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ACIDI ABSCISIK MOLEKULA DHE HORMONI MË I RI NË PARANDALIMIN E DIABETIT MELITUS.

EVIDENCAT E NJË STUDIMI KLINIK PLACEBO KONTROLL

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Përmbledhje

Sindroma metabolike ku përfshin një rreth vicioz midis diabetit, hipekolesterolemisë dhe hipertensionit është shndërruar në një problem global e cila po prek shumicën e popullatës. Sfidat e fundit të industrisë farmaceutike po orientohen drejt gjetjes së molekulave të reja kosto efektive dhe pa efekte anësore. Nisur nga evidencat shkencore mbi rolin e Acidi Abscisic (ABA) si fitomolekulë, por edhe si hormon endogjen i prodhuar nga mamiferrët, në përmirësimin e parametrave metabolik, qëllimi i këtij studimi, ishte formulimi për herë të parë, i një nutraceutiku pilot dhe testimi placebo-kontroll në 70 vullnetarë të rastësishëm në fazën prediabetike për një periudhë 3 mujore, i glicemisë esëll, glicemisë pas ngrëniës, i përgjigjes inflamatore, hemoglobinës së glukozuar dhe lipidemisë. Rezultati më i rëndësishëm i këtij studimi ishte ulja e nivelit të proteinës C-reaktive PCR, markuesi kryesor i inflamacionit, në masën 28% ± 0,1 krahasuar me placebo. Për më tepër mungesa e luhatjeve të niveleve të insulinës në prani të hormonit ABA, dëshmon veprimin e tij si "insulin like" përmës nxitjes së numërit dhe ndjeshmërisë së receptorëve GLUT-4 në miocite dhe hepatocite duke mbrojtur në këtë mënyrë qelizat β-ta të pankreasit. Një rënie e ndjeshme në indeksin FPG (nivelin plazmatik të glicemisë esëll) e në PPG(nivelin plazmatik të glicemisë pas ngrënies) u vu re në grupin e trajtuar me ABA në krahasim me grupin placebo ($p < 0.05$ kundrejt placebo). Të gjitha këto evidenca hedhin dritë për studime më të zgjeruara në numër më të madh pacientësh e nxisin zbulime të mëtejshme në shkencat mjekësore.

Fjalë çelës: *acidi abscisik, nutraceutikë, sindroma metabolike,, faza prediabetike,*

ABSCISIC ACID, THE NOVEL MOLECULE AND ENDOGENOUS HORMONE IN THE PREVENTION OF DIABETES MELLITUS. EVIDENCE FROM A PLACEBO CONTROL CLINICAL TRIAL

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Abstract

Metabolic syndrome, which includes a vicious circle between diabetes, hypercholesterolemia, and hypertension, has become a global problem that affects most of the population. The latest challenges of the pharmaceutical industry are oriented towards finding new molecules that are cost effective and without side effects. Based on the scientific evidence on the role of Abscisic Acid (ABA) as a phytohormone but also as an endogenous hormone produced by mammals, in improving metabolic parameters, the purpose of this study was the formulation for the first time of a pilot nutraceutical and testing by a randomized placebo-control clinical trial in 70 prediabetic volunteers, for a period of 3-month. Clinical end points were, fasting blood glucose, postprandial blood glucose, inflammatory response, glycated hemoglobin, and lipidemia. The most important result of this study was the decrease in the levels of C-reactive protein CRP, the main marker of inflammation, in the range of 28% ± 0.1 compared to placebo. Moreover, the absence changes of the insulin levels in the presence of the ABA hormone proves its action as "insulin like" by promoting the number and sensitivity of GLUT-4 receptors in myocytes and hepatocytes, protecting in this way the β-cells of pancreas. Moreover, a significant decrease in FPG, PPG, FPI, was observed in the ABA group compared to the baseline value ($p < 0.05$ vs baseline) and compared to the placebo group ($p < 0.05$ vs placebo). All this evidence trigger the for further studies in larger numbers of patients and encourage further investigation in medical sciences.

Key words: *Abscisic acid, nutraceuticals, metabolic syndrome, prediabetic stage*

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Introduction

Metabolic syndrome, which includes a vicious circle between diabetes, hypercholesterolemia, and hypertension, has become a global problem that affects most of the population. The latest challenges of the pharmaceutical industry are oriented towards finding new molecules that are cost effective and without side effects. It has been observed that almost all type 2 diabetic patients go across prediabetes phase whose average duration is about 10 years (1). During this long transition step it is possible to avoid the evolution towards diabetes if proper interventions are adopted. Triggered by these evidence, the develop of a sustainable and novel nutraceutical approach, where the treatment goal should be the return to euglycemic condition, represent a challenge exploited in the following study. It has been shown that restoration and maintenance of normal glycemia values during prediabetes and early stages of type 2 diabetes (T2DM) can determine a long-term remission (2,3). It has also been reported that some nutraceuticals are effective and safe in improving insulin sensitivity and glycemic control in patients with dysglycemia (4). Regarding the recent literature, abscisic acid, ABA, a triterpenoid phytohormone, has attracted a considerable interest due to its involvement in management of glucose homeostasis in humans (5). Moreover, at nanomolar concentrations, ABA regulates insulin secretion intensifying its glucose-dependent release and stimulating glucose-independent one (6). It has been reported that hyperglycemia produced an increase of ABA plasma levels in healthy subjects subjected to an oral glucose tolerance test (OGTT), and that this phytormone, at nanomolar concentrations, promoted peripheral glucose uptake in adipocytes and myoblasts, similarly to insulin (7). The following study is addressed to the regression of impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) conditions. To assess this aspect a clinical randomized trial based on sixtyfive patients with IFG or IGT has been conducted and ABA or placebo has been administeredfor 3 months.

Materials and methods

2.1. Sample preparation and nutraceutical formulation

Nectarines thinning waste were collected in June 2020 in the (Vitulazio, Caserta, Italy), at 20-25 days after full bloom, at the fruit thinning stage. Samples were immersed in liquid nitrogen (N_2), and maintained at -80 °C until analysis. Then, were weighed and ground in liquid N_2, and 0.5 g of each homogenized sample was suspended in 15 mL of 80% aqueous methanol containing 1% formic acid for 16 h at 4 °C in darkness under magnetic stirring. The extract was diluted to 25 mL with water, and the pH was adjusted to 2.5 with 1 M aqueous HCl, after which the samples were filtered and eluted. The formulation of nutraceuticals has been obtained as follows.

To determine the presenc of ABA in nectarin thinning waste, chromatographic analyses by HPLC-DAD was conducted as detailed in the previously in the first study of this thesis, according to the procedure suggested by Magnone et al. 2015 (8) with slight modifications. Nectarines (NecP) were extracted with water at 50 °C. After centrifugation, the extract underwent a spray-drying process with maltodextrins as support, obtaining a fine powder containing maltodextrins and NecP, in a 1:1 ratio (w/w) named "ABA nutraceutical". This has been formulated in plastic sachet before the administration per os.

2.2. Study design and patients

A three-months, double-blind, randomized, placebo-controlled, clinical trial has been performed. The study protocol was approved by Institutional Ethical Committee (P-2017000837) and was conducted in accordance with the 1994 Declaration of Helsinki and its amendments and the Code of Good Clinical Practice. All patients provided written informed consent to participate in this study after a full explanation of the study.

We enrolled patients with IFG or IGT, not taking hypoglycemic agents (both pharmaceutical or nutraceutical agents).

Patients were excluded if they had type 1 or type 2 diabetes mellitus, impaired hepatic function (defined as plasma aminotransferase and/or gamma-glutamil transpeptidase (γ-GT) level higher than the three times the upper limit of normal [ULN] for age and sex), impaired renal function (defined as serum creatinine level higher than the ULN for age and sex), or gastrointestinal disorders; current or previous evidence of ischemic heart disease, heart failure, or stroke; weight change of >3 Kg during the preceding 3 months; malignancy, and significant neurological or psychiatric disturbances, including alcohol or drug abuse. Excluded medications (within the previous 3 months) included hypoglycemic agents, laxatives, β-agonists (other than inhalers), cyproheptadine, anti-depressants, anti-serotoninergics, phenothiazines, barbiturates, oral corticosteroids, and anti-psychotics. Women who were pregnant or breastfeeding or of childbearing potential and not taking adequate contraceptive precautions were also excluded.

2.3. Treatments

Patients were randomized to placebo or ABA nutraceutical formulation (2 g of lyophilized extract from dwarf nectarines, corresponding with 14 μg of abscisic acid) for 3 months. Both ABA and placebo were self-administered three times a day, 1 sachet before the breakfast, 1 sachet before the lunch, and 1 sachet before the dinner.

Both ABA nutraceuticals and placebo were supplied as identical sachets with coded to ensure the blind status of the study. Randomization was done using a drawing of envelopes each given a code. Medication compliance was assessed by counting the number of empty sachets returned at the time of specified clinic visits. All ABA containing nutraceuticals were provided free of charge.

2.4. Assessments

At the study beginning, all patients underwent an initial screening assessment including a medical history, physical examination, vital signs (blood pressure and heart rate), a 12-lead electrocardiogram, measurements of height and body weight, calculation of body mass index (BMI), evaluation of fasting plasma glucose (FPG), post-prandial plasma glucose (PPG), glycated hemoglobin (HbA_{1c}), fasting plasma insulin (FPI), homeostatic model assessment of insulin resistance (HOMA index) by Matsuda Index (9) , total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), triglycerides (Tg), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ-GT), creatinine, and high sensitivity C-reactive protein (Hs-CRP).

All parameters were evaluated at baseline and after 3 months since the study start. Moreover, at baseline, and after 3 months, patients underwent an oral glucose tolerance test (OGTT), an euglycemic hyperinsulinemic clamp, and a glucagon test.

2.5. Statistical analysis

An intention-to-treat (ITT) analysis has been conducted in patients who received ≥1 dose of the nutraceutical containing ABA and had a subsequent efficacy observation. A two-way repeated measures analysis of variance (ANOVA) to test continuous variables has been used. Intervention effects were adjusted for additional potential confounders using analysis of covariance. An analysis of variance to assess the significance within and between groups has been conducted. The null hypothesis that the expected mean glycemia change from the end of the study did not differ significantly between placebo, and the nutraceutical was tested using a two-way repeated measures analysis of variance (ANOVA) model. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 14.0 (SPSS Inc., Chicago, Illinois, USA). Data are presented as mean (SD). For all statistical analyses, $p< 0.05$ was considered statistically significant.

Results

3.1. Study sample

On the total of sixty five patients enrolled in the trial, thirty three were randomized to take ABA containing nutraceutical, and thirty two to take the placebo. Sixty patients completed the study; there were 5 patients who did not complete the study and the reasons for premature withdrawal included non-compliance to treatment (1 male in ABA group, and 1 female in placebo group, respectively) or lost to follow-up (1 male in placebo group and 2 females in ABA group, respectively) as shown in Figure 1. The characteristics of the patient population at the time of the start and during the study are shown in Table 1 and Table 2.

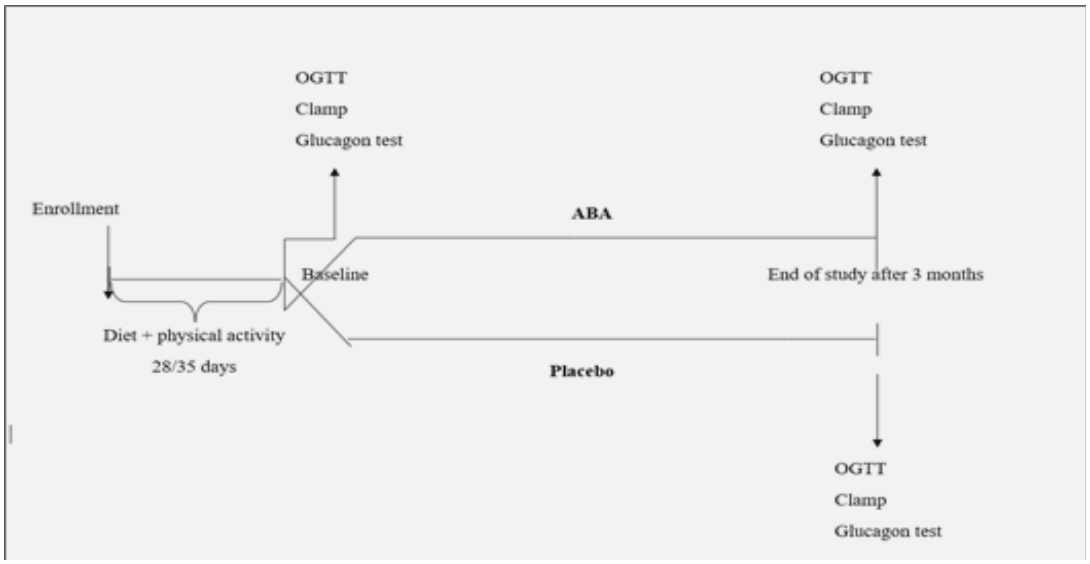

Figure 1. Study design. **OGTT:** oral glucose tolerance test; **Clamp:** euglycemic hyperinsulinemic clamp

Parameters	**ABA**		**Placebo**	
	Baseline	**3 months**	**Baseline**	**3 months**
Patients (n)	33	30	32	30
M/F	15/18	14/16	16/16	15/15
Age (years)	51.9 ± **6.5**	-	52.2 ± **6.8**	-
Smoking status (M/F)	7/6	6/5	8/6	6/6
IFG (n; %)	6/7 (39.4)	3/3 (26.7)	5/6 (34.4)	4/5 (30.0)
IGT (n; %)	9/11 (60.6)	3/10 (46.7)	11/10 (65.6)	10/9 (63.3)
EU from IFG (n; %)	-	5/3 (61.5)	-	0/0
EU from IGT (n; %)	-	0	-	0/0
IFG from IGT (n; %)	-	0/0	-	1/0 (4.8)
IGT from IFG (n: %)	-	3/0 (23.1)	-	0/0
D from IFG (n; %)	-	0/0	-	0/0
D from IGT (n; %)	-	0/0	-	1/1 (9.5)
Lost to FU from IFG (n; %)	-	1/1 (15.4)	-	0/1 (9.1)
Lost to FU from IGT (n; %)	-	0/1 (7.7)	-	1/0 (4.7)

Table 1. Baseline, and 3-month general data of patients during ABA nutraceutical (ABA) treatment and placebo. M: males; F: females; IFG: impaired fasting glycemia; IGT: impaired glucose tolerance; EU: euglycemia; D: diabetes; FU: follow-up.

Parameters	ABA		Placebo	
	Baseline	3 months	Baseline	3 months
Height (cm)	169 ± 0.05	-	168 ± 0.04	-
Weight (Kg)	77.4 ± 6.1	76.5 ± 5.9	77.1 ± 5.9	76.2 ± 5.7
BMI (Kg/m^2)	27.1 ± 1.3	26.8 ± 1.1	27.3 ± 1.5	27.0 ± 1.2
WC (cm)	86.5 ± 4.9	86.4 ± 4.8	86.8 ± 5.0	86.7 ± 4.9
HC (cm)	89.2 ± 5.2	89.0 ± 5.0	88.9 ± 4.9	88.7 ± 4.7
AC (cm)	97.2 ± 5.8	97.0 ± 5.6	97.4 ± 6.0	97.2 ± 5.8
FPG (mg/dl)	109.4 ± 6.5	104.5 ± 6.1*^	112.8 ± 5.6	110.7 ± 5.5
PPG (mg/dl)	144.0 ± 12.8	130.1 ± 12.8*^	153.3 ± 18.4	149.7 ± 18.5
HbA_{1c} (%)	5.9 ± 0.4	5.5 ± 0.2*	5.8 ± 0.3	5.7 ± 0.2
FPI (μU/ml)	10.3 ± 6.7	9.2 ± 5.8*^	10.1 ± 6.5	10.5 ± 6.9
Homa index	2.80 ± 0.7	2.39 ± 0.4*^	2.84 ± 0.8	2.89 ± 0.9
TC (mg/dl)	215.1 ± 15.8	211.0 ± 14.2	218.6 ± 16.9	220.2 ± 18.1
LDL-C (mg/dl)	146.9 ± 18.4	143.4 ± 17.7	150.9 ± 19.2	152.6 ± 20.7
HDL-C (mg/dl)	43.8 ± 5.0	44.0 ± 5.1	43.6 ± 4.9	43.7 ± 4.8
Tg (mg/dl)	122.1 ± 24.2	117.3 ± 22.0	120.4 ± 23.5	119.5 ± 23.1
AST (UI/l)	18.8 ± 10.8	18.5 ± 10.4	18.2 ± 10.3	18.4 ± 10.5
ALT (UI/l)	28.3 ± 14.2	28.9 ± 14.5	26.8 ± 13.1	26.1 ± 12.8
γ-GT (UI/l)	24.5 ± 8.1	24.1 ± 7.7	25.8 ± 8.7	25.3 ± 8.4
Creatinine (mg/dl)	0.6 ± 0.2	0.7 ± 0.3	0.7 ± 0.3	0.8 ± 0.4
Hs-CRP (mg/l)	1.3 ± 0.5	1.0 ± 0.2*^	1.3 ± 0.5	1.4 ± 0.6

Table 2. Baseline, and 3-month anthropometric and biochemical parameters of patients during ABA containing nutraceutical (ABA) treatment and placebo.
Data are expressed as mean ± standard deviation. *$p < 0.05$ vs baseline; ^$p < 0.05$ vs placebo. M: males; F: females; BMI: body mass index; WC: waist circumference; HC: hip circumference; AC: abdominal circumference; FPG: fasting plasma glucose; PPG: postprandial plasma glucose; HbA_{1c}: glycated hemoglobin; FPI: fasting plasma insulin; HOMA index: homeostatic model assessment of insulin resistance; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; Tg: triglycerides; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γ-GT: gamma-glutamyl transpeptidase; Hs-CRP: high sensitivity C-reactive protein.

3.3 Glyco-metabolic parameters

A significant decrease in FPG, PPG, FPI, and Homa index was observed in the ABA group compared to the baseline value ($p < 0.05$ vs baseline) and compared to the placebo group ($p < 0.05$ vs placebo). The HbA_{1c} value was significantly reduced compared to baseline ($p < 0.05$ vs baseline) in the group being treated with ABA (Table 2).

3.4. Lipid profile

No significant modification was observed in the lipid profile parameters, although a slight non-significant reduction was seen in TC, LDL-C and Tg in the ABA group (Table 2).

3.5. Inflammation parameter

Hs-CRP decreased significantly from baseline ($p < 0.05$ vs baseline) in the ABA group and also compared to the placebo group ($p < 0.05$ vs placebo) (Table 2).

3.6. OGTT results

At baseline, 39.4% of patients were affected by IFG in the ABA group vs 34.4% in placebo (p not significant), whilst 60.6% of patients were affected by IGT in the ABA group, and 65.6% in placebo group (p not significant). After 3 months, 26.7% of patients returned to a normal glycemic status in the ABA group vs 0 patients in placebo group ($p < 0.05$); at the end of the study, 20.0% were classified as IFG in the ABA group vs 33.3% in placebo group ($p < 0.05$). In the ABA group, 53.3% were classified as IGT vs 63.3 % in placebo group ($p < 0.01$). In placebo group, 6.7 % developed type 2 diabetes mellitus vs 0 patients in the ABA group (Table 1).

4. Discussion

Starting from the impact that the metabolic syndrome, which includes a cluster of health disorders like obesity, diabetes and cardiovascular diseases,and based on the consideration that these conditions are very closelyrelatedto dietary foods habits, there is the need to develop a sustainable and novel nutraceutical approach as a complementary and/or alternative to the conventional pharmacologic treatment. The present study showed that ABA is effective in improving glyco-metabolic and inflammation parameters in patients with IFG or IGT.

Recently, regarding the scientific data in the literature, demonstrated that chronic consumption of a supplement containing low dose of ABA ameliorated the prediabetes markers (FPG -30.2% and HbA_{1c} -8.1%)in subjects with borderline values of FPG (≥ 100 mg dL^{-1}) and HbA_{1c} ($\geq 5.7\%$) defined in the American Diabetes Association (ADA) recommendations for prediabetes (10). It has been also proven that the improvement was greater in these subjects than in healthy ones, suggesting the beneficial effect of low dose ABA supplementation in prediabetics. The absence of increased insulin secretion was probably due to the stimulation by ABA of muscle glucose uptake and the greater sensitivity of human GLUT4-expressing cells to ABA than pancreatic β-cells.

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