

### **Effects of substituting standard eggs for Columbus® eggs in the diet of Spanish postmenopausal female volunteers**

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### **Abstract**

The effect of substituting 0,65 standard eggs by 1,13 Columbus® eggs per day in the normal diet of 40 Spanish female volunteers, all in the postmenopausal phase of their life, was investigated. 80 female subjects were randomly separated into two groups. One group of 40 staying with their normal diet which included an average of 0,65 eggs/day. The other group of 40 subjects had a mean consumption of 1,13 Columbus® eggs per day within their normal daily diet. The study was carried out over a period of 8 weeks. At the end of the study no significant changes were observed in the control group. However it was seen that the group which consumed Columbus® eggs showed a significant drop of 9,6 % in the serum triglycerides ( $p < 0,05$ ) and an almost 20% decrease in the insulin ( $p < 0,001$ ) value. Total cholesterol, HDL and LDL concentrations showed no significant changes. Furthermore, within the Columbus® eggs consuming group, there was noted an almost 5% increase in the mean glycosilated homoglobine ( $p < 0,01$ ) value and a significant decrease in the systolic blood pressure ( $p < 0,01$ ). Finally, a significant weight loss ( $p < 0,01$ ) was recorded in the Columbus® egg group. Together the participants of this group were able to decrease their cardiovascular risk according to Frammingham a 3 %. These results indicate that substituting standard eggs by Columbus® eggs in the diet of postmenopausal women, may help to reduce the most common cardiovascular risk factors found in this important population group, such as hypertriglycerolemia, insulin (diabetes Type II), high blood pressure and over-weight problems.

### **Introduction**

Recent information coming from an international expert committee of the WHO underlines the fact that middle aged women have a special high risk of pathologic heart disease (Leidy, 2001). Even though Spain is a privileged country regarding the incidence of cardiovascular disease, the mortality figures of women between 50 and 70 years due to fatal heart attacks are steadily increasing, being today the number one cause of death in this population group (Grande Covián, 1988).

To the typical cardiovascular risk factors like hypercholesterolemia, high blood pressure and tobacco consume (Arca et al., 1994), there are additional risk factors for the group of postmenopausal women. These are based on the hormonal and metabolic changes in their bodies during this phase of their life.

With the end of the menstruation there is a significant change in the blood lipoprotein composition (Crook, 1999). The concentration of the HDL goes down, whereas the LDL level tends to go up (Knapp & FitzGerald, 1989). This change in the cholesterol composition of postmenopausal women is independent of age, tobacco, blood pressure and body weight (Struk-Ware, 1990)



On the other hand it is well known that body weight, glucose intolerance and insulin levels increases in the postmenopausal phase of women making them even more sensitive towards cardiovascular disease.

Insulin is a strong stimulator of endothelial cell growth regulating the activity of the LDL-receptors. Hence, a reduction of the insulin concentration could be an important tool to control one of the mechanisms involved in cardiovascular disease (Plosker & Faulds, 1999).

Many women experience an increase in systolic and diastolic blood pressure when coming to this phase of their life. It is not clear if this is due to the aging of the body or if it is an effect of the menopause (De la Gándara, 1977; Avis, 1991). A possible correlation between an increased blood pressure and the frequent occurring headaches in postmenopausal women was discussed by Banegas (Banegas et al., 1993) and García-Fantini (García-Fantini et al., 1999).

Finally the steady loss of bone substance which also starts at an age of 40 to 45 years is also a very important issue for this population group. It is well known that in order to prevent or reduce the impact of this loss conducted to osteoporosis morbidity a daily supplement of calcium is necessary. However, it is also known that our body needs sufficient vitamin D to be able to incorporate the calcium of our diet into our bone structure. Vitamin D is mainly found in animal products (coleciferol), however in very small quantities. Milk, egg yolk and liver are typical sources of vitamin D. Milk and milk products are a very good calcium sources but only contains 15 – 30 IU / litre (De Luca, 1988). On the other hand one egg yolk already contains 25 IU vitamin D.

Seeing the egg as a source of high quality protein, at a low cost and one of the best natural sources of vitamin D, this study wanted to examine the effect of substituting standard eggs by Columbus® eggs in the daily diet of Spanish postmenopausal women. Several studies in the past have shown that Columbus® have a positive effect on blood pressure (Suk, 1991), blood cholesterol composition, blood triglyceride levels (Jiang & Sim, 1993; Ferrier et al., 1992) and mother milk composition (Cherian & Sim, 1996). These positive effects were especially noted when the enriched eggs showed a balance of 1:1 between the two groups of polyunsaturated fatty acids Omega-6 and Omega-3.

To our knowledge, no study has so far been reported investigating the effects of Columbus® eggs in the diet of postmenopausal women.

## Subjects

80 Spanish female volunteers were recruited for this study. All subjects had to fulfil following conditions:

- age: between 45 and 60 years
- in the postmenopausal phase
- Born in Spain and all resident in the same village of Navalcarnero / Madrid
- without cardiovascular pathologies nor degenerative metabolic disorders
- no hormone treatment, nor other medication
- no restrictions to the diet

In order to assure a most homogeneous group, the 80 volunteers of this study not only were chosen from the same village but also belonged to the same physical-and women social club of Navalcarnero. In this way all participants of the study had a comparable social background and similar level of physical fitness. Regarding their social- economic situation, the volunteers represented the normal profile of a typical rural Spanish woman



between 45 and 60 years of age.

All subjects signed a consent form approved by the Autonomia University of Madrid (UAM) stating their volunteer participation in this study. No subject rejected from this study.

## Experimental Design

The 80 subjects were randomly divided in to two groups of 40 volunteers:

- GROUP 1: "Columbus Group"; subjects that included 1 to 2 Columbus® eggs per day in their normal diet. Effectively they consumed a mean of 1,13 eggs per day. Excluding all other sources of possible of standard egg intake.
- GROUP 2: "Control Group"; the subjects of this group simply had to keep on consuming their normal day-to-day diet which contained an average of 0,65 eggs per day. Initially it was foreseen that the Control Group also had to consume a comparable number of standard eggs per day as the Columbus Group. However, due to the delicate health situation of women in this age, where body weight, cholesterol, triglycerides and insulin values tend to rise and cause cardiovascular problems, it was decided on behalf of ethical reasons not to force the control group to ingest the same number of standard eggs as the Columbus Group.

The study was carried out for a period of 8 weeks, between February and April 2002, avoiding the impact of a not representative diet pattern during the Christmas and Easter vacation.

At the beginning and at the end of the study the subjects were asked to give a blood sample, their blood pressure was taken and their somatic data were recorded.

All subjects had to record on a weekly basis their type and quantity of food ingested, giving us all the necessary information regarding the diet of each subject participating in the study. This data pool enabled us to study habits, deficiencies, excesses, preferences, preferred way of preparation and the pattern of consumption of each subject. This was extremely important for the correct interpretation of the numerous data collected during this study. After analysing the daily diet records of all 80 participants of this study, it was calculated that the GROUP 1 (Columbus group) consumed a mean of 1,13 omega-3 eggs per day. The GROUP 2 (Control group) consumed an average of 0,65 eggs/day.

## Analysis

The determination of the somatic parameters was carried out according to the Protocol of the International Biological Program (Weiner & Lourie, 1983; Tejerizo et.al., 1999). Only anthropometrically homologated materials and tools were used for the measurements.

The blood samples were analysed by the laboratory of Dr. Vallejo / Madrid, which is certified according to ISO 9002, AEFA-AEBM and to the European Roche Quality Standard. The biochemical analyses were done by AAS Spectrometry (mg/dl) and the endocrinologic analysis of the insulin curve was determined according to the M.E.I.A.-Method (Mui/ml). The laboratory was not informed of the names of the participants nor to which group (Columbus- / Control Group) the subjects belonged to.



## Statistical Analysis

The statistical data processing in this study was done with a SPSS-Program. All data given in Tables 1 to 4 are shown as mean  $\pm$  SD, giving their minimum and maximum value recorded. In order to examine the significance of differences in means between the data collected before and after the 8 weeks of the study, the paired t-test was applied.

The exact control of the daily diet of each subject enabled the determination of the Key Nutrients consumed. The statistical evaluation of this data was carried out with the NUTRIX Program, optimized by our research team.

The cardiovascular risk of the subjects participating in this study was calculated using the equations of the Framingham-Study (Gordon et al., 1978; Wilson et al., 1998).

## The eggs

The Columbus<sup>®</sup> eggs used in this study were developed by BELOVO S.A. (Belgium) and kindly provided by S.A.T. CAMAR (Toledo/Spain). The egg contains 1.320 mg Omega-3 and 1.320 mg Omega-6 per 100-g. Each egg of 55-g (without shell) contains 725 mg of both Omega-3 and Omega-6 fatty acids.

## Results

Tables 1 to 4 show the results obtained during this study. From these tables can be seen that the only variances with a statistical significance were detected in Columbus Group. No significant change was observed in the investigated blood and anthropometric parameters before and after the study of the Control Group.

In Table 1 and 2 are the values of the blood analyses of the Group 1 (Columbus Group) and Group 2 (Control Group). In the Columbus Group there was a significant decrease observed in the triglyceride level ( $t = 1,85$ ;  $p < 0,05$ ) and in the insulin concentration ( $t = 3,50$ ;  $p < 0,001$ ). The concentration of glucosilated hemoglobine was significantly higher ( $t = -3,10$ ;  $p > 0,01$ ) after the study in the Columbus Group than at the beginning of the study. The average total cholesterol concentration in both groups is slightly higher after the 8 weeks of the study, however without any statistical significance.

In the Control Group (Table 2) we see that both HDL and LDL levels show higher values after the study. In the Columbus Group (Table 1) an increase in LDL and a slight decrease in HDL were detected. Nevertheless these variations had no statistical significance, so no conclusion can be taken from them.

The blood glucose concentration in both groups decreased slightly but again without significance.

Table 3 and 4 reflect the mean anthropometric data of the two groups before and after the study. Again there was no significant change recorded within the Control Group. However the Columbus Group showed a significant decrease in body weight ( $t = 2,67$ ;  $p < 0,01$ ) which explains also the slightly reduced mean Body-Mass-Index (B.M.I.) of the subjects. Furthermore a significant decrease in the systolic blood pressure was detected ( $t = 2,97$ ;  $p < 0,01$ ) whereas no change was found regarding the diastolic blood pressure.

The average waist-to-hip ratio of the volunteers in the Columbus Group slightly increased due to a significant increase ( $t = -2,87$   $p < 0,01$ ) of the waist perimeter and a significant



decrease ( $t = 4,04$ ;  $p < 0,001$ ) of the hip perimeter. Graphic 1 reflects the summary of the principal changes detected in the Columbus Group after the period of intake of the balanced (Omega-6:Omega-3 = 1:1) eggs.

## Discussion

Similar as in other studies reported in the literature (Jiang & Sim, 1993; Ferrier et al., 1992), we have not noted any significant increase of the total cholesterol after a daily consumption of 1,13 Columbus<sup>®</sup> eggs (approx. 8 eggs/week) for a period of 8 weeks. In a paper from Oh (Oh et al., 1991) it was also reported that after a consumption of 4 omega-3 enriched eggs per day for a period of 4 weeks, the total cholesterol concentration remained the same but in the same paper it was found that the LDL fraction decreased and the HDL cholesterol increased. This improvement of the total cholesterol composition could not be confirmed in this study. This may be explainable by the smaller number of omega-3 enriched eggs consumed by each subject in our study (1,13 eggs/day compared to 4 eggs/day) but certainly also by the fact, that women in the postmenopausal phase tend to note a general increase in the LDL cholesterol and a decrease in the HDL (Knapp & FitzGerald, 1995).

Regarding the serum triglyceride concentration, an almost 10% decrease was detected within the Columbus Group. This finding is coherent with other authors who studied the effects of including omega-3 enriched eggs to the diet (Oh et al., 1991; Jiang & Sim, 1993, Ferrier et al., 1992). For the in this study investigated population group, a 10 % decrease in the triglyceride value is of extreme importance, as this parameter normally tends to rise together with the cholesterol in postmenopausal (Knapp & FitzGerald, 1995).

In the Control Group and in the Columbus Group there was a slight but insignificant decrease recorded in the glucose concentration.

The insulin showed a 20 % decrease in the group of ladies that consumed a mean of 1,13 Columbus<sup>®</sup> eggs per day. In the Control Group no significant change was noted. To our knowledge this is the first study showing the positive effects of the consumption of omega-3 enriched eggs on the insulin concentration within the important population group of postmenopausal women. It is well known that glucose intolerance and insulin levels increase in this phase of life and represent an important cardiovascular risk. This study shows that a consumption of only 1,13 Columbus<sup>®</sup> egg (approx 820 mg Omega-3) per day can be an important tool to reduce the risk of diabetes and the cardiovascular diseases.

The glycosilated hemoglobine value which also frequently represents a problem for postmenopausal women was significantly higher (4,7 %) after the consumption of the omega-3 eggs. This could be explained by the observed reduction in circulating insulin.

From the mean anthropometric data in Tables 3 and 4 can be seen, that again there was no significant variance within the control group (GROUP 2). However a significant decrease (approx. 6%) in the systolic blood pressure was observed within the Columbus group (GROUP 1). No change in the diastolic blood pressure was detected. It is interesting to note, that all participants of the Columbus Group had a slightly too high systolic blood pressure before the study. After 8 weeks of consuming 1,13 omega-3 enriched eggs per day all subjects were within the range of a normal blood pressure. Our study confirms the paper of Oh (Oh et al., 1991) who also report in their paper of the fact that the omega-3 fatty acids seem to only actuate on the systolic blood pressure but do not seem to have any effect on the diastolic value.

The Body-Mass-Index (B.M.I.) in the Columbus Group is slightly lower after the 8 week



study (Table 3). This is due to a significant mean decrease (approx 1 %) in the body weight of the subjects in this group and rejects the common opinion that eggs make you fat. At least in the case of the here studied Columbus<sup>®</sup> eggs this is not the case. It is well known that highly unsaturated fatty acids (like the omega-3 fatty acids contained in the enriched eggs) are not so easily converted by our body into adipose tissue and that they generally increase our metabolic rate and energy production. They also are converted in series 3 prostaglandins which have a diuretic effect and help to eliminate excess water. Finally they increase our muscle tone which helps to burn off more calories. Thus, this study indicates that adding only 1,13 omega-3 egg, or approx. 1640 mg of polyunsaturated fatty acids (sum of Omega-3 and Omega-6) to a normal Spanish diet may help to control the general observed body weight problem often found in the population group of postmenopausal women between 45 and 60 years. Further studies will have to be made to verify this finding. Interesting is also the fact, that there was a significant shift in the fat tissue coming from the hip (hip perimeter  $p < 0,001$ ) and going to the waist (waist perimeter  $p < 0,01$ ). The individual cardiovascular risk factor was calculated according to the Framingham study (Gordon et al., 1978) for all 80 subjects of this study. The mean values and their standard deviation for the Columbus Group and the Control Group are given in Table 3 and 4. It can be seen that by excluding all standard egg sources and adding only 1,13 Columbus<sup>®</sup> eggs (or ca. 820 mg Omega-3) per day to an absolutely normal diet\* of a rural Spanish woman in her postmenopausal phase of her life, can lower her cardiovascular risk according to the Framingham equation an average of 3 %. The fact that the here studied ladies between 45 and 60 years represent a large part of the total Spanish population (ca. 35 %) and thus represent a large part of the public expenses for medical care and rehabilitation, justifies the need to carry out more studies in this field to confirm and expand the results of this paper. Using the egg as a natural vector for polyunsaturated fatty acids, especially the ones of the omega-3 family, but also as a vitamin D/E and as a protein source, could be ideal as an easy and economical way of prevention of many diseases related to the postmenopausal phase in the life of women.

\* the normal diet of the participants in this Spanish study is not to be misunderstood as the Mediterranean Diet, which unfortunately loses more and more influence in this country.

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Table 1. Blood analysis data of Group 1 (Columbus Group), before (1) and after (2) the 8 weeks of the study

Variable (n = 40♀) Group 1 (Columbus)	Mean value	± σ	Min	Max	t; p-value
Glucosa 1 (mg/dl)	91,84	14,44	67,0	142,0	n.s.
Glucosa 2 (mg/dl)	90,48	14,25	70,0	152,0	n.s.
Cholesterol total 1 ((mg/dl)	228,18	32,74	177,0	307,0	n.s.
Cholesterol total 2 (mg/dl)	230,16	34,01	154,0	302,0	n.s.
HDL-1 (mg%)	57,00	12,97	33,0	89,0	n.s.
HDL-2 (mg%)	55,00	12,81	31,0	85,0	n.s.
LDL-1 (mg%)	150,55	26,15	106,0	210,0	n.s.
LDL-2 (mg%)	156,25	31,38	94,0	235,0	n.s.
Triglycerides 1 (mg/dl)	103,02	55,91	30,0	270,0	t = 1,85
Triglycerides 2 (mg/dl)	94,21	63,85	22,0	349,0	p ≤ 0,05
Glycosilated Hemoglobine 1 (%)	5,25	0,49	5,0	7,0	t = -3,10
Glycosilated Hemoglobine-2 (%)	5,50	0,51	5,0	6,0	p ≤ 0,01
Insulin 1 (mUI/L)	10,37	4,60	3,08	26,43	t = 3,50
Insulin 2 (mUI/L)	8,32	4,53	3,37	31,94	p ≤ 0,001





Table 2. Blood analysis data of Group 2 (Control Group), before (1) and after (2) the 8 weeks of the study

Variable (n = 40♀) Group 2 (Control)	Mean Value	±σ	Min	Max	t; p-value
Glucosa 1(mg/dl)	90,81	8,69	79,0	114,0	n.s.
Glucosa 2 (mg/dl)	89,30	18,45	70,0	167,0	n.s.
Cholesterol total 1 (mg/dl)	218,84	32,10	155,0	282,0	n.s.
Cholesterol total 2 (mg/dl)	220,80	38,82	135,0	296,0	n.s.
HDL-1 (mg%)	55,03	11,51	33,0	83,0	n.s.
HDL-2 (mg%)	56,30	10,32	34,0	81,0	n.s.
LDL-1 (mg%)	145,36	30,79	86,0	223,0	n.s.
LDL-2 (mg%)	151,90	41,23	79,0	265,0	n.s.
Triglycerides 1 (mg/dl)	92,32	47,58	33,0	207,0	n.s.
Triglycerides 2 (mg/dl)	86,70	48,78	35,0	265,0	n.s.
Glycosilated Hemoglobine 1 (%)	5,23	0,43	5,0	6,0	n.s.
Glycosilated Hemoglobine-2 (%)	5,30	0,47	5,0	6,0	n.s.
Insulin 1 (mUI/L)	14,18	11,67	5,07	46,61	n.s.
Insulin 2 (mUI/L)	10,44	5,04	2,93	19,55	n.s.



Table 3. Mean anthropometric data from the 40 subjects of the Group 1 (Columbus Group); before (1) and after (2) the 8 weeks of the study

Variable (n = 40♀) Group 2 (Control)	Mean Value	± σ	Min	Max	t; p-value
Height (cm)	153,52	6,53	138,1	166,4	---
Weight (Kg) 1	72,39	10,42	56,0	105,0	t = 2,67
Weight (Kg) 2	71,78	10,17	54,5	103,5	p ≤ 0,01
B.M.I. 1	30,81	4,26	23,78	43,31	n.s.
B.M.I. 2	30,49	4,24	23,59	42,69	n.s.
Waist perimeter 1 (cm)	92,21	10,27	74,8	121,9	t = -2,87
Waist perimeter 2 (cm)	94,49	10,94	70,0	123,0	p ≤ 0,01
Hip perimeter 1 (cm)	108,63	9,94	91,6	137,4	t = 4,04
Hip perimeter 2 (cm)	105,38	9,09	90,0	135,0	p ≤ 0,001
Waist/Hip ratio 1	0,842	0,052	0,738	0,966	n.s.
Waist/Hip ratio 2	0,904	0,082	0,690	1,140	n.s.
Vital capacity 1 (l)	1,966	0,392	1,0	2,8	n.s.
Vital capacity 2 (l)	2,187	0,450	1,3	3,1	n.s.
Systolic blood pressure 1 (mm/Hg)	131,61	14,94	98,0	160,0	t = 2,97
Systolic blood pressure 2 (mm/Hg)	123,71	13,50	78,0	150,0	p ≤ 0,01
Diastolic blood pressure1(mm/Hg)	74,28	8,91	60,0	98,0	n.s.
Diastolic blood pressure2(mm/Hg)	74,22	6,92	60,0	86,0	n.s.
Framingham 1	8,41	6,63	1	32	n.s.
Framingham 2	8,17	7,12	1	32	n.s.



Table 4. Mean anthropometric data from the 40 subjects of the Group 2 (Control Group); before (1) and after (2) the 8 weeks of the study.

Variable (n = 40 ♀) Group 2 (Control)	Mean Value	± σ	Min	Max	t; p-value
Height (cm)	153,52	6,53	138,1	166,4	---
Weight (Kg) 1	69,63	14,31	49,5	100,0	n.s.
Weight (Kg) 2	70,82	14,32	49,0	102,0	n.s.
B.M.I. 1	28,55	5,33	21,14	39,06	n.s.
B.M.I. 2	29,08	5,48	22,10	40,35	n.s.
Waist perimeter 1(cm)	88,34	12,70	70,4	111,0	n.s.
Waist perimeter 2(cm)	89,75	12,81	71,0	109,0	n.s.
Hip perimeter 1(cm)	106,75	10,79	90,9	128,3	n.s.
Hip perimeter 2(cm)	105,16	11,34	85,0	128,0	n.s.
Waist/Hip ratio 1	0,825	0,051	0,728	0,919	n.s.
Waist/Hip ratio 2	0,844	0,068	0,720	0,962	n.s.
Vital capacity 1 (l)	1,966	0,392	1,0	2,8	n.s.
Vital capacity 2 (l)	2,187	0,450	1,3	3,1	n.s.
Systolic blood pressure 1 (mm/Hg)	118,29	13,79	90,0	140,0	n.s.
Systolic blood pressure 2 (mm/Hg)	120,58	12,04	102,0	142,0	n.s.
Diastolic blood pressure 1(mm/Hg)	73,29	7,20	55,0	85,0	n.s.
Diastolic blood pressure 2 (mm/Hg)	72,00	7,12	60,0	84,0	n.s.
Framingham 1	5,30	4,86	1	32	n.s.
Framingham 2	5,22	5,00	1	32	n.s.



*Figure 1. Mean changes detected in the Columbus Group after consuming 1.13 Omega-3 enriched eggs per day for a period of 8 weeks*

