IBERIAN CURED-HAM CONSUMPTION IMPROVES ENDOTHELIAL FUNCTION IN HEALTHY SUBJECTS

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Abstract: ***Objectives:*** Previous studies have shown that dietary components such as oleic acid or polyphenols exert beneficial effects on endothelium. We aimed to assess the impact of regular consumption of Iberian cured-ham (ICH) on endothelial function. *Design:* An open-label, randomized controlled parallel study. *Setting:* Volunteers recruited through advertisements at a hospital in Madrid, Spain. *Participants:* 102 Caucasian adults (76.8% females) aged 25–55 years, and free from cardiometabolic disease. *Intervention:* Participants were randomized to an ICH-enriched ad libitum diet or an ad libitum diet without ICH for 6 weeks. Subjects in ICH group were randomly provided with either acorn- or mixed-fed ICH, and followed up for an additional 6-week period under their usual diet. *Measurements:* Clinical parameters, biomarkers of endothelial function and oxidative stress, microvascular vasodilatory response to hyperemia and arterial stiffness were measured before and after the intervention. *Results:* After 6 weeks, a larger decrease in PAI-1 was observed in subjects consuming ICH compared to the Control group (-6.2±17.7 vs. 0.3±1.4 ng/ml; p=0.020). Similarly, microvascular vasodilatory response to hyperemia showed a significant increase (112.4±391.7 vs. -56.0±327.9%; p=0.007). However, neither oxidative stress, hemodynamic nor clinical parameters differed significantly over the study. Additionally, after stopping ICH consumption, improvements in PAI-1 remained for 6 additional weeks with respect to baseline (p=0.006). *Conclusion:* The present study demonstrates, for the first time, that regular consumption of ICH improves endothelial function in healthy adults. Strategies aimed to preserve or improve the endothelial function may have implications in vascular aging beyond the prevention of the atherothrombotic disease.

Key words: Iberian cured-ham, endothelial function, fibrinolysis, vascular aging, polyphenols**.**.

INTRODUCTION

The endothelium is a metabolically active organ playing a critical role in the regulation of vascular wall homeostasis (1). Loss of balance between endothelial-derived vasodilatory and vasoconstrictory factors is associated with progressive changes (2) including pro-inflammatory, pro-oxidant,

proliferative and prothrombotic status, as well as an abnormal modulation of vascular tone, all of which characterize the endothelial dysfunction (ED) (3). ED is recognized as a critical, early, modifiable step in the development and progression of atherosclerosis (4).

A large number of studies (5-8) have investigated the role of diet and dietary components on the endothelial function. These include the Mediterranean diet (MD), a traditional food pattern which has been associated with a lower incidence of cardiovascular disease (9). Constituents of MD such as virgin olive oil and nuts, both high in unsaturated fatty acids and phenolic compounds, have been demonstrated to favorably impact on oxidative stress and endothelial function (10-12).

Dry-cured ham is a traditional product based on preservation of pork food through salting and curing, with a strong presence in countries in the Mediterranean area since ancient time.

Iberian cured-ham (ICH) is produced from Iberian pigs, a free-range reared genotype native to South-western Spain and South-eastern Portugal. Nutritional characteristics of ICH depend mainly on pig feed, which can be based only on acorn and pasture ("acorn-fed") or also including compound feeds ("mixed-fed"). ICH is particularly rich in unsaturated fatty acids, especially in oleic acid (from 50% to 55% of its fat content in mixed- and acorn-fed types respectively), and polyphenols (mainly gallic and ellagic acid derivatives and quercetin) (13). Only a few studies have attempted to assess the effects of regular consumption of dry-cured ham on atherogenic risk factors, reporting beneficial actions on lipid panel (14) and lipid peroxidation (15). More recently, consumption of dry-cured ham was not associated with a higher risk of cardiovascular disease, hypertension or weight gain (16).

The current study is therefore the first to assess the effects of regular consumption of ICH on endothelial function. Within the context of a regular dietary pattern in healthy adults, it would provide value in understanding how ICH affects early cardiovascular risk markers.

The primary aim of the current study was to assess whether regular consumption of ICH improves PAI-1, a circulating marker of endothelial function, compared with a control group following their usual diet. Secondary objectives were to identify its effects on microvascular endothelial function,

oxidative stress biomarkers and arterial stiffness. We also aimed to describe whether there was a lasting effect on endothelium after stopping the consumption of ICH.

Materials and methods

Experimental design

This study was an open-label, randomized controlled parallel study conducted at a cardiovascular risk unit of a university hospital in Madrid, Spain.

Participants were asked to stop the consumption of ICH, as well as to maintain their previous intake of foods high in oleic acid or polyphenols, such as crude virgin olive oil, green tea or red berries from one week before baseline, and thereafter for the duration of the study. At baseline, participants were randomized to either ICH group, consisting of an ICH-enriched ad libitum diet for 6 weeks, or Control group, following an ad libitum diet without ICH. For subjects in ICH group, one of two possible types of ICH, acorn-fed or mixed-fed, were randomly assigned and provided as daily servings (50g). They were also counseled by a nutritionist about strategies for equivalently substituting calories from ICH in their regular diet. All participants were advised to follow their otherwise usual dietary and physical activity patterns throughout the study. Within 3 weeks after the randomization, participants were contacted to ensure compliance with the study protocol. After the 6-week intervention period, subjects in ICH group were followed up for an additional 6-week period under their usual diet, without ICH. Study measurements were made at baseline and after 6 weeks for all participants, and after 12 weeks only for ICH group.

Subjects

A total of 142 healthy volunteers, free from cardiometabolic disease, were recruited through advertisements at Ramon y Cajal University Hospital in Madrid, Spain. Participants were required to be Caucasian adults aged 25–55 years with a body mass index (BMI) between 18.5 and 30 kg/m2, willing to daily consume ICH and to comply with study protocol, and able to provide written informed consent. Participants were excluded if they had been diagnosed with atherosclerotic vascular disease, diabetes, hypertension, dyslipidemia, metabolic syndrome (17), hyperferritinemia, or having at least

one of the following risk factors: HDL-cholesterol ≤35 mg/dL, LDL-cholesterol ≥130 mg/dL, triglycerides ≥150 mg/dL, hemoglobin A1c ≥5,7%, fasting plasma glucose ≥100 mg/dL. Moderate to heavy smokers (>10 cigarettes/day) were also excluded. Additionally, any other condition that could interfere with study participation, such as pregnancy, alcohol or drug abuse, mental disorders, anemia, kidney, pulmonary or liver disease, was excluded. Of the 142 respondents, 38 failed to complain with incursion/exclusion criteria: 29 lipid profile, 8 BMI, 1 age. Two participants withdrew before randomization, leaving 102 to be randomized.

Clinical measurements

Height and body weight were measured while wearing light clothing and no shoes, and BMI (kg/m2) was calculated. Waist circumference was measured in the standing position, midway between the lowest rib and iliac crest, directly on the skin. Body fat mass percentage was measured using the bioelectrical impedance method (Omron BF300). Systolic (SBP) and diastolic (DBP) blood pressure were measured in the sitting position after 5 min of rest, using an automated sphygmomanometer (Omron 705CP), the mean of three measurements was used. Medical history, 1st-degree family history of cardiovascular disease (parents or siblings <55 years in men and <65 in women), physical inactivity (<90 min/week of walking), smoking status and food frequency (18), were recorded at baseline.

Laboratory measurements

Overnight fasting blood samples and first-void urine samples were collected and processed. Concentrations of total cholesterol, triglycerides, HDL-C, LDL-C, blood glucose, uric acid and urine creatinine were analyzed in the certified local laboratory using standard procedures. Aliquots for biomarker assessment were stored at -80ºC until all participants completed the study. Biomarkers were measured using commercial cytokine enzyme-linked immunosorbent assays: Plasminogen activator inhibitor-1 (PAI-1) (ng/ml), Meranini; Thiobarbituric acid reactive substances (TBARS) (µM/L), Cayman; F2-isoprostanes (ng/ml), Northwest.

Microvascular endothelial function

Laser-Doppler flowmeter DRT4 (Moor Instruments, UK) was used to measure ischemic reactive hyperemia. With the subject lying in the supine position in a room with stable temperature (20-22°C), the laser probe was placed close to the wrist, distal from a blood pressure cuff placed above the elbow. Skin temperature was controlled using a probe heated to a thermoneutral temperature (33ºC). After a 5-min resting period, basal capillary flow (arbitrary perfusion units) was measured for 3 min. Thereafter, 3-min distal ischemia was induced by inflating the cuff to suprasystolic pressure. Subsequently, the cuff was deflated and the flow during reactive hyperemia was recorded for 3 min. Ischemic reactive hyperemia index (IRHi) was calculated as: 100 x (peak hyperemic flow – baseline flow) / (baseline flow), and expressed as a percentage.

Central pressure and arterial stiffness

Central blood pressure parameters were measured by applanation tonometry using the SphygmoCor system (AtCor Medical, Australia) over the radial artery, with the subject in the sitting position. The augmentation index (AIx@75), an indirect measure of arterial stiffness, was calculated as augmentation pressure divided by pulse pressure ×100 to give a percentage, and normalized to heart rate at 75 bpm (19).

Sample size The main outcome variable was the difference between baseline and after 6 weeks for PAI-1 (ng/ml). Based on previous data, a sample size of 52 subjects in each group was needed to have 80% power to detect a difference in PAI-1 levels of 5 units (SD: 9 units), using the Student's t-test for independent samples, considering a statistical significance value of 0.05. ***Analysis of total phenolic content*** 6 samples (2.5g) of each of both acorn- and mixed-fed ICH were assessed for total phenolic content, according to Folin- Ciocalteu method (20). Gallic acid was used as a standard and ellagic acid as a reference, since ellagitannins are located deeper in ICH. ***Ethics*** Protocol and consent form were approved by the Ramon y Cajal Hospital Clinical Research Ethics Committee (Madrid, Spain). Signed informed consent was obtained from all study participants before study enrolment. Those who completed the study received non-monetary compensation for their participation (a batch of ICH). ***Statistical analyses*** Statistical analyses were performed using SPSS 15 for Windows (SPSS, Inc., Chicago, IL, USA). Values were expressed as percentages or as mean ± standard deviation (SD).

Prior to hypothesis testing, data were examined for normality. Differences at baseline between groups were assessed by student t-test for independent samples. Changes within ICH after 12 weeks were tested according to t-test for paired samples. Primary and secondary outcomes at 6-weeks were tested using univariate analysis of variance, adjusting for baseline values. The alpha level of significance was $p<0.05$. **Results** ***Baseline characteristics*** A total of 102 participants started the study; however two participants withdrew from the ICH group (n=1 withdrew consent; n=1 became pregnant during the trial), leaving 100 participants to complete the study. One was excluded from data analysis due to incomplete biological samples. Finally the analyzed study population included 99 subjects (48 in the ICH group and 51 controls). Subjects from the ICH group were also randomized to consume either acorn-fed (n=26) or mixed-fed (n=22) ICH. The mean age was 40.2 ± 8.7 years, with a predominance of females (76.8%). Sedentary subjects constitute 13.1% of the study population, and 33.3% had 1st-degree family history of cardiovascular disease. 21.2% were current light smokers and 6.1% ex-smokers (>1 year). At baseline, there were no statistically significant differences between the control and the intervention (ICH) group in age (40.5 ± 8.9 versus 39.9 ± 8.5 years), gender distribution (82.4% vs. 70.8% females) or clinical characteristics (Table 1). In addition, the mean age of the women was also similar in both groups. ***Changes after 6 weeks*** When assessing the mean adjusted change in PAI-1 over 6 weeks, there was a diet effect (p=0.020). Those in the ICH group had a larger decrease in PAI-1 (-6.2 ± 17.7 ng/ml) compared to controls (0.3 ± 1.4 ng/ml) (Table 2). Similarly, subjects in the ICH group showed a significant increase (p=0.007) in the ischemic reactive hyperemia index (IRHi) (112.4 ± 391.7%), when compared to the Control group (-56.0 ± 327.9%). Otherwise, neither oxidative biomarkers nor central hemodynamic parameters differed significantly between both groups over the study. Similarly, none of the clinical variables reported in Table 1 showed significant differences between subjects in both groups over 6 weeks.

Secondary analyses: type of ICH and follow-up period With regard to the type of ICH, different behaviors were observed in the main endothelial outcomes between subjects eating acorn- and mixed-fed ICH (Figure 1). Similar trends were seen in PAI-1 after 6 weeks for both ICH (-7.7 ± 19.2 vs. -4.4 ± 16.1 ng/ml) despite of different baseline values, while only subjects who consumed acorn-fed ICH exhibited an improving trend according to IRHi (259.5 ± 358.6 vs. -43.8 ± 373.6%). After the

end of regular consumption of ICH, PAI-1 values remained significantly lower for 6 additional weeks with respect to baseline (15.8 ± 12.1 ng/ml at week 12, p=0.006). IRHi also showed an improving trend (522.3 ± 317.8% at w12), though this did not reach statistical significance (Figure 1).

Total phenolic content in ICH The analysis for total phenolic content in ICH showed that the acorn-fed samples were higher in polyphenols than the mixed-fed (1.43 ± 0.09 vs. 1.06 ± 0.20 μg/g ICH).

Discussion To the best of our knowledge, this is the first study to demonstrate that regular consumption of Iberian cured-ham improves endothelial function in healthy adults. One of the most important observations in this study involves a significant decrease in PAI-1 levels, the primary endpoint, after regular consumption of ICH. Plasminogen activator inhibitor-1 is an anti-fibrinolytic (21) peptide which, under physiological conditions, is produced by hepatocytes, adipose tissue and endothelium. In response to a variety of noxious stimuli, endothelial synthesis of PAI-1 increases markedly, leading to a hypofibrinolytic and prothrombotic state which characterizes the endothelial dysfunction (3). Beneficial effects of ICH on endothelial function, and particularly on PAI-1 levels, could be related to its high content in monounsaturated fatty acids (MUFA), mainly oleic acid (13). Previous studies using MUFA support findings of a fall in PAI-1 (22, 23). Moreover, Mediterranean diet (MD), an acid oleic-rich diet, has been also demonstrated to lower basal levels of PAI-1 (24). Interestingly, most of the fat content of genuine Mediterranean diet (MD) is derived from virgin olive oil, which, like ICH, also contains a range of non-fat micronutrients with a high biological potency, including phenols (12). Presence of phenolic compounds in virgin olive oil has been associated with antithrombotic properties (24) and improvements in endothelial function (10).

With regard to microvascular vasodilatory response to post-ischemic hyperemia, our results are in line with the currently available evidence supporting beneficial effects of regular consumption of several dietary compounds, as unsaturated fatty acids and polyphenols, on endothelium-dependent vasodilation (5-8). It should be noted however that almost all of these studies are based on a macrovascular measurement of endothelial function, flow-mediated dilatation (FMD) (25). Because of the different physiological role of conduit arteries and small vessels, important differences should be considered between micro- and macrovascular endothelial function, as both only show a weak

correlation with each other (26). Even so, Ruano et al. (10) documented short-term improvements in microvascular function during the postprandial state after the intake of virgin olive oil. It is worth noting that differences in endothelial function between genders have been reported (27). Even though a greater FMD in females may be accounted for by their smaller vessel size (28), in premenopausal women, HDL and estrogen have been related to endothelial nitric oxide synthase (eNOS) stimulation (29). In our study, despite a predominance of women, the results do not seem to be influenced by gender distribution, as both the percent of females and the mean age of the women were similar in the two groups. Thus, our biochemical (PAI-1) and hemodynamic (IRHi) findings jointly provide evidence of a role for ICH on vascular dysfunction in small vessels.

When both endothelial measurements (PAI-1 and IRHi) are shown separately according to the type of ICH, apparently different responses were observed. Improvements in PAI-1 were consistent with a slightly higher content of phenols and MUFA in acorn-fed ICH. Meanwhile, unexpectedly, only participants who consumed acorn-fed ICH seemed to improve IRHi, even though the study was not designed to find differences between both types of ICH. A comprehensive appraisal would be required to clarify this point, considering that vasodilation of the microvasculature involves not only nitric oxide, but also other vasodilatory factors such as prostaglandins (30).

The assessment of the effect of ICH on oxidative stress (OE) was also among our secondary objectives. Neither TBARS nor F2-isoprostane/creatinine ratio showed significant differences between groups after 6 weeks. Although there is much evidence supporting that OE contributes to atherogenesis (31), the association between consumption of antioxidant-rich foods and reduction in OE markers is less overt. Previously, a small study (15) conducted in older adults showed that including ICH in the diet reduced lipid peroxidation. However, our results are consistent with several other well-conducted studies that failed to demonstrate dietary effects on OE (32, 33). Self-regulatory mechanisms as cellular and circulating enzymes and antioxidants could be involved in this relationship (34), making specifically designed studies necessary. In addition, our study took place in a Mediterranean country where, on average, diet is high in fruits and vegetables, what could contribute to explain the discrepancies between findings regarding endothelial function and OE.

However, consumption of antioxidant-rich foods has shown to decrease OE in individuals with low dietary fruit and vegetable intake (35).

Although endothelial function is a determinant factor of large artery hemodynamics (36), in our study neither central blood pressure nor augmentation index significantly differed between groups. Arterial stiffness depends not only on endothelium, but also, even more importantly, on structural elements within the arterial wall as well as on vessel pressure. Thus, in our study, changes in constituents such as collagen and elastin might be limited due to a short period of intervention. Moreover, ICH is high in salt, estimated at 1200mg/100g (13), which could adversely impact on blood pressure. However, as blood pressure changes did not differ between both groups, the possibly expected hypertensive response might have been offset by a favorable action at vascular level (37).

Given ICH's fatty acid composition and energy profile (13), there is a common concern about whether ICH consumption leads to undesired weight gain. In our study, participants' anthropometric measures did not change significantly from baseline, considering that the inclusion of ICH in the diet – 50g/daily representing less than 200 kcal- was framed within the total caloric content. Moreover, Ruiz-Canela et al. (16), after 6 years of follow-up among a 13,293 initially healthy subjects, reported no evidence of any association between the consumption of dry-cured ham and weight gain. Moreover, in our study metabolic parameters including lipid profile also remain unaffected. A previous study (14) reported a favorable impact of ICH on LDL and HDL cholesterol. However, in that case an additional source of the oleic acid – olive oil- was included, which could explain these apparently discordant findings. It is also noteworthy that triglycerides and uric acid remained unaltered.

Furthermore, we observed that the improvement in endothelial function persisted for at least 6 weeks after completion of ICH consumption. Likewise it has been previously described that an "endothelial memory" exists following vascular stress (38, 39), our findings may suggest that this memory could conversely act as a sustained benefit after stopping the intervention. This fact would be in line with the metabolic legacy described, in the longer term, for statin therapy (40) and antidiabetic treatment (41). In this regard, it has been hypothesized that bioactive food components can influence epigenetic

phenomena, either by inhibiting or modifying enzymatic reactions involved in DNA methylation or histone alterations (42).

Overall, the improvement in endothelial function, as observed after regular ICH consumption, may have exciting implications in slowing down the process of aging beyond the prevention of the atherothrombotic disease, according to the modern vascular theory of aging (43). Current evidence (44) supports vascular oxidative stress and inflammation as the major mechanisms by which aging leads to age-associated endothelial dysfunction, even in the absence of clinical cardiovascular disease or its major risk factors (45). A number of interrelated processes underlie these mechanisms, such as increased bioactivity of endothelial vasoconstrictor and hypofibrinolytic molecules, activation of nuclear factor kappa B (NF-κB) signaling pathway and formation of advanced glycation end-products (AGEs) (46-50).

We acknowledge several potential limitations to be considered in our study. First, given the nature of the tested food, the use of an active or placebo comparator was not possible. Similarly, the compliance assessment was limited. Even though a prescribed standardized diet could have allowed us to control for dietary variables, we decided to interfere as little as possible in their life-style patterns throughout the study in order to strengthen the real-life applicability of our findings. Second, the results regarding the two types of ICH should be carefully examined, as the study was not designed to assess differences between both types, but rather to provide additional insights on the role of different sources and compositions. Finally, the study was performed solely in Spanish healthy subjects, mostly women, so it is uncertain whether our findings could be extrapolated to different populations. The longer-term effects of ICH on endothelial function in patients with cardiovascular disease also remain to be examined in future studies.

Conclusions

The present study demonstrates for the first time that regular consumption of Iberian cured-ham (ICH) improves endothelial function in healthy adults. These improvements in PAI-1 levels and microvascular response to hyperemia were independent of classical cardiovascular risk factors, and

seemed to remain for a 6-week period after stopping ICH consumption. Moreover, our study also indicates that ICH may be included in the diet without weight gain or increasing triglyceride levels at least in the short term.

Given the increasing numbers of older adults and associated health care burden, effective strategies are needed to preserve or improve the endothelial function. In this regard, our results would seem particularly compelling, even though further and longer-term studies are needed to clarify the mechanisms involved in the relationship between nutrients, epigenetics, ageing and endothelium.

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Ethical standard: The study complies with the current ethical standards for investigation involving human participants in Spain

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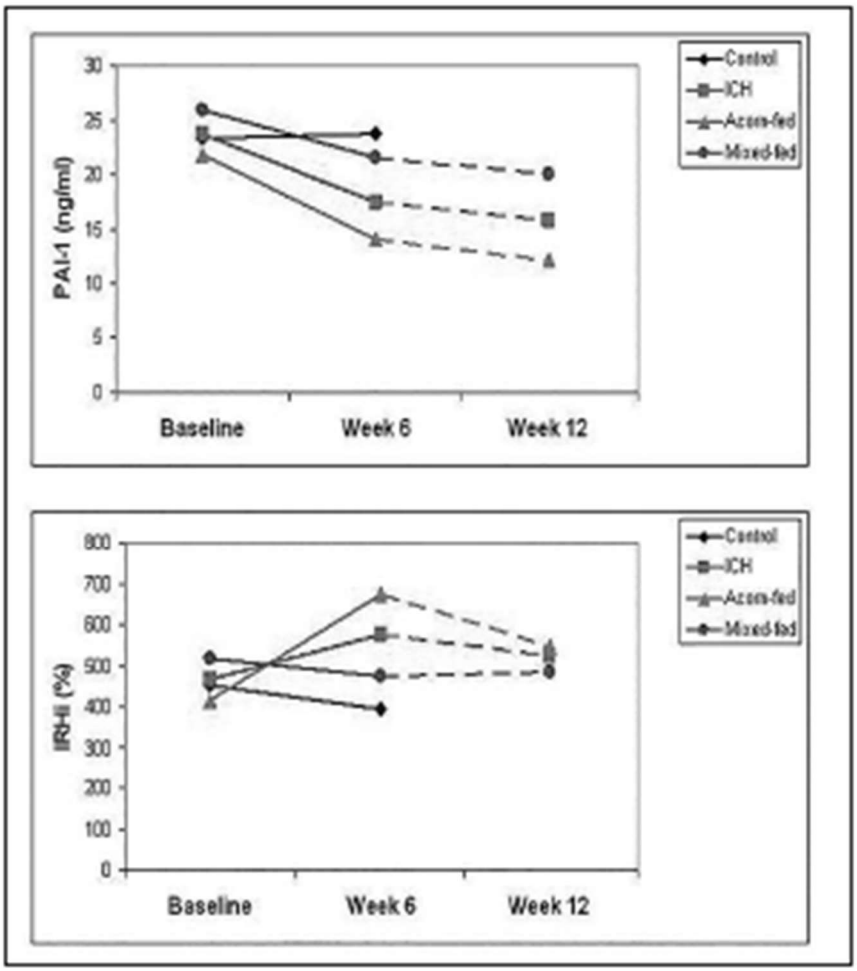
Figure 1

Behavior of the two endothelial outcomes (PAI-1 and IRHi) throughout the entire study period, including the follow-up period for ICH group. Results given as mean for the two main groups, and also separated within the ICH group according to the type of ICH (acorn- or mixed-fed). PAI-1, plasminogen activator inhibitor-1; ICH, Iberian cured ham; IRHi, ischemic reactive hyperemia index



TABLES

Table 1
Baseline and 6-week measurements amongst the study population

	Baseline		Week 6	
	Control	**ICH**	**Control**	**ICH**
Weight (kg)	65.7 ± 10.8	65.3 ± 12.1	65.4 ± 10.8	65 .1 ± 12.3
BMI (kg/m2)	23.8 ± 2.9	23.6 ± 3.2	23.6 ± 3.0	23.5 ± 3.2
Waist circumference (cm)	80.9 ± 9.3	83.7 ± 8.0	81.6 ± 8.3	82.5 ± 8.4
Body fat (%)	27.6 ± 6.3	27.4 ± 6.8	27.2 ± 6.9	26.3 ± 6.0
SBP (mmHg)	110.3 ± 11.4	112.7 ± 9.5	109.0 ± 9.9	109.0 ± 12.4
DBP (mmHg)	72.6 ± 8.4	75.3 ± 7.8	69.9 ± 7.9	70.7 ± 6.7
Total cholesterol (mg/dl)	176.4 ± 24. 9	183.1 ± 21.6	174.9 ± 23.2	179.9 ± 27.1
HDL-cholesterol (mg/dl)	57. 7 ± 12.2	56.9 ± 13.0	57.6 ±11.8	56.1 ± 12.9
LDL-cholesterol (mg/dl)	105.0 ± 18.9	110.9 ± 19.1	103.2 ± 19.7	106.8 ± 20.1
Triglycerides (mg/dl)	67.2 ± 27.5	74.7 ± 34.8	69.5 ± 25.0	73.2 ± 35.2
Fasting plasma glucose (mg/dl)	77.8 ± 8.5	76.4 ± 8.7	77.4 ± 7.8	76.0 ± 8.7
Uric acid (mg/dl)	4.5 ± 1.2	4.5 ± 1.1	4.5 ± 1.3	4.6 ± 1.2

Values are mean ± standard deviation. ICH, Iberian cured ham; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2
Main outcomes at baseline and week 6

	Group	Baseline	Week 6
Biomarkers			
PAI-1 (ng/ml)*	Control	23.3 ± 16.1	23.7 ± 15.8
	ICH	23.7 ± 18.7	17.5 ± 16.5
TBARS (μM)	Control	6.3 ± 3.4	6.7 ± 4.6
	ICH	6.3 ± 4.4	6.5 ± 3.6
F2-isoprostane/creatinine ratio (ng/mmol)	Control	134.6 ± 76.5	130.3 ± 78.4
	ICH	133.7 ± 76.2	151.7 ± 82.8
Microvascular endothelial function			
IRHi (%)†	Control	449.1 ± 244.0	393.1 ± 205.6
	ICH	463.9 ± 183.2	576.3 ± 355.6
Pulse wave analysis			
Central SBP (mmHg)	Control	102.9 ± 11.1	100.3 ± 11.2
	ICH	104.1 ± 9.7	98.2 ± 11.0
Central DBP (mmHg)	Control	73.9 ± 8.9	69.8 ± 8.9
	ICH	76.0 ± 8.5	69.6 ± 6.8
Augmentation Index at 75 bpm - AIx@75 (%)	Control	23.0 ± 13.6	23.5 ± 10.4
	ICH	24.5 ± 11.4	24.6 ± 15.2

Values are mean ± standard deviation. PAI-1, plasminogen activator inhibitor-1; ICH, Iberian cured ham; TBARS, thiobarbituric acid reactive substances; IRHi, ischemic reactive hyperemia index; SBP, systolic blood pressure; DBP, diastolic blood pressure. * $p \leq 0.05$, † $p \leq 0.01$ between groups (analyzed using univariate analysis of variance, adjusting for baseline values).