

Research Article

Effects of Consumption of Beef from Variably Fed Cattle on Anthropometric Measurements, Serum Parameters and Fatty Acid Composition in Healthy Men and Women¹⁻³

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Abstract

Background: Despite the large body of convincing evidence and the leaner beef cuts now available, controversy over the relationship between red meat consumption and coronary heart disease risk factors continues. Several reports have shown that, as compared with grain-fed beef, pasture-fed beef contains increased concentrations of β -carotene and α -tocopherol, high levels of long chain n-3 PUFAs, a more desirable n-6/n-3 ratio and increased levels of CLA all substances reported to have favourable biological effects on human health.

Objective: The objective of the present study was to investigate the effects of lean beef from three different cattle production systems, pasture-fed beef [P], pasture and grain supplemented-beef [M] and feedlot-beef [F] on anthropometric measurements, serum parameters and fatty acid composition in healthy men and women.

Design: Forty-eight apparently healthy subjects [24 men and 24 women], were randomly assigned to consume a balanced diet [from a catering service] each of the three beef types for four weeks. Each participant consumed each diet with a period of wash up of 21 days between the different diets. Three groups of 16 subjects [8 men and 8 women] rotated through the three experimental periods.

Results: In men systolic blood pressure was lower in P [109.8 vs 115.8 mmHg] than in F. The other anthropometric measurements were not affected by the beef type in either sex. However, the three diets caused significant changes from the baseline values of weight, systolic pressure, BMI and PM in both men and women. No significant differences were detected in the serum parameters and fatty acid proportion between the three diets. However, significant differences with the baseline values were found.

Conclusions: No significant differences were found in the different variables studied between the three experimental diets. However, all the diets significantly affected the baseline values of the variables studied which followed a similar trend but with variable intensity according to the beef diet and sex

INTRODUCTION

Argentina is a great beef consuming country [60kg/year IPCVA 2014]. Despite the large body of convincing evidence and the leaner beef cuts now available, controversy over the relationship between red meat consumption and coronary heart disease (CHD) risk factors continues and patients with hypercholesterolemia are often told to adopt diets in which chicken or fish replaces red meats because of the lower saturated fat content [1,2].

Although beef lipids are one of the few sources of dietary n-3 and n-6 highly polyunsaturated fatty acids (HPUFAs), they are considered an unhealthy component. Health related concerns about beef are due to its relatively high concentrations of hypercholesterolemic, saturated fatty acids [SFAs] and low concentration of hypocholesterolemic polyunsaturated fatty acids (PUFAs). Consumption of SFAs with 12-16 carbon atoms and cholesterol has been associated with increased serum low-density lipoprotein, a risk factor for (CHD) [3-5]. Recommendations for n-6 and n-3 classes of PUFAs are also important because scientists

recognize differences in the metabolism and physiological function between these fatty acids (Scientific Review Committee, 1990). According to the Department of Health (1994) the n-6/n-3 fatty acids ratio is an important index to evaluate the nutritional value of a fat.

Studies with isoenergetic low-fat diets with a high ratio of polyunsaturated to saturated fatty acids have shown that the replacement of lean beef with chicken causes similar decreases in plasma total and LDL cholesterol in hypercholesterolemic subjects [6,7]. Studies comparing the effects of red meat or fatty fish have suggested that the decrease in plasma total, VLDL, and LDL cholesterol is due to the lower levels of saturated fats in fish, and that the decrease in total VLDL and triacylglycerols is due to the higher levels of n-3 PUFAs [8,9]. Lean beef, poultry without skin and lean fish found in an American Heart Association diet with a high polyunsaturated to saturated fatty acid ratio and high fiber content induced numerous favourable changes in coronary artery disease risk factors in hypercholesterolemic men, regardless of the protein source [10].

A great number of studies have shown that grass-fed beef, as compared with grain-fed beef, contains increased concentrations of β -carotene and α -tocopherol, increased levels of n-3 PUFAs, a more desirable n-3/n-6 ratio and increased levels of conjugated linoleic acid [CLA], all substances reported to have favourable biological effects on human health [11-15]. Of particular interest are the long-chain n-3 eicosapentaenoic acid [C20:5n-3; EPA] and docosahexaenoic acid [C22:6 n-3; DHA]. Although their action is not fully understood, these fatty acids probably account for the inverse relation between fish consumption and the risk of developing CHI and stroke that has been observed in epidemiological studies [16]. Several researches have shown that grass systems have positive effects in the amounts of [CLA] in ruminant lipids [17]. CLA refers to a mixture of positional and geometric isomers of conjugated dienoic derivatives of linoleic acid. The main dietary sources of CLA for humans are beef and dairy products. There is a great interest in CLA because of its anticarcinogenic and antiatherogenic properties and its ability to reduce body fat while enhancing lean body mass [18-20]. Fatty acid composition in serum lipids reflects dietary fatty acids and can be used as a biomarker of fat quality, but also as an indicator of disease risk [21].

The objective of the present study was to investigate the effects of lean beef from three different cattle production systems, pasture-fed beef [P], pasture and grain supplemented-beef [M] and feedlot-beef [F] on anthropometric measurements, serum parameters and fatty acid composition in healthy men and women.

SUBJECTS AND METHODS

Subjects

This study was a randomized, controlled, dietary intervention trial conducted at the Instituto Cardiovascular de Buenos Aires, [ICBA] Argentina. Of the 200 subjects screened, 24 men [22-43 years] and 24 women [22-51 years] met all inclusion criteria: they were > 21 years had < 240mg/dl of cholesterol, had no major medical or psychiatric problems, and were judged to be

reliable in maintaining the dietary requirements of the protocol. All subjects provided written informed consent, and all clinical procedures were under control by the ICBA. The subjects were counselled to not eat or drink any additional foods or beverages other than those provided or described in the protocol.

During the first three weeks of the study, the subjects were under a self-selected diet. During the four week of the study, they were divided in three groups of 16 subjects each [8 men and 8 women] receiving the three diets adjusted to satisfy energy demands. The weights of the subjects were monitored during the study, and individual energy intake was adjusted to maintain their original weight. A balanced diet, from a catering service, was offered to the participants in their homes.

The diet included 150 g and 280 g of lean beef, respectively for women and men. The diets differed only in the source of beef: pasture-beef [P], pasture and grain-fed beef [M] or feedlot-fed beef [F] and were 1800 kcal for women and 2400 kcal for men in the first period and 2800 kcal for the other last periods. 21 days of wash up between diets was established. Representative data of the fatty acid composition of the three types of beef consumed in Argentina are presented in Table 1.

Clinical assessment

Weight [kg], waist circumference [cm] measured between the lowest rib and the iliac crest, blood systolic and diastolic pressure, body mass index [BMI], resting metabolic rate [RMR],

Table 1: Intramuscular fat and fatty acid composition of beef from the three systems (14). Mean \pm SD.

%	Pasture	Pasture + Corn	Feedlot
IMF	2.8 \pm 0.78 a	3.9 \pm 0.99 ab	4.3 \pm 0.71 b
C14:0	2.3 \pm 0.31	2.3 \pm 0.34	2.6 \pm 0.51
C14:1+C15:0	2.2 \pm 0.34 a	1.6 \pm 0.81 a	1.9 \pm 0.40 a
C16:0	23.9 \pm 1.91 a	23.5 \pm 1.77 a	20.9 \pm 2.07b
C16:1	3.1 \pm 0.32	3.3 \pm 0.40	3.8 \pm 0.46
C17:0	1.9 \pm 0.31 ab	1.7 \pm 0.52 a	2.5 \pm 0.33 b
C17:1	0.9 \pm 0.08 a	0.9 \pm 0.16 a	1.5 \pm 0.08 b
C18:0	13.7 \pm 0.98 a	11.9 \pm 1.39 bc	10.5 \pm 0.95 c
C18:1 trans	2.8 \pm 0.64 a	2.4 \pm 0.39 a	4.4 \pm 0.76 b
C18:1 n-9	28.3 \pm 2.13 a	32.6 \pm 1.19 b	29.7 \pm 2.15 ab
C18:2 n-6	3.3 \pm 0.41 a	3.8 \pm 0.73 a	5.3 \pm 1.40 b
C18:3 n-6	0.12 \pm 0.04	0.13 \pm 0.06	0.13 \pm 0.05
C18:3 n-3	1.24 \pm 0.09 a	0.86 \pm 0.14 c	0.27 \pm 0.04 d
CLA	0.57 \pm 0.10 a	0.55 \pm 0.07 a	0.30 \pm 0.02 b
C20:2	0.08 \pm 0.01	0.09 \pm 0.02	0.12 \pm 0.10
C20:3 n-6	0.38 \pm 0.11 a	0.43 \pm 0.09 ab	0.59 \pm 0.11 b
C20:4 n-6	1.20 \pm 0.30 ab	1.04 \pm 0.28 ab	1.33 \pm 0.50 b
C20:5 n-3	0.54 \pm 0.09 a	0.26 \pm 0.05 cb	0.16 \pm 0.06 b
C22:4 n-6	0.08 \pm 0.07 a	0.12 \pm 0.04 ab	0.18 \pm 0.03 b
C22:5 n-3	0.78 \pm 0.14 a	0.43 \pm 0.11cb	0.26 \pm 0.12 b
C22:6 n-3	0.24 \pm 0.06 a	0.05 \pm 0.01 b	0.04 \pm 0.01 b

a b c Means within a row with different letters are significantly different ($p < 0.05$)

fat free mass [FFM], fat mass [FM], total body fat [TBW], protein mass [PM], muscular mass [MM] and glycogen, were measured. The body composition was determined with a Maltron Bio Scan to measure and collect raw data to further develop scientific equations used in body composition.

Serum measurements

Samples of fasting blood [12 hs] were obtained from the antecubital vein of each subject. The serum was separated and dispensed into different tubes for the different analyses. For the determination of vitamins and serum fatty acids composition, samples were frozen at -70°C until they were analyzed. Serum total cholesterol, and HDL-, LDL- cholesterol, α , β and pre- β lipoproteins and triacylglycerol concentrations were determined according to the accepted lipid clinical chemistry methodology of the ICBA. Total serum cholesterol was measured by the reaction of cholesterol esterase/cholesterol oxidase/peroxidase. Total serum triglycerides were measured through the reaction of glycerol-3-phosphate oxidase and peroxidase, cholesterol with an enzymatic cholesterol oxidase/peroxidase, HDL and LDL-cholesterol with a selective precipitation with Dextran/Mg, triacylglycerol with enzymatic GPO/PAP, and α , β and pre- β lipoproteins with agarose gel electrophoresis. Hematocrit and hemoglobin were analyzed with a Coulter MAXM-Analyzer with an autoloader, PN 4235934 J, ERS by the Westergren method, glucose with an enzymatic [glucose oxidase] auto analyzer, urea and creatinine with an enzymatic kinetic auto analyzer, and uric acid with an enzymatic [uricase] auto analyzer. K, Na, Cl were determined with the ion selective method and serum glucose concentration was measured by the enzymatic glucose oxidase method.

Serum fatty acid composition

Aliquot samples of 2.6 ml of serum were extracted using the Folch, Lees and Sloane method [22]. The chloroform extract was used for fatty acid analysis. Fatty acid methyl esters [FAMES] were prepared according to the method of Pariza, Park and Cook [23] and measured using the Chrompack CP 900 equipment fitted with a flame ionization detector. FAMES were separated with a fused silica capillary column CP-Sil 88 [100m x 0.25mm i.d [Chrompack Inc., Middleburg, The Netherlands], with N_2 as the carrier gas. The oven temperature was programmed at 70°C for 4 min, increased from 70 to 170°C at a rate of $13^{\circ}\text{C}/\text{min}$, and then increased from 170°C to 200°C at $1^{\circ}\text{C}/\text{min}$. Individual fatty acids were identified by comparing relative retention times with individual fatty acids standards [PUFA-2 Animal Source. Supelco]. Analytic results were expressed as percentages of total fatty acids.

Determination of α -tocopherol and β -carotene

α -tocopherol and β -carotene were determined by high-performance liquid chromatography [HPLC] with electrochemical detection. After incubation, serum samples [100ul] were precipitated with ethanol [1ml]. Hexane [4ml] was added and the samples were mixed vigorously for 1 min, the tubes were then centrifuged at $300 \times g$ for 10 min. A 3-ml aliquot from the upper organic phase was dried under a N_2 stream, and the residue was re dissolved in methanol: ethanol [1:1, v/v]. Before

HPLC injection, the samples were filtered through a $0.22 \mu\text{m}$ nylon membrane. Isocratic reverse phase chromatography was performed using a C-8 column, and 20 mM LiClO_4 in methanol: H_2O [99:1, v/v] as mobile phase. For electrochemical detection, an amperometric BAS LC4C detector [Bioanalytical Systems Inc., West Lafayette, IN. USA] was used and a potential of $+0.6\text{V}$ was applied.

Determination of Vitamin B12, B6 and zinc

Vitamin B12 was determined by electro chemical luminescence [ECLIA Roche Diagnosis], vitamin B6 by HPLC and Zn by flame atomic absorption spectrometry [24].

Experimental design

A balanced sequential design and an extra period, based on an eight Latin square 3×3 for each sex, were used. The columns represent the subjects whereas the rows represent the periods of application of the diet. A healthy subject between 20 and 50 years old was considered the experimental unit because he/she was sequentially under all the diets and one extra period. The extra period was always a repetition of the last diet received. The 3×3 [t = 3 treatments p = 3 periods and k = 3 subjects] Latin square basic design, requires two Latin squares with different aleatorizations. This allowed representing the frequency of two successive diets. The extra period also allowed representing residual effects of a diet upon the next diet. The changes regarding the baseline values were established creating a variable $d = [\text{diet value}] - [\text{baseline value}]$ for each subject and diet. We assumed that the periods of three weeks between diets, removed the residual effects of the previous diets. The t student test allows testing the null hypothesis of "no diet effect".

RESULTS

Anthropometric measurements

Systolic blood pressure and waist circumference decreased in men according to the beef type diet (Table 2), being lower in P than in F. The other anthropometric measurements were not affected by the beef type in either sex. The three diets produced, however, some significant decreases in weight, systolic pressure and BMI and, increases in protein mass compared with the baseline values.

Serum parameters

No significant differences in the serum parameters between the three beef types were detected [Table 3]. Small but significant differences [p < 0.05] were detected only for urea in men [30.1 vs. 33.3 and 33.3 for F versus P and M respectively] and vitamin B6 in women [20.7 vs. 15.9 and 18.5 for M versus P and F]. Most basal blood parameters were affected, following a similar trend but with variable intensity according to the beef type and sex [Table 3]. Hemoglobin, glucose, zinc, vitamins B12 and B6, total cholesterol, LDL and HDL cholesterol, α and β lipoprotein decreased. Sodium increased significantly only in men.

α -tocopherol and β -carotene increased in men and women, however, β -lipoprotein increased significantly only in women.

Serum fatty acids

Serum lipid fatty acids proportions values were quite similar

Table 2: Anthropometric measurements, average values and differences between basal values in men and women according to beef type. P: pasture-fed beef, M: pasture & corn-fed beef and F: feedlot-fed beef. BMI: body mass index, RMR: resting metabolic rate, FFM: fat free mass, FM: fat mass, TBW: total body fat, PM: protein mass, MM: muscular mass.

Item		Men	Women	Men Differences with basal	Women Differences with basal
Weight kg	P	78.2	60.0	-1.3**	-0.46
	M	78.6	59.3	-0.7	-0.99*
	F	78.2	59.9	-1.0*	0.53
Waist cm	P	87.4b	77.4	-0.46	0
	M	88.0a	77.0	0.13	-0.44
	F	87.5ab	77.4	-0.31	-0.88
Blood pressure					
Systolic mmHg	P	109.8b	104.7	-5.6**	-5.2*
	M	111.8ab	102.9	-2.3	-7.3**
	F	115.8a	103.1	1.5	-7.6***
Diastolic mmHg	P	70.7	68.7	-2.5	-0.4
	M	73.6	66.6	0.7*	-2.2
	F	73.7	65.4	0.7	-4.1*
BMI, kg/m ²	P	24.7	23.1	-0.47***	0.35
	M	24.8	22.8	-0.25*	-0.55**
	F	24.7	23.1	-0.34*	-0.36**
RMR	P	1803	1320	-30.7	40.0
	M	1808	1318	-25.1	37.9
	F	1806	1320	-26.0	40.5
FFM, kg	P	61.7	42.4	-1.63	-4.15
	M	61.9	42.2	-1.48	-4.32
	F	61.9	42.4	-1.52	-4.08
FM	P	16.5	17.5	0.20	-0.57
	M	16.7	17.0	0.77	-6.06
	F	16.4	17.5	0.48	-5.71
TBW	P	43.9	31.4	-3.71	-3.77
	M	44.0	31.2	-3.79	-3.89
	F	44.0	31.5	-3.84	-3.62
PM	P	13.2	7.8	1.52*	-0.05
	M	13.3	7.8	1.69*	-0.09
	F	13.3	7.8	1.70*	-0.11
MM	P	31.3	19.3	2.67	-5.97
	M	31.4	19.2	2.82	-6.04
	F	31.4	19.3	2.76	-5.92
GLYCOGEN	P	560.8	385.1	-14.8	7.7
	M	562.5	383.0	-13.4	6.1
	F	562.0	385.3	-13.9	8.3

a b c Means within a column with different letters are significantly different ($p < 0.05$)

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

with the three diets [Table 4]. Significant differences with the baseline values were founded. No changes were detected in C14:0, C16:1, C18:2 n-6 and C18:3 n-6 and small and irrelevant changes in C17:1 and CLA. The odd fatty acids C15:0 and C17:0 decreased significantly with all diets and in both sexes. C18:0 decreased in women with the F meal, C18:1 Trans decreased in women with all diets and C18:1 increased in women with the F diet. C20:3 n-6 and C20:4 n-6 increased in women but C22:4 n-6 decreased only in men. C20:5 n-3 increased with the P diet in women and in men with the M diet. C22:5 n-3 increased only in men with the P diet and in C22:6 n-3 no changes were detected. Significant increases in the baseline of n-3 HPUFAs were found in men with the P diet and in women with all de diets, n-6 HPUFA

increased only in women with the P and M diets. Baseline values of SFAs were not affected whereas those of MUFAs increased in women with the F diet. Total PUFAs increased in all diets only in women.

DISCUSSION

We measured several anthropometrics and serum parameters in healthy men and women under similar diets differing only in the type of beef. Beef from three different production systems was evaluated: pasture only, pasture supplemented with corn and feed lot in both men and women. The daily intake of 150 and 280 g of beef as a part of a balanced diet induced small differences in all the parameters studied between the three beef types but

Table 3: Serum parameters according to the diet and differences with basal values for men and women. P: pasture-fed beef, M: pasture & corn-fed beef and F: feedlot-fed beef.

Item		Men	Women	Men Differences with basal	Women Differences with basal
Hemoglobin g/%	P	14.9	12.9	-0.267	-0.223
	M	14.9	12.8	-0.019	-0.369*
	F	14.9	12.8	0.067	-0.296*
Hematocrit (%)	P	45.6	39.1	-0.202	0.246
	M	45.6	38.8	0.092	-0.079
	F	45.8	38.9	0.796	0.069
Glycemia mg/%	P	79.3	79.5	-10.4**	-5.2
	M	81.5	77.4	-5.4*	-7.3**
	F	82.7	77.8	-4.6*	-6.8**
Urea mg/%	P	33.3b	29.3	0.438	2.333*
	M	33.3b	29.4	0.729	2.396
	F	30.1a	29.0	-2.21	1.792
Uric acid mg/%	P	4.84	3.2	-0.021	0.144
	M	4.92	3.1	0.090	0.063
	F	4.80	3.3	-0.040	0.217
Creatinine mg/%	P	1.0	0.84	-0.019	-0.008
	M	1.0	0.85	0.023	0.006
	F	1.0	0.83	-0.002	-0.011
Sodium mE/L	P	145.9	143.9	1.292*	0.188
	M	146.9	144.0	1.875**	0.165
	F	146.1	144.0	1.271*	0.313
Potassium mE/L	P	4.4	4.4	-0.052	-0.027
	M	4.5	4.4	-0.035	-0.104
	F	4.3	4.4	-0.146	-0.040
Zinc ug/dl	P	123.1	113.6	-43.31**	-51.9*
	M	119.8	113.5	-39.21**	-48.0
	F	131.5	106.1	-28.52	-58.3*
Vitamin B12 pg/ml	P	360.4	422.6	-84.2**	-71.6
	M	363.5	404.8	-55.7*	-102.9*
	F	362.8	471.8	-55.1	-23.7
Vitamin B6 pg/ml	P	17.1	15.9	-4.74	-4.05*
	M	15.6	20.7	-6.54**	0.560
	F	15.9	18.5	-6.62*	-1.09
α -tocopherol mug/dl	P	30.7	33.0	7.13***	5.28***
	M	31.1	32.4	6.47***	4.43***
	F	30.4	32.4	5.92***	4.56***
β -carotene umol/L	P	1.8	2.2	0.948***	0.835***
	M	1.8	2.2	0.681***	0.819***
	F	1.7	2.3	0.631***	0.871***
Cholesterol mg/dl	P	183.3	194.1	-20.9***	-20.3***
	M	185.9	191.3	-12.5*	-23.3***
	F	186.8	194.2	-11.9*	-21.2***
HDL-Chol. mg/dl	P	50.3	58.1	0.042	-5.375**
	M	48.6	60.2	-1.688	-2.958
	F	49.7	59.7	-0.396	-4.083**
LDL-Chol. m/dl	P	115.4	122.8	-21.0***	-11.5*
	M	119.6	116.4	-11.2*	-17.9**
	F	119.7	120.0	-11.7**	-15.0***
Triacylglycerol mg/ml	P	90.2	83.7	1.52	-5.021
	M	91.6	77.6	4.458	-12.042*
	F	90.0	87.6	3.271	-0.917

α -lipoprotein %	P	26.3	27.2	-0.167	-0.958*
	M	26.3	27.5	-0.271	-0.625
	F	26.4	27.2	-0.250	-1.146***
β -lipoprotein %	P	56.1	55.8	-0.771	-0.771
	M	55.9	56.1	-0.500	-0.500
	F	55.9	55.6	-0.042	-1.000*
Pre β %	P	17.6	17.0	0.938	1.729**
	M	17.8	16.5	0.771	1.250*
	F	17.2	17.2	0.292	2.146***

a b c Means within a column with different letters are significantly different (p<0.05)
* p< 0.05 ** p<0.01 *** p<0.01

Table 4: Serum fatty acid composition. Differences with basal values in men and women according to beef type. P: pasture-fed beef, M: pasture & corn-fed beef and F: feedlot-fed beef.

Fatty acid %		Men	Women	Men Differences With basal	Women Differences With basal
C14:0	P	0.87	0.86	-0.054	-0.073
	M	0.90	0.85	0.066	-0.091
	F	0.92	0.86	0.010	-0.068
C15:0	P	0.60	0.58	-0.294***	-0.240***
	M	0.56	0.65	-0.214**	-0.181**
	F	0.56	0.60	-0.199**	-0.219**
C16:0	P	21.3	21.4	0.091	-0.578
	M	21.4	21.4	0.560*	-0.570
	F	21.4	21.5	0.577	-0.473
C16:1	P	1.9	2.04	0.127	0.033
	M	1.8	1.98	0.095	-0.017
	F	1.8	2.14	0.117	0.142
C17:0	P	0.42	0.39	-0.304***	-0.364***
	M	0.40	0.37	-0.236***	-0.388***
	F	0.35	0.38	-0.280***	-0.371***
C17:1	P	0.33	0.28	-0.057	-0.045*
	M	0.32	0.33	-0.014	-0.001
	F	0.30	0.32	-0.039	-0.004
C18:0	P	8.1	8.1	-0.156	-0.330
	M	7.9	8.3	-0.068	-0.232
	F	7.8	8.0	-0.169	-0.467**
C18:1 t	P	0.42	0.41	-0.032	-0.112**
	M	0.44	0.43	-0.006	-0.097*
	F	0.41	0.41	-0.024	-0.102**
C18:1	P	18.9	17.6	0.433	0.328
	M	19.3	18.0	0.612	0.789
	F	20.0	18.4	1.293	1.111**
C18:2	P	31.7	32.3	1.164	1.686
	M	31.5	31.4	0.297	0.859
	F	31.5	31.3	0.314	0.621
C18:3n-6	P	0.52	0.51	-0.010	-0.033
	M	0.52	0.46	0.001	-0.085
	F	0.52	0.54	-0.014	0.008
C18:3 n-3	P	0.36b	0.41	0.010	0.105
	M	0.34b	0.46	-0.008	-0.003
	F	0.30a	0.35	-0.052	0.052
CLA	P	0.36	0.37	-0.058	-0.028
	M	0.35	0.37	-0.055*	-0.043
	F	0.37	0.42	-0.037	0.016
C20:3 n-6	P	1.95	1.89b	0.197*	0.071

M	1.88	1.96ab	0.083	0.137
F	1.82	2.08a	0.021	0.244**
<i>C20:4 n-6 P</i>	7.5	8.0	0.170	0.533**
M	7.6	8.3	0.079	0.802***
F	7.6	8.1	0.046	0.553**
<i>C20:5 n-3 P</i>	0.48b	0.43	0.148	0.132**
M	0.42a	0.40	0.100*	0.090
F	0.38a	0.37	0.051	0.065
<i>C22:4 n-6 P</i>	0.18	0.21	-0.101**	-0.059*
M	0.19	0.24	-0.079*	-0.037
F	0.18	0.26	-0.093**	-0.021
<i>C22:5 n-3 P</i>	0.63	0.58	0.113**	0.105
M	0.56	0.53	-0.010	0.055
F	0.57	0.58	0.006	0.084
<i>C22:6 n-3 P</i>	1.58	1.52	0.158	0.097
M	1.51	1.57	0.039	0.137
F	1.37	1.59	-0.100	0.172*
<i>n-3 P</i>	3.1	2.9	0.430*	0.439***
M	2.8	2.8	0.116	0.279*
F	2.6	2.9	-0.100	0.373**
<i>n-6 P</i>	41.9	42.9	1.439	2.198*
M	41.7	42.4	0.382	1.677*
F	41.6	42.2	0.275	1.406
<i>SFAs P</i>	30.3	30.4	-0.077	-0.981
M	30.2	30.5	0.558	-0.897
F	30.1	30.3	0.508	-1.008
<i>MUFAs P</i>	20.8	19.6	0.559	0.403
M	21.1	20.0	0.707	0.814
F	21.8	20.5	1.410	1.295**
<i>PUFAs P</i>	44.9	45.8	1.869	2.634**
M	44.5	45.2	0.498	1.956*
F	44.2	45.1	0.176	1.779*
a b c Means within a column with different letters are significantly different (p<0.05)				
* p< 0.05 ** p<0.01 *** p<0.01				

significant changes respect to the baseline values. The three diets induced significant decreases in weight, systolic pressure, BMI and increases in protein mass compared with the baseline values. In contrast to the decrease observed in systolic pressure, a previous study reported increased systolic blood pressure with 250 g of beef per day added to the neither diets of normotensive vegetarians for 4 weeks [25].

However, other similar studies found no effect on blood pressure [26-28]. The INTERMAT study found that vegetable and animal protein intake, adjusted for age and sex, was inversely correlated with blood pressure [29].

The differences in the serum parameters between the three beef types were also small but most baseline serum parameters were affected, following a similar trend but with variable intensity according to the beef type and sex. Total cholesterol, LDL and HDL cholesterol, and α and β lipoprotein decreased. A short-term controlled study and a long-term study [30] also showed the cholesterol-lowering effects of lean red meat incorporated into an AHA diet.

It has been recently concluded that maintaining or even increasing beef fat consumption has no effect on serum

LDL cholesterol in men [31] results that are consistent with earlier studies [32]. Several studies comparing beef and other animal protein sources with those of lean white fish [33] have shown that beef and other animal protein sources induced lower concentrations of plasma LDL apolipoprotein B than did the consumption of lean fish [34].

Several studies comparing beef, pork, chicken and fish have shown similar results in subjects with normal or high cholesterol [35- 39]. Non-HDL-cholesterol and the ratio of total cholesterol to HDL-cholesterol were found to be good as or better than apolipoprotein fractions in the prediction of future cardiovascular events [40]. Increased beef consumption increased apolipoprotein A-1 but not serum cholesterol of mildly hypercholesterolemic men with different levels of habitual beef intake. In the present study the vitamin B12 decreased respect to the baseline values but the values were relatively high and normal in meat eaters. The results from a study in Australia showed that the serum levels of vitamin B12 in men in his thirties were lower in lacto-ovo-vegetarians [mean 211 pmol/l] than in omnivores [334 pmol/l] and in vegans, being the mean value [145 pmol/l] bordering the deficient range and that high meat eaters averaged 402 pmol/l [41].

The serum fatty acid composition found in the present study was similar to the values reported by others for meat eating subjects [42]. The proportions of fatty acids in plasma partly reflect the composition of fatty acids in the diet; the fatty acids found in triacylglycerol's reflect the dietary intake in the past few hours, the fatty acids found in phospholipids and cholesterol esters reflect the dietary intake in the past few days, and free fatty acids are those released from adipose tissue [21]. The pasture-fed beef type diet increased the proportions of C18:3 n-3 and C20:5 n-3 in men and decreased the proportions of C20:3 n-6 in women. The higher n-3 PUFAs in pasture-fed beef and higher n-6 PUFAs in feedlot-fed beef [15] could explain the differences. The odd fatty acids C15:0 and C17:0 decreased significantly in both sexes. These fatty acids could be indicators of the milk lipid consumption in the diets studied [43]. The small decreases in C18:0 and C18:1 trans and the significant increase in C18:1 in women with the F diet could be related to the lower amounts of C18:0 and higher amounts of C18:1 in feedlot-fed beef than in pasture-fed beef [15]. SFAs were not affected but MUFAs baseline values increased in women with the F diet.

Generally, all n-3 HPUFAs were higher in the P diet compared with the others but the differences were not always statistically significant. The higher contribution of C18:3 n-3 in pasture-fed beef and the higher concentrations of C20:3n-6 in feedlot-fed beef could explain the differences detected. Significant increases were detected in the baseline values of n-3 and n-6 PUFAs in women but not in men. Differences in the n-3 and n-6 PUFAs in men and women could be due to sex differences in PUFAs metabolism [44-46]. There is evidence that increased consumption of n-3 fatty acids protect from CHD and that excessive consumption of n-6 fatty acids at the expense of n-3 ones may promote CHD and other chronic diseases. If the recommended intake of n-3 PUFAs is 100-200 mg /day [Department of Health, 1994], the pasture-fed beef contribution could be important. The amount of PUFAs in muscle tissue of lean meat needs to be taken into account when determining dietary PUFAs intakes, whereas previous estimates of n-3 HPUFAs have often been based on consumption of n-3 HPUFAs of sea-foods only [47]. These changes in fatty acid composition affect the nutritional value of fat because n-3 PUFAs have beneficial effects on human physiology and health preventing the occurrence of CHD, hypertension, inflammatory and immune disorders and neurological dysfunctions [48]. These differences reflect the lipid composition of the diet, as grass contains a high concentration of C18:3 precursors of the n-3 series while concentrates contains high levels of C18:2 precursor de la n-6 fatty acid family. Consuming red meat from pasture-fed animals compared with grain-fed animals as part of the habitual diet can significantly increase plasma and platelet n-3 HPUFAs [49].

In the present study, no changes were detected in the CLA concentration. Cattle rose on pasture usually present higher CLA levels than those rose on feedlot but these differences were not detected in this study. Cattle raised on pasture have a positive impact on the fatty acid tissue profile, mainly due to an increase in the proportion of n-3 fatty acids and CLA. Other sources of CLA in our diets such as milk, butter, cream can also contribute to the CLA intake. Meat and meat products contribute about 25-30% of the total human CLA intake in Western populations [50].

CONCLUSIONS

No significant differences were found in the different variables studied between the three experimental diets. However, all the diets significantly affected the baseline values. These values decreases, following a similar trend but with variable intensity according to beef type and sex, systolic blood pressure, weight, glucose, zinc, vitamin B12 and B6, total cholesterol, LDL and HLD cholesterol and increased sodium, vitamin E, β -carotene and n-3 PUFAs. In spite the relatively small contribution of a lean beef portion to the total diet kcal it was possible to detect some healthy effects such as the decreases in glucose, and total and LDL cholesterol and the increases in alfa-tocopherol and beta carotene. The pasture-beef contribution to all these parameters was generally higher compared with M and F beef, especially its contribution to the n-3 PUFAs. The consumption of moderate amounts of lean red meat, as part of a balanced diet, contributed positively to a healthy diet.

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