



Original/Síndrome metabólico

Glucomannan and glucomannan *plus* spirulina-enriched squid-surimi added to high saturated diet affect glycemia, plasma and adipose leptin and adiponectin levels in growing fa/fa rats

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Abstract

Type 2 diabetes is a very prevalent chronic disease. Among dietary factors for its prevention and treatment, interest has grown in satiating fibre (konjac glucomannan) and spirulina. Our previous studies suggest that glucomannan itself and/or in conjunction to spirulina displayed hypolipemic and antioxidant effects when incorporated to squid surimi as functional ingredients. The present study aims to determine whether glucomannan-enriched or glucomannan *plus* spirulina-enriched squid-surimi improve plasma glucose and insulin levels in Zucker fa/fa rats fed a high saturated fat diet. Twenty four growing rats, divided into three groups, were given modified AIN-93M diets for seven weeks: 30% squid-surimi control diet (C), 30% glucomannan-enriched squid-surimi diet (G) and 30% glucomannan *plus* spirulina-enriched squid-surimi diet (GS). All rats became hyperglycemics and hyperinsulinemics, but G and GS diets induced significantly lower glucose levels (20%; $p < 0.05$) but did not modify insulinemia with respect to C diet. GS animals showed higher HOMA-D ($p < 0.05$) than C ones suggesting increased insulin availability. Plasma leptin and adiponectin decreased in G and GS vs. C group ($p < 0.05$). Adipose adiponectin increased significantly in G and GS vs. C rats (16-20 times, $p < 0.01$). Leptin in adipose tissue was higher in GS vs. G group ($p < 0.05$). In conclusion, both glucomannan-diets were

LAS DIETAS HIPERSATURADAS QUE CONTIENEN SURIMI DE CALAMAR ENRIQUECIDO CON GLUCOMANANO Y GLUCOMANANO/ESPIRULINA AFECTAN A LA GLUCEMIA Y A LOS NIVELES PLASMÁTICOS Y ADIPOSOS DE LEPTINA Y ADIPONECTINA EN RATAS FA/FA EN CRECIMIENTO

Resumen

La diabetes tipo 2 es una enfermedad crónica de gran prevalencia. Entre otros factores dietéticos para su prevención y tratamiento, la fibra saciante (p. ej. glucomanano de konjac) y la espirulina han experimentado un interés creciente. Estudios previos sugieren que el glucomanano solo o en conjunción con la espirulina, incorporados a surimi de calamar, como ingredientes funcionales, induce efectos hipolipemiantes y antioxidantes. En el presente estudio tratamos de determinar si dichos surimis de calamar mejoran la glucemia y la insulinemia en ratas Zucker fa/fa alimentadas con una dieta hipersaturada. Veinticuatro ratas en crecimiento, divididas en tres grupos, fueron alimentadas durante siete semanas con las siguientes dietas AIN-93M modificadas: dieta control con 30% de surimi de calamar (C), dieta glucomanano con 30% de surimi de calamar enriquecido con 15% glucomanano (G) y dieta glucomanano/espirulina con 30% de surimi de calamar enriquecido con 15% glucomanano y espirulina 3 g/kg dieta (GS). Todos los grupos presentaron hiperglucemia e hiperinsulinemia, pero las dietas G y GS redujeron significativamente los niveles de glucosa (20%; $p < 0,05$) con respecto a la dieta C, aunque no lograron modificar la insulinemia. Los animales alimentados con GS tuvieron valores de HOMA-D ($p < 0,05$) más elevados respecto al grupo C, sugiriendo un aumento de la disponibilidad de insulina. La leptina y la adiponectina plasmáticas disminuyeron en la dieta G y GS respecto a la C ($p < 0,05$). La adiponectina en tejido adiposo aumentó significativamente en G y GS vs. C (16-20 veces, $p < 0,01$). La leptina en tejido adiposo fue mayor en GS vs. G ($p < 0,05$). En resumen, ambas dietas con glucomanano fueron capaces de reducir la hiperglu-

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able to reduce hyperglycemia and increase adipose tissue adiponectin levels in fa/fa rats, suggesting an anti-hyper-trophic and insulin-sensitizing adipokine effect in this tissue. Spirulina inclusion increased insulin availability. Although results are promising, the utility of consuming glucomannan surimis as part of usual diets demands future studies.

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Key words: *Functional foods. Glucomannan. Obesity. Spirulina. Type 2 diabetes mellitus.*

Abbreviations

HOMA, homeostatic model assessment
QUICKI, quantitative insulin sensitivity check index
T2DM, type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic disease with an increasing prevalence all around the world¹. Its treatment is mainly focused on hypoglycemic drugs, which cause high costs in health systems², so new strategies in prevention such as healthy diets and exercise are strongly recommended³. Different foods such as dairy products or cereal fibre have been shown to be effective in regulating plasma glucose levels^{4,5}. In addition, emerging technologies in food processing have made it possible to add ingredients with known health benefits and/or remove potentially undesirable components to obtain functional foods, and to modify any properties to improve consumer satisfaction^{6,7}.

Glucomannan, a dietary fibre extracted from *Amorphophallus konjac* tubers, commonly consumed in Japan and Taiwan, forms highly viscous solutions when dissolved in water, due to its high water-holding capacity. This fibre is known to have satiating, laxative, and hypocholesterolemic properties but also to produce flatulence, abdominal pain, and esophageal or lower gastrointestinal obstruction at high doses⁸. In addition, our research team has found that its consumption improved plasma lipoprotein profile and antioxidant status in fa/fa rats⁹⁻¹¹. Vuksan *et al.*¹² reported that diabetic patients consuming glucomannan improved their glycemic control and peripheral insulin sensitivity in T2DM glycemia/insulinemia status.

Spirulina platensis is a microalga rich in minerals and antioxidant compounds such as carotenoids and phycocyanin¹³. Data in rodents point to its antidiabetic activity^{14,15}, and metabolic syndrome ameliorating¹⁶ but as yet there is no clear scientific evidence in humans¹⁷.

cemia e incrementar la adiponectina en tejido adiposo, sugiriendo para este tejido efectos antihipertróficos y sensibilizantes a la insulina. La inclusión de espirulina incrementó la disponibilidad de insulina. Aunque los resultados son prometedores, la conveniencia del consumo de surimis con glucomanano como parte de una dieta normal requiere futuros estudios.

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Palabras clave: *Alimentos funcionales. Diabetes mellitus tipo 2. Espirulina. Glucomanano. Obesidad.*

According to Campo-Deaño *et al.*¹⁸ squid-surimi is a healthy and safe food and may substitute for other conventional protein sources due to its low fat content and high protein quality. It has also been recommended as a suitable matrix to add different compounds in order to create potential functional foods. To the best of our knowledge, few studies have been performed to date investigating the effects of squid-surimi on T2DM^{6,19} and none at all with regard to those of glucomannan- or glucomannan *plus* spirulina-enriched surimi.

Zucker fa/fa rat results from a spontaneous mutation on chromosome 5, which encodes leptin receptors, decreasing its functionality. In this animal, hyperphagia and obesity appear from the first week of age along with increased growth of subcutaneous fat depot, as happens at the early stage of human T2DM. Those rats show moderated hyperglycemia, insulin resistance, mild glucose intolerance, hyperlipidemia, hyperinsulinemia, and moderate hypertension²⁰, making them a widely used model for diabetes, obesity and cardiovascular disease studies²¹.

Adiponectin is an anti-inflammatory and insulin-sensitizing adipokine. In sharp contrast to most adipokines, adiponectin expression and serum concentrations are not increased but reduced in a variety of obese and insulin-resistant states^{22,23}. Leptin is a pro-inflammatory adipokine and both leptin-deficient and leptin-resistant obese rodents exhibit severe insulin resistance²¹. This condition is rapidly ameliorated by leptin administration²⁴. To the best of our knowledge no information relating glucomannan consumption and adiponectin is available.

The hypothesis of the present study is that glucomannan-enriched and glucomannan *plus* spirulina-enriched squid-surimi act as functional foods by improving glycemia/insulinemia status and the leptin and adiponectin rate in plasma and adipose tissue. The present study, thus, aims to determine the effects of large amounts of glucomannan- or glucomannan *plus* spirulina-enriched squid-surimi in high saturated fat and hyperenergetic rat diets on a) fasting glucose, insulin levels, and on most commonly used indexes for insulin resistance (HOMA, QUICKI); and b) plasma and adipose tissue leptin and adiponectin levels and their respective ratio.

Material and methods

Diet preparation and experimental design

All experiments were performed in compliance with Directive 86/609/EEC of 24th November 1986 (modified by Directive 2003/65/CE of 22nd July 2003) for the protection of scientific research animals. The present study was approved by the Spanish Science and Technology Advisory Committee (project AGL 2008-04892-C03-02 and Consolider Ingenio 2010, CSD 2007-00016) and by an ethics committee of the Universidad Complutense of Madrid (Spain). A total of twenty-four male growing Zucker fa/fa rats with an initial body weight of approximately 120 g were obtained from Harlan Laboratories Models (Harlan, SL, Barcelona, Spain). The animals were housed individually in metabolic cells in a temperature-controlled room (22.3 ± 1.9 °C) with a 12 h light-12 h dark cycle. The rats were fed commercial rat pellets (Panlab, Barcelona, Spain) during a one-week adaptation period to environmental conditions and then distributed into three groups of eight animals each, according to their average body weight.

Three experimental semi-synthetic diets were prepared in a room under appropriate environmental conditions (4 °C and low enlightenment) to reduce changes in their antioxidant properties blending AIM-93M diets and surimi. Vitamin and mineral contents of AIN-93M diets were designed to cover requirements for rats once the whole diet was prepared (Table I). Control diet (C) was composed of a homogeneous mixture of 70% rodent diet (AIN-93M

#102634 purified rodent diet; Dyets, Inc., Bethlehem, PA, USA) and 30% freeze-dried restructured squid-surimi (with 15% microcrystalline cellulose); glucomannan diet (G) consisted of a mixture of AIN-93M #102635 feed (70 %) and freeze-dried, restructured glucomannan-enriched squid-surimi (30%, 15% of glucomannan into surimi) and glucomannan *plus* spirulina diet (GS) consisted of a mixture of AIN-93M #102635 feed (70%) and freeze-dried, restructured glucomannan *plus* spirulina-enriched squid-surimi (30%, 15% glucomannan into surimi and 3 g/kg diet of spirulina). Water and food were provided ad libitum over the 7-week experimental period. At the end of the experiment, in order to avoid inter-assay variations that could affect the comparison of data from the different groups, fasting rats were taken, one at a time from each of the six groups, anesthetized and euthanized by extracting blood from the descending aorta.

Fasting glucose and insulin levels, insulin resistance indexes

Fasting plasma glucose levels were determined by the hexokinase/glucose-6-phosphate dehydrogenase method, using a commercial kit according to the manufacturer's instructions (kit HK/G6P-DH #10127825001, Roche diagnostics, Mannheim, Germany) in a Hitachi 737 Automatic Analyzer (Hitachi Ltd., Tokyo, Japan). Insulin levels were determined in plasma using a rat insulin ELISA kit #10-1250-01 from Mercodia AB (Uppsala, Sweden). Then, following indexes were calculated using the *formulae*:

Table I
Composition (g/kg) of the experimental diets^a

	C diet	G diet	GS diet
Cornstarch	213.49	254.66	254.66
Microcrystalline cellulose	49.41	8.23	8.23
Squid-surimi	300.00	255.00	255.00
Glucomannan	0.00	45.00	45.00
Spirulina	0.00	0.00	3.00
Energy content (MJ/kg diet)	13.33	14.18	14.23

C: AIN-93M (70%) + Control squid-surimi (30%); G: AIN-93M (70%) + Glucomannan enriched squid-surimi (30%); GS: AIN-93M (70%) + Glucomannan *plus* spirulina enriched squid-surimi (30%). ^aOther ingredients (g/kg diet): casein, 105.0; palm olein, 115.29; dyetose (carbohydrate composition: monosaccharides, 10; disaccharides, 40; trisaccharides, 50; tetrasaccharides and higher, 900), 108.5; sucrose, 70.0; AIN-93M mineral mix, 29.75; AIN-93VX vitamin mixture, 12.16; choline bitartrate, 3.06; L-cystine, 1.26; t-butylhydroquinone, 0.02. Mineral mix #210050 24.5; Vitamin mix #310025, 162.07. AIN-93M mineral mix (g/kg): calcium carbonate, 357.00; potassium phosphate monobasic, 250.00; potassium citrate.H₂O, 28.00; sodium chloride, 74.00; potassium sulfate, 46.60; magnesium oxide, 24.00; ferric citrate U.S.P., 6.06; zinc carbonate, 1.65; manganous carbonate, 0.63; cupric carbonate, 0.30; potassium iodate, 0.01; sodium selenate, 0.01025; ammonium paramolybdate.4H₂O, 0.00795; sodium metasilicate.9H₂O, 1.45; chromium potassium sulfate.12H₂O, 0.275; lithium chloride, 0.0174; boric acid, 0.0815; sodium fluoride, 0.0635; nickel carbonate, 0.0318; ammonium vanadate, 0.0066; finely powdered sucrose, 209.806. AIN-93VX vitamin mixture (g/kg): niacin, 3.00; calcium pantothenate, 1.60; pyridoxine HCl, 0.70; thiamine HCl, 0.60; riboflavin, 0.60; folic acid, 0.20; biotin, 0.02; vitamin E acetate (500 IU/g), 15.00; vitamin B₁₂ (0.1 %), 2.50; vitamin A palmitate, (150000 µg/g), 0.80; vitamin D₃ (10000 µg/g), 0.25; vitamin K₁-dextrose mix (10 mg/g), 7.50; sucrose, 967.23.

HOMA-IR = fasting insulin (mUI/L) x fasting glucose (mmol/L) / 22.5

HOMA-IS = 1 / HOMA-IR

HOMA-%B = fasting insulin (mUI/L) x 20 / (fasting glucose (mmol/L) - 3.5)

HOMA-D = HOMA-IS x HOMA-%B

QUICKI = 1 / [log (fasting insulin (mUI/L)) + log (fasting glucose (mg/dL))]

Leptin and adiponectin levels

Leptin and adiponectin in plasma or white adipose tissue homogenates were determined by the ELISA method using the mouse/rat leptin kit #K1006-1 and mouse/rat adiponectin kit #K1002-1 of B-Bridge International, Inc. (Cupertino, California, USA).

Statistical analyses

Statistical analyses were performed using the SPSS version 19.0 statistical analysis package (SPSS, Inc., Chicago, IL, USA). Results were expressed as means and standard deviations. One way ANOVA followed by the Bonferroni test was used to assess the effect of the diet. Where variances were assumed to be unequal the T2 of Tamhane post hoc test was applied. Results were accepted as significant when $p < 0.05$.

Results and discussion

Fasting glucose and insulin levels, insulin resistance indexes

Table II shows detailed information about weight gain, daily food intake, glucose, insulin and homeo-

static assessment models (HOMA and QUICKI) for the study of insulin resistance or sensitivity.

As a characteristic of the Zucker fa/fa model, rats develop hyperphagia, obesity, glucose intolerance, hyperinsulinemia and insulin resistance at 11-12 weeks²¹. All these facts were found in C fa/fa rats. According to the standard cut-off points for diabetes, all G and GS rats presented severe hyperglycemia, although treatments reduced glycemia by 20% in comparison to the C rats ($p < 0.05$). Vuksan *et al.*¹² described glycemia reduction in diabetic patients consuming konjak flour. The most probable mode of action of glucomannan appears to be *via* retention of carbohydrates absorption through its sequestration into the high viscous gel generated or slowing gastric emptying, and thus, its bioavailability in gut²⁵.

As 6 mUI/L insulin has been proposed as the cut-off point for hyperinsulinemia^{26,27}, we can assume that all C rats were insulin-resistant. In contrast to other studies, glucomannan or spirulina did not significantly decrease insulinemia^{12,28,29}. Nonetheless, G and GS groups showed a non-significant tendency (17% and 13%, respectively; both $p > 0.1$) to decrease insulin levels with respect to the C one. In fact, 25% of G rats and 12.5% of GS showed insulinemia ≤ 6 mUI/L; suggesting that some rats ameliorated their insulin by the consumption of G and GS diets. Several factors can be implicated in those differences: length of experiments, age and animals models, type of diet. It should be emphasized that, in our case, growing fa/fa rats were used and these animals consumed hyperenergetic and high-saturated fat diets for relatively long time.

HOMA-IR is an index related to insulin resistance³⁰. As a consequence of the glucose and insulin levels observed, insulin resistance in Zucker fa/fa rats tended to ameliorate after feeding with G and GS diets with respect to the C one (29% and 24%, respectively; both $p > 0.1$).

Table II
Fasting glucose and insulin levels, and HOMA and QUICKI indexes- (n=8 per group).

	Control		Glucomannan		Glucomannan + Spirulina		ANOVA
	Mean	SD	Mean	SD	Mean	SD	
Intake (g/day)	34.18	2.55	33.55	2.80	34.22	2.35	0.135
Weight gain (g)	217.08 a	14.77	195.16 b	26.63	193.26 b	15.97	<0.001
Fasting glucose (mg/dL)	331.50 a	29.20	284.38 b	32.44	278.25 b	47.52	0.018
Insulin mUI/L	10.82	2.04	8.96	2.99	9.33	2.12	0.23
HOMA-IR	8.73	1.64	6.23	2.36	6.61	1.95	0.13
HOMA-IS	0.12	0.03	0.16	0.06	0.15	0.04	0.25
HOMA-%B	14.93	3.63	15.04	5.20	15.59	10.07	0.63
HOMA-D	1.73 a	0.42	2.25 ab	0.75	2.43 b	0.68	0.034
QUICKI	0.28	0.01	0.29	0.01	0.29	0.01	0.41

a, b: Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$).

Insulin sensitivity or β -cell function, according to HOMA-IS, QUICKI and HOMA-%B indexes did not change by experimental treatment; however, HOMA-D, increased significantly ($p < 0.05$) in GS *vs.* C rats. No data on rat HOMA-D was available on references, making the discussion of the result difficult; however, taking into account the meaning of the HOMA-D, an increased bioavailability of insulin in the added spirulina diet can be suggested.

Total fat, leptin and adiponectin levels

Table III shows adipose tissue weight, and plasma and adipose tissue leptin and adiponectin levels. The total amount of fat found in our experimental animals is consistent with other studies in Zucker diabetic rats^{31, 32}. Although there were non-significant changes among groups for the total fat amount and the fat index (gonadal + perirenal fat/body weight), major changes with regard to leptin and adiponectin levels were observed.

Data on both plasma adipocytokines suggest that C rats were obese, as low plasma levels of adiponectin and high on leptin has been found in obesity³³. A cross-talk between fat cells and adipose tissue macrophages seems to be a key driving force for the initiation and progress of inflammation in obesity. Some of the macrophages are “normal” residents of the adipose tissue but others are recruited presumably to serve originally as scavengers of dead macrophages and other “toxic lipid remnants”³⁴. Adipose hypertrophy leads to macrophage recruitment and elevated release of free fatty acids, worsening insulin resistance in mature adipocyte³⁵.

The increment of adiponectin in adipose tissue by G and GS diets (16 and 20-fold, respectively, both $p < 0.01$) is not easy to explain. However, as adiponectin

has been found to exert anti-inflammatory properties³⁶, it can be speculated that its increase could be linked to a decrease of adipocyte hypertrophy and, in turn, to inflammation of this tissue, even conditioning an adiponectin retention in adipose tissue, reducing plasmatonic levels. Future studies should be addressed to understand the adiponectin balance between plasma and adipose tissue.

In lean Zucker rats, leptin circulated at approximately 4ng/ml plasma whereas levels were elevated more than 6-fold in fa/fa rats³⁷. Plasma leptin, which is known to be directly related to adipose tissue mass³⁸, was lower (both $p < 0.05$) in G and GS (both around 40%) rats *vs.* C ones. As leptin has been found to exert negative effects on health^{39,40}, the decrease found in plasma following glucomannan-surimi diets should be interpreted as relevant. Leptin inside of adipose tissue was higher ($p < 0.05$) in GS *vs.* G. These differences seem related to the total fat content or fat index found in G *vs.* GS rats; however, other interpretations can be suggested⁴¹.

While the plasma adiponectin/leptin ratio did not change by the G and GS diets, in adipose tissue this ratio was increased at least 16-fold in G and GS rats *vs.* their C counterparts, reinforcing again the role of these functional ingredients on the adipose tissue hormone levels and its hypertrophy.

Conclusions

In summary, the inclusion of glucomannan and/or glucomannan *plus* spirulina surimis in a high saturated hyperenergetic diet did not affect food intake but reduced weight gain and improved glycemia. Results in adiponectin and the adiponectin/leptin ratio in adipose tissue are promising and suggest an anti-hypertrophic and insulin-sensitizing effect in this tissue. However,

Table III
Total fat, leptin and adiponectin levels. (n=8 per group).

	Control		Glucomannan		Glucomannan + Spirulina		ANOVA
	Mean	SD	Mean	SD	Mean	SD	
Total fat* (g)	21.00	2.12	18.41	3.17	20.61	3.29	0.184
Fat index (Total fat* / Final weight *100)	6.50	0.51	6.18	0.61	6.56	0.84	0.484
Plasma leptin (ng/mL)	10.88 a	0.69	7.76 b	1.58	7.84 b	1.72	0.002
Adipose tissue leptin (ng/mg protein)	7.05 ab	0.84	6.11 a	1.25	8.66 b	1.71	0.063
Plasma adiponectin (μ g/mL)	8.63 a	0.29	5.63 b	0.57	6.81 b	0.98	0.003
Adipose tissue adiponectin (ng/mg protein)	18.6 a	14.5	312.8 b	142.5	386.3 b	145.9	0.009
Plasma adiponectin/leptin ratio	796.3	59.2	725.4	85.5	812.9	174.6	0.546
Adipose tissue adiponectin/leptin ratio	2.76 a	2.25	54.00 b	40.70	44.89 b	17.43	0.043

a, b: Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$). *Gonadal *plus* perirenal fats.

more studies are needed to understand the results obtained and to ascertain the utility of consuming glucomannan-surimi as part of regular diets in individuals with obesity and/or metabolic syndrome.

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The authors declare that there are no conflicts of interest.

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