Effect of rooster comb extract, rich in hyaluronic acid, on isokinetic parameters in adults with mild knee pain

Glòria Bernal[1,2], Rosa Mª Solà[1,2], Maria C. Casajuana[1,2], Laura Pérez-Merino[1,2], Jenny Faba[1,2], Roser González[1,2], Ana E. Astilleros[1,2], Montserrat Giralt[2]

[1]Hospital Universitari Sant Joan de Reus. Servei de Fisioteràpia, Rehabilitació i Logopèdia. [2]Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili. Tarragona.

Received: 28.02.2018
Accepted: 14.03.2018

Summary

Background: Knee osteoarthritis manifests itself in a first phase as a mild gonalgia and produces a decrease in muscle strength, which can be objectively assessed with isokinetic testing. In the treatment of knee osteoarthritis, should be considered the hyaluronic acid orally.
Objective: Assess the evolution of isokinetic parameters of muscle strength in the knee joint with mild gonalgia before and after the consumption of RCE, rich in hyaluronic acid, orally.
Methodology: Nutritional intervention trial, double-blind, randomized, controlled with placebo and in parallel with two treatment groups: the active group with a low-fat yogurt with 80 mg of roster comb extract (RCE) and the control group with a low-fat yogurt without RCE. The main variables of the study were the peak torque, total work and mean power of the isokinetic valuation at the speed of 180 º/seg and 240 º/seg by the movements of flexion and extension of the knee joint.
Results: After 12 weeks of consumption of yogurt, men of the active group, compared with those in the control group, obtained statistically significant differences (p <0.05) and clinical improvement (>10%) at the speed of 180º/seg in the movement extension in the PT variable (p=0.048) (19.33%), TT (p=0.020) (37.97%) and PM (p=0.029) (47.25%), and in the flexion movement in the variable PT (p=0.007) (25.41%), TT (p=0.014) (42.98%) and PM (p=0.022) (48.90%).
Conclusions: The intake of a low-fat yogurt with RCE rich in hyaluronic acid improves the muscle strength of the knee in men with mild gonalgia.

Key words:
Knee. Pain. Isokinetic. Hyaluronic acid.

Efecto del extracto de cresta de gallo, rico en ácido hialurónico, sobre los parámetros isocinéticos en personas con gonalgia leve

Resumen

Introducción: La artrosis de rodilla se manifiesta en una primera fase como una gonalgia leve y produce una disminución de la fuerza muscular, que puede ser valorada objetivamente con la prueba isocinética. Dentro de su tratamiento se debe considerar el ácido hialurónico por vía oral.
Objetivo: Valorar la evolución de los parámetros isocinéticos de fuerza muscular en la articulación de la rodilla con gonalgia leve antes y después del consumo de un extracto de cresta de gallo (ECG), rico en ácido hialurónico, por vía oral.
Metodología: Ensayo de intervención nutricional, doble ciego, aleatorizado, controlado con placebo y en paralelo con dos grupos de tratamiento: grupo activo con ingesta de un yogur bajo en grasa con 80 mg de ECG rico en ácido hialurónico y grupo control con ingesta de un yogur bajo en grasa sin ECG. Se valoraron los parámetros isocinéticos de pico torque (PT), trabajo total (TT) y potencia media (PM) a las velocidades de 180º/seg y 240º/seg para los movimientos de flexión y extensión de la rodilla.
Resultados: Después de 12 semanas del consumo del yogur, los hombres del grupo activo, en comparación con los del grupo control, obtuvieron diferencias estadísticamente significativas (p<0,05) y mejora clínica (>10%) a la velocidad de 180º/seg en el movimiento de extensión en la variable PT (p=0,048) (19,33%), TT (p=0,020) (37,97%) y PM (p=0,029) (47,25%), y en el movimiento de flexión en la variable PT (p=0,007) (25,41%), TT (p=0,014) (42,98%) y PM (p=0,022) (48,90%).
Conclusión: La ingesta de un yogur bajo en graso con extracto de cresta de gallo rico en ácido hialurónico mejora la fuerza muscular de la rodilla en hombres con gonalgia leve.

Palabras clave:
Gonalgia leve. Isocinéticos. Ácido hialurónico.

Special Award in Oral Communication at the 7th National Sports Medicine Conferences. Zaragoza, 24th - 25th November 2017

Correspondence: Gloria Bernal
E-mail: gloria.bernal@urv.cat

Introduction

Osteoarthritis (OA) of the knee is a major public health issue[3], as it causes chronic disability in older people[2]. There are no obvious injuries or associated anomalies in the initial phases, and it presents itself as mild gonalgia[3].

The stability of this joint depends largely on the ligaments and the power of the muscles that surround it, mainly the quadriceps and the hamstrings[4]. It is vital to understand the muscle function as objectively as possible, as alterations to the skeletal-muscle system are reflected in stability disorders[5]. Isokinetic devices enable us to assess muscle performance and provide quantitative, objective and documented data regarding muscle capacities[5-7]. With the isokinetic method, a movement is performed at a constant speed covering the entire range of movement (ROM) and with an adjustable resistance throughout the entire ROM of a joint[5,8,9]. The most cited measured parameter in literature – as the most reliable and easy to establish – is the peak torque (PT) [10], though there are others, such as total work (TW) and the average power (AP)[11,12].

PT corresponds to the maximum value of the Moment registered and is expressed in Newtons per metre (N m). TW is the total muscle strength to repeat with a greater work load and is expressed in Joules (J). AP represents the relationship between the work performed and the time needed to complete the duration of the test, and is expressed in Watts (W)[6].

Hyaluronic acid (HA) is a polysaccharide of high molecular weight formed of b-1.4- D-glucuronic acid and b-1.3-N-acetyl-D-glucosamine. It is the largest component of synovial liquid and of cartilage, responsible for their viscoelastic properties. Its unique viscoelasticity has led to its use in diverse biomedical applications, such as viscosupplementation in OA treatment[13]. Joint pain leads to restricted movement, and consequently a reduction of HA concentration, which worsens joint pain as it increases the friction on the surface of the cartilage. In the case of OA, it has been proven that HA concentration reduces drastically[14].

It has recently been shown that HA administered orally is absorbed and distributed to organs and joints[15]. This knowledge opens up the possibility of developing therapies with HA taken orally to treat joint pain. In a preliminary nutritional intervention study, low-fat yoghurts were administered, supplemented with Mobilee TM (natural rooster comb extract (RCE) rich in HA) for people with joint pain. The results showed improvements in muscle strength in the affected joint, as well as less pain[16].

The main aim of this study is to assess the evolution of the isokinetic parameters of muscle strength (PT, TW and AP) of the different muscle groups involved in a knee joint with mild gonalgia, before and after the oral consumption of RCE rich in HA compared to non-consumption. The secondary target is to study the influence of sex, age and body mass index on the isokinetic parameters assessed.

Material and method

Nutritional intervention double-blind randomised study, controlled with a placebo and parallel study comprising a control group and an active group.

The study demographic comprised participants (n=89) suffering from mild gonalgia, with a VAS score over 3 cm and below 5 cm, with a minimum evolution of 6 months. Some inclusion and exclusion criteria were established so the study participants could be selected (Figure 1). All the participant gave their written informed consent before starting the study, in accordance with the Helsinki Declaration (South Africa review from 2000) and the applicable Spanish regulation regarding nutritional intervention studies, biomedical research and personal data protection, and Good Clinical Practices (GCP) from the International Conference on Harmonisation (ICH).

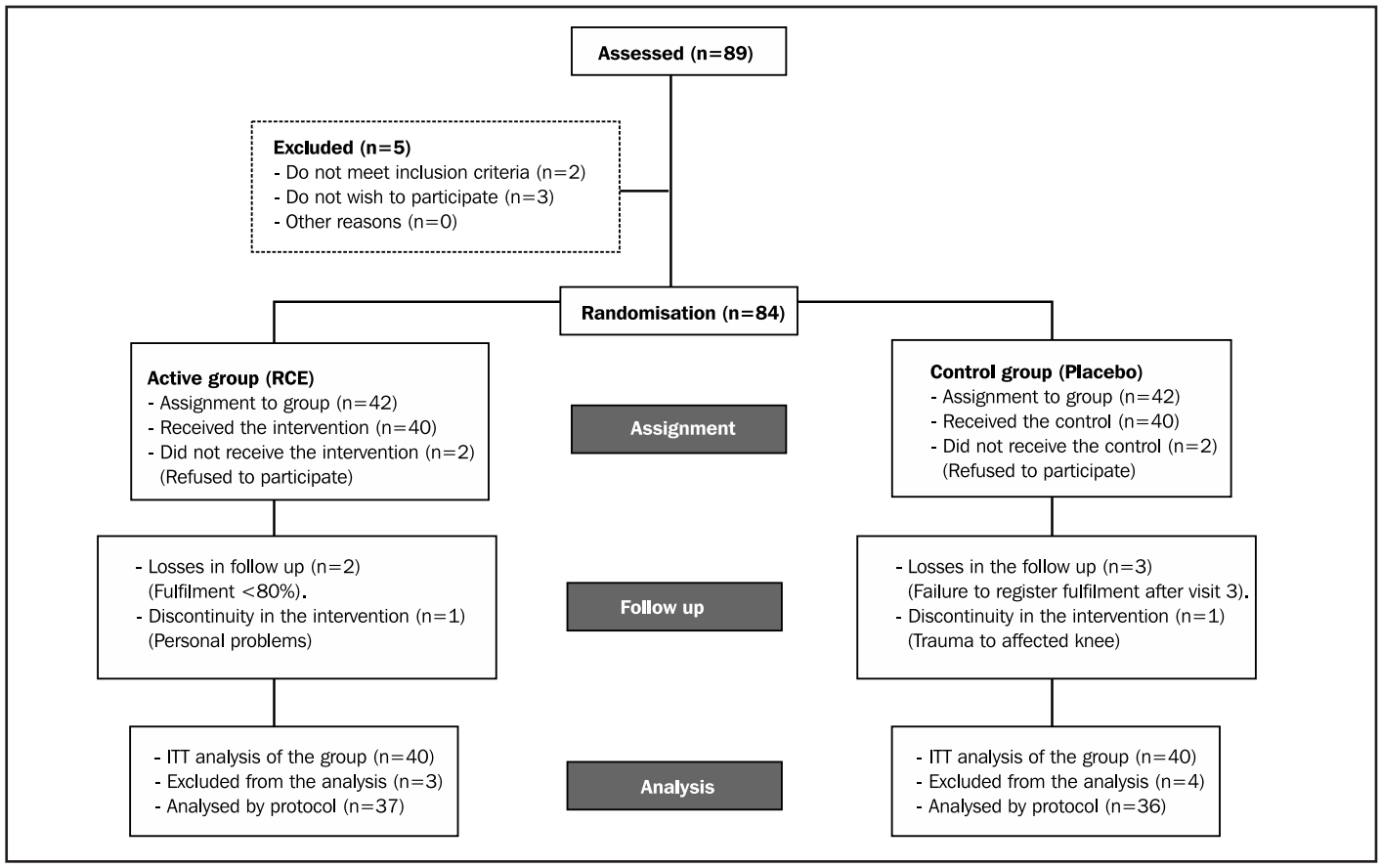
The study was conducted between September 2010 and March 2011 at the Sant Joan University Hospital (Reus, Spain).

The participants were randomly distributed into two treatment groups: one of the groups (RCE group) received a low-fat yoghurt (125 ml/day) with 80 mg of RCE added (Mobilee™; Bioibérica SA, Palafolls, Spain), whilst the other group (placebo group) received the same low-fat yoghurt without the added RCE. The treatment lasted for 12 weeks for both groups.

Figure 1. Study criteria.

Inclusion criteria
1. Adults aged between 20 and 70 years.
2. Subject that suffer from mild gonalgia (VAS value higher than 3 and lower than 5 for a minimum duration of 6 months).
3. Subjects that after understanding the protocol and the study process, provided their written informed consent to participate in the study.
4. Subjects considered to be in good general health according to their clinical history, physical examinations and available laboratory analyses.
Exclusion criteria
1. Subjects that require habitual treatment with paracetamol or other pharmaceutical drugs to control their joint pain.
2. Subjects that suffer from active rheumatoid arthritis and any inflammatory arthritic state that the researchers consider to be exclusionary.
3. Subjects undergoing oral corticosteroid treatment in the 4 weeks leading up to the selection.
4. Subjects undergoing intra-articular corticosteroid treatment in the study joint in the 3 months leading up to the selection.
5. Subjects with significant injury to the study joint in the 12 months leading up to the study (determined according to clinical history).
6. Subjects that are consuming medication or supplements for osteoarthritis at the time of selection.
7. Subjects that depend on medical prescription to control pain.
8. Subjects that are participating in any clinical trials or that have received a study product within the thirty days prior to the selection/inclusion in the study.
9. Subjects with allergies to dairy products.
10. Subjects following a hypocaloric diet for weight loss.
11. Pregnant or breastfeeding subject.
12. Subjects that take nutraceuticals containing HA or other muscle regeneration products.
13. Subjects that present axes alteration and inflammatory problems.

Figure 2. Flow chart.



Each 100 g low-fat yoghurt contained: 25.3% protein, 0.2% fat, 4.45% carbohydrates and 30 kcal of energy. The only difference between the products given to the RCE group and those given to the placebo group, was the RCE supplement (80 mg/ unit). The RCE group's yoghurt contained 65% HA.

A diet log was kept of 3 days before starting the study and at 12 weeks. Furthermore, participants were given a list of foods rich in mucopolysaccarides and/or in HA with instructions to avoid these elements, so as to anticipate any influence on measuring the research substance.

All the participants underwent an isokinetic assessment, which consisted in measuring their dynamic muscle strength with an isokinetic dynamometer (Biodex4Pro).

The muscle isokinetic test was implemented by physiotherapists at the Rehabilitation, Physiotherapy and Speech Therapy Service at the Sant Joan de Reus University Hospital. The first and final isokinetic test could be carried out up to 5 days before visit 2 (V2) of inclusion and visit 8 (V8) of finalisation, respectively. The main variables were those of the isokinetic test (PT, TW and AP), at two angular speeds, 180º and 240º/second, both in flexion (hamstring muscles) and in extension (quadriceps) of the knee. These variables were collected during V2 and V8. A standardised work protocol (SWP) was established, detailing the way that the isokinetic tests should be carried out. The isokinetic test consisted in performing one set of five repetitions at 180º/sec, and one set of five repetitions at 240º/sec, of the extension/flexion movement of the knee. A 2-minute break was given between sets.

As the main efficiency parameter, the evolution of the maximum strength was studied: the PT of the most affected joint in the extension movements at 240º/sec. As secondary efficiency parameters, the PT, TW and AP of both joints in extension and flexion at 180º/sec were studied, as well as the PT, the TW and the AP of both joints in flexion at 240º/sec, and the TW and AP of the two joints in extension at 240º/sec.

Prior to carrying out the study, the inter-evaluator and intra-evaluator reliability of the measurements performed with the isokinetic test were assessed. The results of this study reveal that the Biodex System 4 dynamometer has a reliability rating of good to very good in the assessment of the knee joint.

The flow chart (Figure 2) displays the selection and recruitment of the study demographic, the distribution of the participants in the control group with a placebo and the active group with RCE, and the reasons for excluding subjects.

All the data collected was introduced into a database created for the study. The descriptive results were expressed as average ± standard deviation (SD) or percentages, in accordance with the measurement value.

To compare the effects of the two products on the efficiency of the main variable, as well as on the main secondary efficiency variables, an analysis of covariance (ANCOVA) was performed, with the baseline value as the covariate. For the remaining efficiency variables, the hypothesis was tested using the exact Fisher test for the categorical variables, the Student *t* test for the continuous variables, and the Mann-Whitney U test for the ordinal variables. Based on the results of the previous trials, the efficiency of the main variables was tested in the sub-group of sex, participants > 50 years of age, and BMI. All the statistical tests were carried out using the SPSS package. A two-tailed significance trial of $p < 0.05$ was considered statistically significant.

Results

A descriptive analysis of the entire sample was performed, differentiating between the two treatment groups of the demographic by protocol (DP). The random distribution of the sample in the different treatments determined n=36 in the placebo treatment and n=37 in the RCE treatment. Table 1 displays the characteristics of each group in terms of the variables of age, weight, height, body mass index, sex and race. The two treatment groups were homogeneous in these characteristics, thus avoiding imbalances in the treatment groups.

The effectiveness of using RCE as a nutritional support was assessed according to the isokinetic variables in the two treatment groups. Table 2 displays the overall results of the isokinetic variables PT, TW and AP, in flexion and extension movement of the knee joint, at the speeds of 240º/sec and 180º/sec, in the two treatment groups, on the first visit with the baseline and the difference between the baseline and the final value, represented in the table in the "Changes at 12 weeks" after treatment column. No statistically significant differences were found at 12 weeks compared to baseline values in any of the two treatment groups, regardless of the movement and speed, nor in any of the isokinetic variables.

All the isokinetic variables for both treatment groups were assessed but the participants of each group were differentiated by sex.

Table 1. Demographic and baseline characteristics.

Variable	Placebo	RCE
Age; years	42.50±13.18	42.95±10.35
Weight; Kg	68.81±13.78	70.63±14.18
Height; cm	166.06±8.61	165.19±10.86
Body mass index; Kg/m^2	24.84±3.88	25.97±4.94
Sex; female, n (%)	21 (58.3%)	25 (67.6%)
male, n (%)	15 (41.7%)	12 (32.4%)
Race; Caucasian, n (%)	36 (100.0%)	37 (100.0%)

Table 2. Overall results of the isokinetic variables of each study product.

	Movement	Speed	Product	Baseline	Changes at 12 weeks	*p*
Peak torque (N m)	Extension	240º/sec	Placebo (n=36)	64.87 ± 32.85	7.60 [4.00 ; 12.16]$_a$	0.466
			RCE (n=37)	68.23 ± 32.14	5.40 [2.66 ; 11.41]$_a$	
		180º/sec	Placebo	72.86 ± 37.70	7.15 [4.45 ; 14.12]$_a$	0.631
			RCE	63.10 [60.94 ; 85.07]	6.60 [6.88 ; 19.91]$_a$	
	Flexion	240º/sec	Placebo	38.06 ± 19.48	4.55 [1.98 ; 8.79]$_a$	0.834
			RCE	33.40 [32.72 ; 48.67]	5.00 [0.11 ; 8.10]$_a$	
		180º/sec	Placebo	38.24 ± 20.44	5.30 [1.86 ; 8.74]	0.211
			RCE	38.34 ± 20.03	8.14 [5.13 ; 11.16]	
Total work (J)	**E**xtension	240º/sec	Placebo	283.85 [277.48 ; 416.18]	36.95 [7.76 ; 75.82]$_a$	0.408
			RCE	292.00 [271.87 ; 399.56]	47.20 [37.17 ; 116.41]$_a$	
		180º/sec	Placebo	370.75 ± 216.64	58.40 [31.81 ; 98.76]^a	0.289
			RCE	345.51 ± 187.22	66.60 [66.53 ; 161.73]$_a$	
	Flexión	240º/seg	Placebo	138,45 [118,50 ; 208,74]	33,73 [12,22 ; 55,24]	0,450
			ECG	171,84 ± 126,47	44,69 [24,84 ; 64,54]	
		180º/seg	Placebo	160,90 [126,01 ; 215,31]	46,15 [24,04 ; 68,94]$_a$	0,195
			In investigation	167,41 ± 122,88	51,10 [49,08 ; 100,34]$_a$	
Average power (W)	Extension	240º/sec	Placebo	127.08 ± 77.01	21.45 [10.77 ; 38.12]$_a$	0.667
			RCE	110.30 [99.76 ; 152.42]	19.50 [16.65 ; 51.4]$_a$	
		180º/sec	Placebo	109.73 ± 67.34	29.15 [20.12 ; 42.89]$_a$	0.938
			RCE	105.45 ± 64.99	22.30 [25.51 ; 59.27]$_a$	
	Flexion	240º/sec	Placebo	47.55 [41.46 ; 76.44]	16.78 [8.82 ; 24.73]	0.655
			RCE	61.54 ± 48.24	19.30 [11.08 ; 27.52]	
		180º/sec	Placebo	46.25 [37.10 ; 66.60]	17.65 [11.33 ; 26.17]$_a$	0.795
			RCE	51.85 ± 41.55	16.50 [15.64 ; 32.94]$_a$	

RCE: rooster comb extract. The sub-indexes "a" indicate not normal distribution.

Table 3. Results of the isokinetic variables of each study product by sex.

Sex	Movement	Speed	Product	Baseline	Changes at 12 weeks	*p*
Peak torque (N m)						
Male	Extension	240º/sec	Placebo (n=15)	86.05 ± 38.54	5.68 [-1.25 ; 12.61]	0.126
			RCE (n=12)	101.50 ± 30.36	14.63 [3.89 ; 25.37]	
		180º/sec	Placebo	94.69 ± 46.14	7.68 [0.23 ; 15.13]	0.095
			RCE	119.85 [86.17 ; 132.25]	21.87 [4.09 ; 39.64]	
	Flexion	240º/sec	Placebo	50.34 ± 21.36	4.60 [-1.32 ; 7.83]$_a$	0.300
			RCE	64.58 ± 23.17	7.65 [-8.57 ; 16.12]$_a$	
		180º/sec	Placebo	47.51 ± 25.26	6.66 [1.82 ; 11.50]	**0.039**
			RCE	58.70 ± 19.71	14.28 [8.34 ; 20.23]	
Female	Extension	240º/sec	Placebo (n=21)	49.74 ± 16.48	9.80 [4.46 ; 15.14]	**0.043**
			RCE (n=25)	52.26 ± 17.56	3.38 [-0.41 ; 7.18]	
		180º/sec	Placebo	57.27 ± 19.60	7.90 [3.56 ; 17.29]$_a$	0.683
			RCE	55.62 ± 19.79	5.90 [4.08 ; 14.57]$_a$	
	Flexion	240º/sec	Placebo	29.28 ± 12.27	6.91 [1.84 ; 11.97]	0.322
			RCE	29.23 ± 13.79	4.26 [1.56 ; 6.96]	
		180º/sec	Placebo	31.61 ± 13.19	4.33 [-0.76 ; 9.43]	0.756
			RCE	28.57 ± 10.82	5.20 [2.15 ; 8.25]	
Total work (J)						
Male	Extension	240º/sec	Placebo	463.93 ± 247.74	51.90 [-17.68 ; 108.64]$_a$	0.200
			RCE	523.43 ± 188.91	61.10 [44.65 ; 260.31]$_a$	
		180º/sec	Placebo	481.59 ± 271.43	80.10 [21.80 ; 138.40]	**0.053**
			RCE	579.100 [390.74 ; 645.49]	203.63 [73.73 ; 333.54]	
	Flexion	240º/sec	Placebo	242.63 ± 161.65	30.52 [-5.97 ; 67.01]	0.188
			RCE	300.63 ± 117.66	69.30 [16.45 ; 122.15]	
		180º/sec	Placebo	234.87 ± 166.13	64.63 [27.40 ; 101.86]	0.076
			RCE	277.14 ± 138.57	121.85 [62.34 ; 181.36]	
Female	Extension	240º/sec	Placebo	263.19 ± 113.69	39.16 [-3.00 ; 81.32]	0.955
			RCE	245.61 ± 112.10	40.46 [15.20 ; 65.71]	
		180º/sec	Placebo	291.58 ± 121.50	48.20 [11.31 ; 98.09]$_a$	0.421
			RCE	262.65 ± 110.03	55.80 [40.59 ; 101.75]$_a$	
	Flexion	240º/sec	Placebo	107.18 ± 69.31	36.02 [7.16 ; 64.89]	0.840
			RCE	110.02 ± 73.30	32.88 [15.86 ; 49.89]	
		180º/sec	Placebo	124.79 ± 76.51	33.54 [4.33 ; 62.74]	0.303
			RCE	114.74 ± 70.28	52.09 [28.75 ; 75.43]	
Average power (W)						
Male	Extension	240º/sec	Placebo	168.43 ± 93.52	26.07 [0.03 ; 52.11]	0.086
			RCE	106.75 [77.49 ; 136.79]	67.74 [20.85 ; 114.64]	
		180º/sec	Placebo	140.51 ± 86.16	38.14 [18.08 ; 58.20]	0.073
			RCE	161.33 ± 70.89	77.30 [32.51 ; 122.09]	
	Flexion	240º/sec	Placebo	87.56 ± 64.64	15.30 [0.56 ; 28.92]$_a$	0.200
			RCE	214.85 [151.18 ; 248.95]	27.60 [14.24 ; 53.99]$_a$	
		180º/sec	Placebo	71.43 ± 56.61	23.67 [10.95 ; 36.38]	0.108
			RCE	88,28 ± 47.01	41.45 [20.82 ; 62.08]	
Female	Extension	240º/sec	Placebo	97,54 ± 45.36	23.29 [6.84 ; 39.74]	0.564
			RCE	90,58 ± 50.90	17.86 [6.62 ; 29.09]	
		180º/sec	Placebo	87,74 ± 39.01	22.40 [12.33 ; 41.21]$_a$	0.501
			RCE	78,62 ± 41.48	21.60 [14.98 ; 36.27]$_a$	
	Flexion	240º/sec	Placebo	38,51 ± 26.42	18,23 [8.03 ; 28.43]	0.309
			RCE	39,66 ± 30.89	12.19 [5.04 ; 19.35]	
		180º/sec	Placebo	37,87 ± 24.29	13.20 [5.67 ; 24.81]$_a$	0.817
			RCE	34.37 ± 24.40	14.50 [8.80 ; 23.30]$_a$	

RCE: rooster comb extract. The sub-indexes "a" indicate not normal distribution.

Table 3 displays the PT, TW and AP results obtained by sex, at the first visit and the change after 12 weeks of treatment, in both the placebo and RCE treatment groups. In the group of males, statistically significant differences were found at the speed of 180º/sec in the PT variable in flexion movement, and in the TW variable in extension movement. In terms of the females, statistically significant differences were found in the PT variable in extension movement at the speed of 240º/sec. If we display and compare the results of % of clinical improvement (Figure 3), which considers the result to be functionally important if it is ≥ 10%, we can observe that the males from the RCE group – in comparison to the placebo group – obtain more than 10% in all the variables and the movements studied at the speed of 180º/sec, with these results

Figure 3. Percentage of clinical improvement in males.

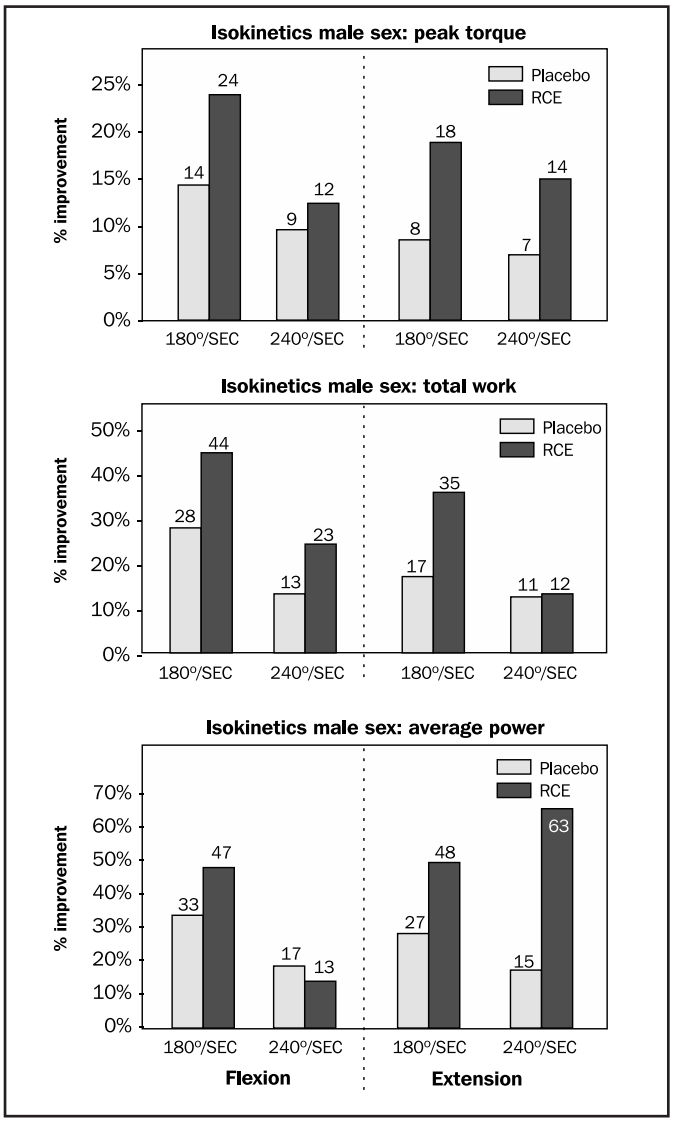
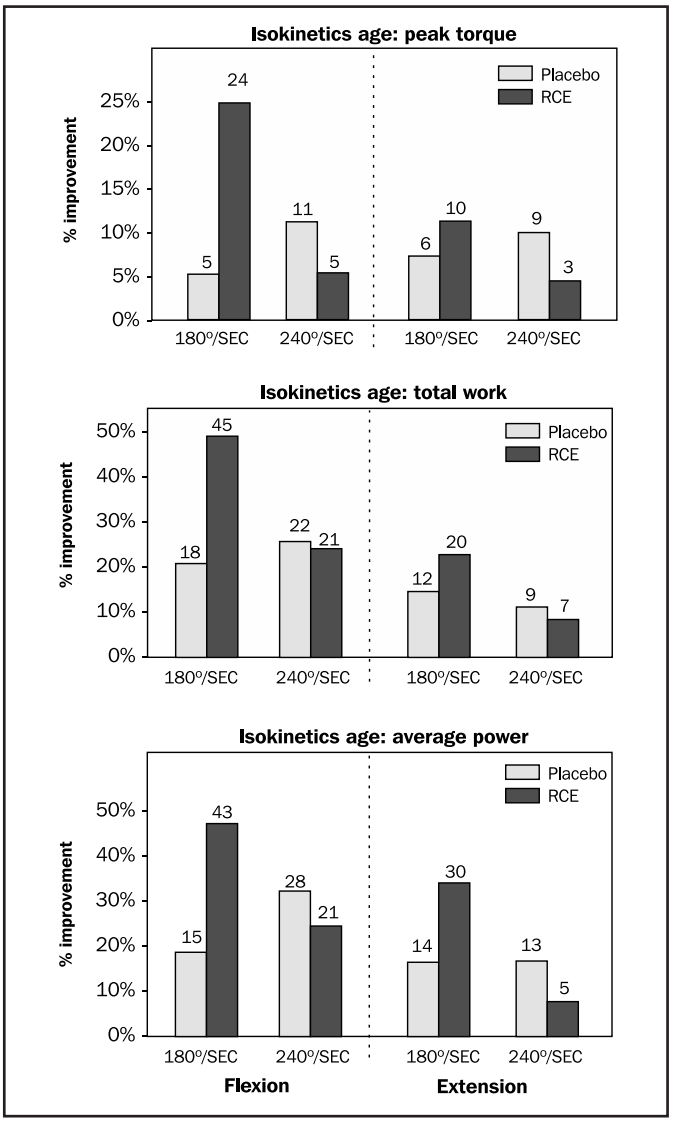


Figure 4. Percentage of clinical improvement in >50 years.



coinciding with statistical significance. At the speed of 240º/sec, there is also clinical improvement, but it is not ≥ 10% in all of them.

The same isokinetic variables were also assessed in the two treatment groups differentiated by age. Table 4 displays the results obtained from the PT, TW and AP variables differentiated by age (> 50 years and ≤ 50 years), at the first visit and the change after 12 weeks of treatment. Among the participants aged > 50 years we found statistically significant differences in the PT variable at the speed of 180º/sec and in the flexion movement. With regards to the participants aged ≤ 50 years, no statistically significant differences were found between groups in any of the variables, movements or speeds studied. When displaying the % of clinical improvement (Figure 4), we can see that those aged > 50 years in the RCE group, compared to the placebo group, obtained a 19% improvement, coinciding with the statistical significance of the difference variable ($p < 0.005$) in the PT in flexion movement at 180º/sec. We can also observe this clinical improvement of over 10% in the TW variable in flexion movement at 180º/sec, and in the AP variable at the same speed but in the two movements. However, for the participants aged ≤ 50 years, no clinical improvement was observed in any of the isokinetic variables.

After analysing the variables with the DP, we can see that the results revealing the most differences between the two treatment groups were those in which the demographics were separated by sex and at the speed of 180º/sec. For this reason we believe it would be advisable to carry out an analysis with the intention to treat (ITT) demographic, following the *Consolidated Standards of Reporting Trials* (CONSORT) regulations guide. Table 5 displays the results of the isokinetic variables in the ITT demographic, divided by sex and specifically at the speed of 180º/sec.

Table 4. Results of the isokinetic variables in each study product by age.

	Age	Movement	Speed	Product	Baseline	Changes at 12 weeks	*p*
Peak torque (N m)							
	> 50	Extension	240º/sec	Placebo (n=11)	56.54 ± 24.20	4.87 [-2.62 ; 12.36]	0.432
				RCE (n=12)	59.99 ± 26.05	1.89 [-2.05 ; 5.83]	
			180º/sec	Placebo	62.92 ± 26.49	3.56 [- 5.69 ; 12.81]	0.577
				RCE	63.83 ± 27.78	6.48 [-0.38 ; 13.33]	
		Flexion	240º/sec	Placebo	36.19 ± 18.50	3.80 [-2.40 ; 10.57]$_a$	0.786
				RCE	36.44 ± 16.98	1.70 [-1.93 ; 6.45]	
			180º/sec	Placebo	34.79 ± 15.27	1.85 [-2.54 ; 6.25]	**0.053**
				RCE	33.30 ± 15.29	7.88 [3.14 ; 12.63]	
	≤ 50	Extension	240º/sec	Placebo (n=25)	63.30 [53.74 ; 83.32]	9.50 [4.38 ; 14.61]	0.999
				RCE (n=25)	72.18 ± 34.48	9.50 [3.36 ; 15.64]	
			180º/sec	Placebo	77.24 ± 41.42	7.40 [6.01 ; 17.60]$_a$	0.587
				RCE	77.41 ± 39.35	7.00 [7.63 ; 25.80]$_a$	
		Flexion	240º/sec	Placebo	37.00 [30.53 ; 47.22]	5.70 [1.67 ; 10.24]	0.954
				RCE	33.40 [31.72 ; 53.76]	6.30 [-0.75 ; 10.72]$_a$	
			180º/sec	Placebo	40.40 [30.48 ; 49.02]	6.82 [2.20 ; 11.44]	0.629
				RCE	40.76 ± 21.81	8.27 [4.20 ; 12.33]	
Total work (J)							
	> 50	Extension	240º/sec	Placebo	305.14 ± 170.23	27.90 [-24.33 ; 83.11]$_a$	0.928
				RCE	301.23 ± 150.87	19.60 [0.29 ; 57.65]	
			180º/sec	Placebo	334.34 ± 159.77	40.50 [-32.43 ; 85.43]	0.347
				RCE	296.61 ± 134.31	58.65 [13.34 ; 117.73]$_a$	
		Flexion	240º/sec	Placebo	134.24 ± 115.46	29.91 [0.23 ; 59.59]	0.929
				RCE	149.69 ± 86.88	31.60 [2.75 ; 60.45]	
			180º/sec	Placebo	153.07 ± 104.24	27.50 [-24.17 ; 62.16]	0.134
				RCE	139.10 ± 93.64	62.45 [29.20 ; 111.16]$_a$	
	≤ 50	Extension	240º/sec	Placebo	285.90 [274.70 ; 455.65]	51.90 [2.06 ; 92.44]	0.290
				RCE	352.27 ± 209.01	54.70 [43.51 ; 155.98]$_a$	
			180º/sec	Placebo	386.77 ± 238.60	76.60 [40.92 ; 123.78]	0.399
				RCE	368.98 ± 206.25	74.90 [71.24 ; 203.68]$_a$	
		Flexion	240º/sec	Placebo	139.30 [118.45 ; 234.65]	35.41 [6.01 ; 64.81]	0.424
				RCE	182.47 ± 142.02	50.97 [24.07 ; 77.87]	
			180º/sec	Placebo	159.60 [119.06 ; 237.73]	49.90 [31.84 ; 85.34]	0.567
				RCE	181.00 ± 134.30	49.10 [42.50 ; 111.28]$_a$	
Average power (W)							
	> 50	Extension	240º/sec	Placebo	109.04 ± 63.50	14.70 [-6.85 ; 29.30]	0.928
				RCE	112.10 ± 65.18	5.40 [-4.46 ; 25.89]$_a$	
			180º/sec	Placebo	95.18 ± 45.76	13.08 [-5.24 ; 31.40]	0.279
				RCE	85.88 ± 51.19	25.81 [8.30 ; 43.32]	
		Flexion	240º/sec	Placebo	41.02 ± 42.73	11.57 [0.10 ; 23.05]	0.938
				RCE	52.07 ± 29.83	10.98 [-1.24 ; 23.19]	
			180º/sec	Placebo	44.67 ± 32.70	6.90 [-6.36 ; 22.88]	0.235
				RCE	41.63 ± 29.77	17.85 [7.72 ; 32.00]$_a$	
	≤ 50	Extension	240º/sec	Placebo	135.05 ± 82.18	26.10 [11.97 ; 48.56]	0.594
				RCE	132.80 ± 85.22	31.00 [21.11 ; 69.35]$_a$	
			180º/sec	Placebo	116.13 ± 74.84	38.80 [25.82 ; 53.41]	0.915
				RCE	114.84 ± 69.65	22.60 [26.66 ; 74.03]$_a$	
		Flexion	240º/sec	Placebo	48.20 [41.44 ; 86.96]	19.06 [8.41 ; 29.72]	0.568
				RCE	66.09 ± 54.93	23.30 [12.45 ; 34.15]	
			180º/sec	Placebo	51.20 [35.25 ; 74.77]	21.90 [14.79 ; 31.94]	0.734

RCE: rooster comb extract. The sub-indexes "a" indicate not normal distribution.

We have added a new column, which represents the change between the RCE group and the placebo group. There are statistically significant differences in all the isokinetic variables, both in the flexion and extension movement for males. No statistically significant differences were found for females. When displaying the % of clinical improvement in PT, TW and AP (Figure 5), it can be observed that the significant differences of the difference variable ($p < 0.005$) coincide with the clinical improvement of over 10% among the males, in the two movements at the

Table 5. Results of the isokinetic variables (PT, TW and AP) in each study product by sex and speed of 180º/second ITT demographic.

Sex	Movement	Speed	Product	Baseline	Changes at 12 weeks	Changes RCE *vs* placebo	p RCE *vs* placebo
Peak torque (N m)							
Male	Extension	180º/sec	Placebo	96.23±45.01	7.20 [0.20; 14.20]	16.14 [0.11; 32.17] (11.85%)	**0.048**
			RCE	112.86±37.28	21.82 [5.64; 38.01]		
	Flexion	180º/sec	Placebo	48.99±25.11	6.24 [1.66; 10.83]	10.21 [2.92; 17.50] (12.67%)	**0.007**
			RCE	60.89±19.58	15.47 [9.47; 21.47]		
Female	Extension	180º/sec	Placebo	55.10±19.21	10.43 [3.57; 17.30]	-1.37 [-8.84; 6.10] (-1.37%)	0.713
			RCE	55.17±19.54	9.69 [4.60; 14.77]		
	Flexion	180º/sec	Placebo	30.43±12.79	4.33 [-0.76; 9.43]	-0.065 [-5.29; 5.16] (4.27%)	0.980
			RCE	28.11±10.85	5.20 [2.28; 8.13]		
Total work (J)							
Male	Extension	180º/sec	Placebo	476.56±262.99	75.09 [19.86; 130.33]	139.1 [23.00; 255.1] (22.21%)	**0.020**
			RCE	539.76±195.15	204.94 [86.61; 323.26]		
	Flexion	180º/sec	Placebo	239.47±161.55	60.59 [24.92; 96.25]	74.53 [15.94; 133.1] (17.68%)	**0.014**
			RCE	294.33±135.03	126.49 [71.36; 181.62]		
Female	Extension	180º/sec	Placebo	278.51±119.02	54.70 [11.31; 98.09]	11.47 [-36.09; 59.03] (9.04%)	0.629
			RCE	260.01±108.65	74.56 [44.42; 104.69]		
	Flexion	180º/sec	Placebo	119.04±75.00	33.54 [4.33; 62.74]	15.03 [-19.26; 49.31] (17.51%)	0.381
			RCE	112.48±69.82	51.39 [28.96; 73.81]		
Average power (W)							
Male	Extension	180º/sec	Placebo	140.34±83.24	35.76 [16.43; 55.08]	46.32 [5.00; 87.64] (21.77%)	**0.029**
			RCE	167.76±67.95	79.26 [38.26; 120.27]		
	Flexion	180º/sec	Placebo	73.04±55.07	22.19 [9.96; 34.42]	25.56 [3.93; 47.19] (18.52%)	**0.022**
			RCE	92.18±44.52	45.08 [24.70; 65.46]		
Female	Extension	180º/sec	Placebo	83.35±38.27	26.77 [12.33; 41.21]	-2.67 [-18.87; 13.54] (2.02%)	0.741
			RCE	77.63±40.95	26.50 [16.14; 36.86]		
	Flexion	180º/sec	Placebo	35.83±23.76	15.24 [5.68; 24.81]	-0.17 [-11.30; 10.96] (4.60%)	0.976
			RCE	33.59±24.24	15.83 [8.86; 22.80]		

RCE: rooster comb extract. The sub-indexes "a" indicate not normal distribution.

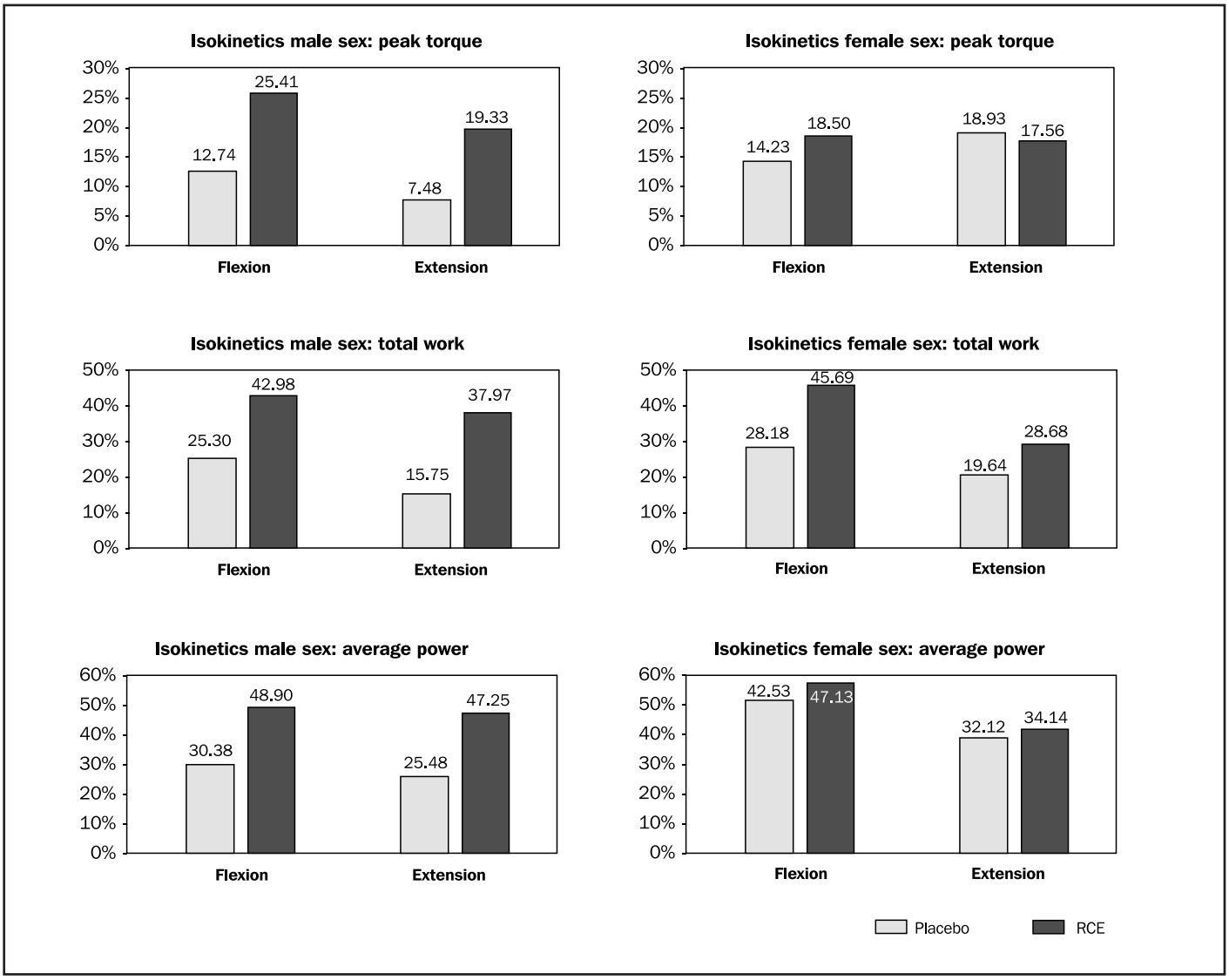
speed of 180º/sec. Regarding the females, no clinical improvement > 10% was found in any isokinetic variable, apart from in the TW variable in the flexion movement and at the speed of 180º/sec.

Discussion

These results suggest that consuming RCE may improve muscle strength deficiencies in knees affected by OA. The state of the muscles surrounding the joint, which give it stability, is very important in OA. The muscles involved are the quadriceps, responsible for the knee extension movement, and the hamstrings, responsible for the flexion movement of the knee[17]. Muscle strength varies by sex and age, and in a healthy population females have less muscle strength than males in all age groups. Male muscle strength diminishes progressively and in a linear fashion with age, whilst female muscle strength diminishes at around 41 years of age[18]. Kasai and co. observed differences in sex and age related to muscle composition and quality. Different studies have also suggested that the loss of ovarian function associated with the reduction of circulating concentrations of 17β-estradiol may be indirectly associated with the accelerated reduction of muscle strength following the menopause[19]. Changes in the sex hormones that affect muscular metabolism may explain why this response is better in males, however, more studies are required to explain the reasons behind these differences between sexes and also to provide more information about muscle functions[20].

The PT, TW and AP variables can be assessed at different angular speeds. In current literature, there is controversy regarding these speeds, yet the manufacturers of the devices suggest recommendations regarding the position and speed to be taken into account when performing the test[11]. In our study we have assessed an average speed (180º/sec) and a quick speed (240º/sec) based on the study by Martinez Puig[16]. The PT and TW allow us to see the strength of a muscle and how this strength can be maintained in a specific work time. That said, a muscle should not just have strength but also the ability to react quickly, which is what we can see with the AP variable. In the study, we can observe that after 12 weeks of consuming RCE-supplemented yoghurt, the isokinetic

Figure 5. Percentage of clinical improvement depending on sex and on the ITT population.



variables improve, and therefore the muscle capacities that will directly protect the joint so the knee can operate correctly.

From our knowledge, there is little information about the clinical importance of improvements to the isokinetic measurements of the knee. Knapik and Dauty suggest that when the comparison between two sets of isokinetic variable data is greater than 10%, it is generally considered to be functionally important[21,22]. In the ITT demographic divided by sex, this study confirms that low-fat yoghurt supplemented with RCE rich in HA (80 mg/d) and consumed for 12 weeks, can improve the muscle condition of knees affected with mild pain in men, in comparison to the baseline value of the same knee and at the speed of 180º/sec. Specifically, the clinical improvement is: 25.41% in the hamstring muscles and 19.33% in the quadriceps in the PT; 42.98% in the hamstrings and 37.97% in the quadriceps in the TW; and 48.90% in the hamstrings and 47.25% in the quadriceps in the AP. Overall it could be said that there is an improvement of at least 11% in males, compared to the control group at 180º/sec, with statistically significant differences obtained in all the isokinetic variables for males and at the speed of 180º/sec. Therefore, the improvement in muscle strength of the affected knee joint that was observed after the RCE treatment, could suggest its importance in clinical practice[23].

Regarding the methodology used, when the study analysis is performed we need to consider whether we are speaking of the ITT population or DP. As the CONSORT guide states in the description of an intervention study, the term "modified intention to treat" is very widely used to describe an analysis that excludes participants that do not adhere adequately to the protocol, in particular those that did not receive a minimum established amount of the treatment. However, the same guide suggests that an alternative terms is "by protocol", in favour of a clear description of exactly what was included in each analysis, those that adhered correctly

to the protocol, and in particular those that did not receive a minimum established amount of the treatmen[t24,25]. In view of this, the majority of our results and statistical analyses were performed on the DP population, despite also including a section that analyses a part of the ITT population.

In this study, the muscle assessments were performed using an isokinetic dynamometer, which is a precise method for assessing muscle activity[20]. Various literary articles highlight the importance of establishing the reliability and validity of these devices, as this way the precise assessment of muscle performance can be guaranteed[9]. Numerous studies have assessed the reliability and validity of isokinetic dynamometers in protocols of the different joints[26-29]. In our study, a regulatory work procedure (RWP) was created beforehand, in which each step of the test was specified, so that the physiotherapists that carried out the assessment could perform it in the same way. We performed a preliminary study to observe reliability and validity using the isokinetic test-retest on the knee joint. The results we obtained in this study revealed that the Biodex System $_4$ dynamometer is reliable for the intra-evaluator test, as well as for the inter-evaluator test on the isokinetic assessment of the knee joint.

Currently, muscle performance studies seem to be based on isokinetic assessment, though to interpret and describe the results more studies are required so a protocol assessment can be used as a reference[30]. In our study we contribute PT, TW and AP reference values of baseline isokinetic tests, for the pathology of mild gonalgia or early OA of the knee.

In terms of treatment with HA, Tashiro and co. performed a study on the effectiveness of the oral administration of HA. They observed 60 people with OA randomly divided into two groups. The first consumed HA (200 mg once a day) and the other was given a placebo for the duration of 12 months. The subjects from both groups performed quadriceps strengthening exercises each day. The HA group tended to improve and this improvement was clearer in subjects aged <70 years. The effect of oral HA on musculature was better in the second and fourth month after consumption in the relatively younger subjects[31].

Balogh and co. indicate that orally administered HA is absorbed and distributed ubiquitously in joints. Results from tests on rats and dogs indicate that orally-administered HA is absorbed and distributed in the skin, bones and synovial joints, including the knee joints, and remains there for prolonged periods of time[32].

Therapeutic effects of HA in patients with OA of the knee do not necessarily require HA absorption. A study by Asari *et al.,* states that a high molecular weight HA can attach to the Toll-like receptor $_4$ (TLR_4) in the intestinal epithelium and exercise biological activity without being absorbed; this union has been proven to increase the secretion of suppressor of cytokine signalling $_3$ ($SOCS_3$), which leads to the suppression of pro-inflammatory cytokines. This union also eliminates the expression of pleiotrophin, which contributes to the suppression of inflammation. The therapeutic effects of HA observed in this study could be the result of these mechanisms, with the HA that remains in the intestines without being absorbed[33].

Another possibility is that the therapeutic effect of HA is obtained via similar mechanisms to glucosamine (GlcN). GlcN is an agent that can modify the OA illness, though its therapeutic effectiveness and mechanism of action continue to be contested[34].

Souich observed the absorption, distribution and mechanism of action of symptomatic slow acting drugs for OA (SYSADOA). In his review he supports data on the oral absorption and corporal distribution of SYSADOA, and discusses its mechanism of action. SYSADOA are absorbed in the small intestine with a bio-availability that ranges from 5 to 45%, and they accumulate in the joint tissues. They comprise three natural components: HA, chondroitin sulphate (CS) and GlcN[35]. The mechanism of action of the HA and CS differ in various aspects from the GlcN. As they are large molecules, HA and CS do not penetrate chondrocytes, synoviocytes, osteoblasts, osteocytes or osteoclasts, and therefore cause an anti-inflammatory effect with the participation of membrane receptors (CD44, TLR4 and ICAM1), with a resulting double-effect: preventing these receptors from participating in the fragments of the extracellular matrix – the cause of inflammatory reaction – and blocking the signal transduction pathways by the fragments, therefore reducing the nuclear translocation of pro-inflammatory transcription factors. GlcN penetrates cells via glucose transporters. Its primary effect is linked to its O-GlcNAcylate protein capacity, and consequentially, modulates its activity, for example reducing the nuclear translocation of the NF-kB. GlcN can also affect the transcription of pro-inflammatory cytokines via epigenetic mechanisms. The characteristics of the mechanism of action support the use of CS combined with GlcN and suggest that HA and CS will be more effective in the initial phases of OA[36].

To conclude, it is possible to establish that the consumption of a nutritional support containing hyaluronic acid (RCE) can help muscle strength and knee functionality in a general population suffering from mild gonalgia, more specifically males, and it can prevent knee arthritis. This line of study should be continued so as to establish assessment protocols, action mechanisms and treatment options for this pathology. The confirmation of these findings in other groups of patients suffering from muscle-originating mild gonalgia could be of socio-economic value.

The European Commission has approved RCE as a new food ingredient (*European Food Safety Authority Journal* 2013).

Conflict of interests

The authors declare to have no conflicts of interest whatsoever.

Bibliography

1. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions. *Bull World Health Organ*. 2003;81(9):646-56.
2. Guccione AA, Felson DT, Anderson JJ, Anthony JM, Zhang Y, Wilson PW, *et al*. The effects of specific medical conditions on the functional limitations of elders in the Framingham Study. *Am J Public Health.* 1994;84(3):351-8.
3. Kon E, Filardo G, Drobnic M, Madry H, Jelic M, van Dijk N, *et al*. Non-surgical management of early knee osteoarthritis. *Knee Surg Sports Traumatol Arthrosc.* 2012;20(3):436-49.
4. Llorente F. Potenciación de la rodilla. En: *Potenciación muscular.* Jornadas Nacionales de Fisioterapia MAPFRE. Madrid: Ed. MAPFRE, S.A; 1988. p. 155-71.
5. Slocker A, Segovia JC. Valoración de la fuerza isocinética. En: Segovia JC, López FJ, Legido JC. *Manual de Valoración Funcional. Aspectos clínicos y fisiológicos*. 2ed. Madrid: Ed. Elseiver; 2008. p. 221-33.
6. Huesa F, Garcia J, Vargas J. Dinamometría Isocinética. En: Sánchez I, Ferrero A, Aguilar JJ, Climent JM, Conejero JA, Flórez MT, Peña A, Zambudio R. *Manual SERMEF de Rehabilitación y Medicina Fisica.* 2º ed. Madrid: Ed. Médica Panamericana; 2008. p. 83-8.
7. Jimenez J. Potenciación muscular con aparatós cinesiterápicos-isocinéticos. En: Fundación Mapfre. *Potenciación Muscular.* Madrid: Ed. Mapfre; 1989. p. 63-74.

8. Oman J. La Isocinética en la Rehabilitación. En: Prentice W. *Técnicas de Rehabilitación en la Medicina Deportiva.* 3ª ed. Barcelona: Ed. Paidotribo; 2001. p. 94-106.
9. Orri JU, Darden Gl. Technical Report: Reliability and validity of the isam 9000 isokinetic dynamometer. *JSCR*. 2008; 22(1):310-7.
10. Amorim M, Leme LE. Isokinetic dynamometry in elderly women undergoing total knee arthroplasty: a comparative study. *Clinics (Sao Paulo).* 2006;61(3):215-22.
11. Alqualo RE, Magalhaes LE, Hiroko SA, Jones AN, Natour JA. Isokinetic assessment of the hip muscles in patients with osteoarthritis of the knee. *Clinics.* 2010;65(12):1253-9.
12. Zawadzki J, Bober T, Siemieński A. Validity analysis of the Biodex System 3 dynamometer under static and isokinetic conditions. *Acta Bioeng Biomech*. 2010;12(4):25-32.
13. Fakhari A, Berkland C. Applications and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment. *Acta Biomater.* 2013; 9(7):7081-92.
14. Brandt KD, Block JA, Michalski JP, Moreland LW, Caldwell JR, Lavin PT. Efficacy and safety of intraarticular sodium hyaluronate in knee osteoarthritis. ORTHOVISC Study Group. *Clin Orthop Relat Res.* 2001;(385):130-43.
15. Balogh L, Polyak A, Mathe D, Kiraly R, Thuroczy J, Terez M, *et al.* Absorption, uptake and tissue affinity of high-molecular-weight hyaluronan after oral administration in rats and dogs. *J Agric Food Chem*. 2008;56(22):10582-93.
16. Martinez-Puig D, Moller I, Fernandez C, Chetrit C. Efficacy of oral administration of yoghurt supplemented with a preparation containing hyaluronic acid (MobileeTM) in adults with mild joint discomfort: a randomized, double-blind, placebo-controlled intervention study. *Med J Nutrition Metab.* 2013;6:63-8.
17. Hafez AR, Al-Johani AH, Zakaria AR, Al-Ahaideb A, Buragadda S, Melam GR, *et al.* Treatment of knee osteoarthritis in relation to hamstring and quadriceps strength. *J Phys Ther Sci.* 2013;25:1401–5.
18. Danneskiold-Samsøe B, Bartels EM, Bülow PM, Lund H, Stockmarr A, Holm CC, *et al.* Isokinetic and isometric muscle strength in a healthy population with special reference to age and gender. *Acta Physiol (Oxf).* 2009;197 Suppl:1-68.
19. Sirola J, Rikkonen T. Muscle performance after the menopause. *J Br Menopause Soc.* 2005;11(2):45-50.
20. Molczyk L, Thigpen LK, Eickhoff J, Goldgar D, Gallagher JC. Reliability of Testing the Knee Extensors and Flexors in Healthy Adult Women Using a Cybex II Isokinetic Dynamometer. *J Orthop Sports Phys Ther.* 1991;14:37-41.
21. Knapik JJ, Bauman CL, Jones BH, Harris JM, Vaughan L. Preseason strength and flexibility imbalances associated with athletic injuries in female collegiate athletes. *Am J Sports Med*. 1991;19:76-81.
22. Dauty M, Dupré M, Potiron-Josse M, Dubois Ch. Identification of mechanical consequences of jumper's knee by isokinetic concentric torque measurement in elite basketball players. *Isokinet Exerc Sci.* 2007;15:37-41.
23. Leung WC. Balancing statistical and clinical significance in evaluating treatment effects. *Postgrad Med J.* 2001;77:201-4.
24. Altman DG, Schulz KF, Moher D, Egger M, Davidoff F, Elbourne D, *et al.* The revised CONSORT statement for reporting randomized trials: explanation and elaboration. *Ann Intern Med.* 2001;134(8):663-94.
25. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, *et al.* CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *Int J Surg.* 2012;10(1):28-55.
26. Tunstall H, Mullineaux DR, Vernon T. Criterion validity of an isokinetic dynamometer to assess shoulder function in tennis players. *Sports Biomech.* 2005;4(1):101-11.
27. Aydoğ E, Aydoğ ST, Cakci A, Doral MN. Reliability of isokinetic ankle inversion- and eversion-strength measurement in neutral foot position, using the Biodex dynamometer. *Knee Surg Sports Traumatol Arthrosc*. 2004;12(5):478-81.
28. Meeteren JV, Roebroeck ME, Stam HJ. Test-retest reliability in isokinetic muscle strength measurements of the shoulder. *J Rehabil Med.* 2002;34(2):91-5.
29. Lund H, Søndergaard K, Zachariassen T, Christensen R, Bülow P, Henriksen M, *et al.* Learning effect of isokinetic measurements in healthy subjects, and reliability and comparability of Biodex and Lido dynamometers. *Clin Physiol Funct Imaging.* 2005;25(2):75-82.
30. Nerin MA, Montaño JA, Carrasco L, Martínez Romero JL. Evaluación isocinética de la musculatura flexoextensora de la rodilla en universitarios: estudio preliminar. *Rev S And Traum y Ort.* 2007;24-25:24-31.
31. Tashiro T, Seino S, Sato T, Matsuoka R, Masuda Y, Fukui N. Oral administration of polymer hyaluronic acid alleviates symptoms of knee osteoarthritis: a double-blind, placebo-controlled study over a 12-month period. *ScientificWorldJournal.* 2012;2012:167928.
32. Balogh L, Polyak A, Mathe D, Kiraly R, Thuroczy J, Terez M, *et al.* Absorption, uptake and tissue affinity of high-molecular-weight hyaluronan after oral administration in rats and dogs. *J Agric Food Chem.* 2008;56(22):10582-93.
33. Asari A, Kanemitsu T, Kurihara H. Oral administration of high molecular weight hyaluronan (900 kDa) controls immune system via Toll-like receptor 4 in the intestinal epithelium. *J Biol Chem*. 2010;285:24751–8.
34. Torrent A, Ruhí R, Theodosakis J, Blanco F. Comparison of the efficacy of two products sold as orally-adminsitered hylauronic acid supplements, ib0004 and id386 on the endogenous in vitro synthesis of hyaluronic acid by human synoviocytes. *Osteoarthr Cartil.* 2009;17:S277–8.
35. Zhang W, Nuki G, Moskowitz RW, Abramson S, Altman RD, Arden NK, *et al.* OARSI recommendations for the management of hip and knee osteoarthritis: part III: Changes in evidence following systematic cumulative update of research published through January 2009. *Osteoarthritis Cartilage.* 2010;18(4):476-99.
36. du Souich P. Absorption, distribution and mechanism of action of SYSADOAS. *Pharmacol Ther.* 2014;142(3):362-74.