. Differences in responses between individuals may originate from differences in Cr status where a deficiency [15] or a state of insulin resistance may influence the metabolic response to Cr-supplementation [28]. Furthermore, the number of responders and non-responders to Cr was investigated in the present study. A responder was defined as a subject showing more than 5 % decrease in postprandial glucose iAUC (0-180 min) compared to the corresponding meal with the Ref drink. Nine out of the 18 subjects were defined as responders to Cr, with this number being considered too low to proceed with any statistical evaluation. It should also be noted that Cefalu et al [29] proposed that the division of subjects into responders or non-responders based on glucose responses may conceal the ones benefiting from Cr on insulin sensitivity, which thereby falsely characterizes them as non-responders. It should be noted that the potential insulin sensitizing effects of Cr in the present study may have been masked by the insulinogenic effects of the AA. Furthermore, most studies have been conducted over a longer time period, and it cannot be excluded that a longer-term intervention could have revealed differences between the 5AA and 5AA+CrPic drinks.

Both whey proteins and Cr have been suggested to modulate appetite and attenuate body weight gain [12, 30, 31]. In this study, hunger, satiety, and the desire to eat were measured but no differences were found between any of the meals. It should be noted though that the study was not powered to detect differences in appetite ratings.

CONCLUSIONS

Drinks containing 5AA (with and without CrPic) attenuated postprandial glycemia to a challenging sandwich breakfast by 25-30%. An early insulin increase seems to be of importance for the benefits on postprandial glycemic regulation and a combination of 5AA with CrPic improved insulin economy. Therefore, it is concluded that the 5AA+CrPic drink, when co-ingested with a high glycemic breakfast meal, affected the glycemic response in healthy "at risk" subjects beneficially, and thereby may circumvent pro-oxidative and/or pro-inflammatory conditions associated with oscillatory glycemic episodes as described in acute and longer term studies.

Abbreviations

CrPic, Chromium picolinate; 5AA, 5 amino acids (leucine, isoleucine, valine, lysine and threonine); Ref, Reference meal; iAUC, incremental area under the curve; iPeak, incremental peak; GP, glycemic profile

Competing interests

Elin Ostman and Inger Bjorck jointly own a patent application describing the combination of protein and mineral that has been studied. Double Good AB holds a license to the IPR. Rickard Oste is a co-inventor of the patent application and adjunct professor at Food for Health Science Centre, at part-time from his employment at Aventure AB. Anna Forslund declares no conflict of interests.

Authors' contributions

E. Ostman, R. Oste, and I. Bjorck designed the study; AF performed the practical part of the study, in addition to the statistical analysis of the data with supervision from EO; EO and AF drafted the manuscript and all authors engaged in the manuscript work. EO had primary responsibility for the final content. All authors have read and approved the final manuscript.

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