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Title: Horse meat consumption affects iron status, lipid profile and fatty acid composition of red blood cells in healthy volunteers

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Horse meat, compared to others, is high in iron, low in fat and cholesterol and a good source of polyunsaturated fatty acids such as linoleic and α-linolenic acid. The aim of the study was to investigate the effect of moderate consumption of horse meat on iron status, lipid profile and fatty acid composition of red blood cells in healthy male subjects. Fifty-two subjects were randomly assigned to two groups of 26 subjects each: a test group consuming two portions of 175 g/week of horse meat, and a control group that continued their usual diet abstaining from eating horse meat during the 90 days trial. At the onset of the study and after 90 days, blood samples were collected for analysis. Horse meat consumption significantly ($P \leq 0.05$) reduced serum levels of total and LDL cholesterol (-6.2% and -9.1% respectively) and transferrin (-4.6%). Total n-3, LCPUFA n-3 and DHA content in erythrocytes increased ($P \leq 0.05$) by about 7.8%, 8% and 11% respectively. In conclusion, the regular consumption of horse meat may contribute to the dietary intake of n-3 PUFA and may improve lipid profile and iron status in healthy subjects.

Keywords: horse meat, iron status, lipid profile, red blood cells, fatty acid composition, healthy subjects.

Abbreviations: BHT, 2,6-di-tert-butyl-4-methylphenol; BMI, body mass index; C, control group; CRI, collision reaction interface; CVDs, cardiovascular diseases; DHA, docosahexeanoic acid; EPA, eicosapentonoic acid; GC, gas chromatography; HDL, high density lipoprotein; HM, test group; ICP-MS, inductively coupled plasma mass spectrometry; LC-PUFA, long chain polyunsaturated fatty acid; LDL, low density lipoprotein; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; RBCs, red blood cells; SFA, saturated fatty acid.

Introduction

Several studies have correlated the high consumption of red meat with an increased risk for CVDs and colon cancer (Kelemen *et al.,* 2005; Kontogianni *et al.*,2008; Iqbal *et al.*, 2008; Zyriax *et al.,* 2008; Cross *et al.,* 2007; Wei *et al.,* 2004). Nowadays, dietary recommendations provided by the Italian National Research Institute for Food and Nutrition (INRAN), the American Heart Association (AHA) and the United States Department of Agriculture (USDA) Center for Nutrition Policy and Promotion, and the Word Cancer Research Funds suggest limiting the intake of red meat to less than 500g cooked weight per week and to encourage the intake of white meat (including chicken, game and turkey) because they are low in fat and cholesterol. This message is particularly important for Mediterranean populations whose traditional diet does not include a high intake of red meat.

Recent research has emphasized the nutritional value of horse meat. Compared to other meats, it is very low in fat and cholesterol (about 20% less) and it is a good source of PUFA and low in SFA, indicating that the consumption may be beneficial for health (Lanza *et al.,* 2009; Lee *et al.,* 2007). In addition, the horse meat is very high in iron, one portion (175 g) providing about one-third of daily recommended dietary intake. Insufficient iron intake results in the deficiency condition anemia, impaired cognitive performance and reduced immune function (Geissler & Singh, 2011).

The aim of the present study was to investigate the effect of regular moderate consumption (twice per week) of horse meat on iron status (hemoglobin, sideremia, transferrin, transferrin saturation and ferritin), serum cholesterol, triglyceride levels and on fatty acid composition of red blood cells in free-living healthy subjects. To our knowledge, this is the first study that attempts to evaluate the effect of regular horse meat consumption on the above markers in humans.

Materials and Methods

Chemicals and dietary ingredients

Omegawax test mix standard, Cholesterol, 6-ketocholestanol, BHT, sodium chloride were from Sigma-Aldrich (St.Louis, MO, USA). Chloroform, acetyl chloride, potassium carbonate, toluene, methanol, acetonitrile and hexane were purchased from Merck (Darmstadt, Germany). Water was obtained from a Milli-Q apparatus (Millipore, Milford, MA). Horse meat was provided by NabaCarni Spa (Rezzato, BS, Italy); pork and beef meat were purchased from local market.

Experimental design

Fifty-two male healthy and free-living volunteers (ages 20-50 years, BMI 24.4± 3.8 kg/m^2), were enrolled from the staff and student population of the University of Milan. Only male subjects were considered for the study in order to exclude bias related with changes in iron status and other parameters that occur in premenopausal women due to the hormonal fluctuation during the various phases of the menstrual cycle.

The selection of the volunteers was performed based on the assessment of anthropometric characteristics, clinical and biochemical parameters concerning iron status and lipid profile. Exclusion criteria included: smoking (>10 cigarettes/day), hypertension (diastolic pressure ≥ 90 mm Hg and systolic ≥140 mm Hg), high serum cholesterol (≥ 5.9mmol/L; 230 mg/dL), high triglycerides (≥ 2.3mmol/L; 200 mg/dL), obesity (BMI › 30). A medical history questionnaire was used to exclude diseases such as diabetes, renal insufficiency, dysfunctions related to iron metabolism, known food allergies or other diseases. The selection of the volunteers was performed also by considering their eating behaviors evaluated by means of a questionnaire about food intake and preferences, specifically evaluating the frequency of fish and meat, but also nuts and seeds, and pulses, intake targeting horse and beef products in order to have a homogeneous group of volunteers for meat consumption habits.

Volunteers who did not eat meat and those who followed specific diets were excluded. Additionally subjects were excluded from the study if they were taking drugs, supplements or medications. The experimental protocol was conducted according to the guidelines reported in the Declaration of Helsinki and all the procedures involving subjects were approved by the Ethics Committee of the University of Milan (March 26th 2009 - all.4). A written informed consent was signed by each participant. The randomized parallel dietary intervention study was carried out for a period of 90 days. Subjects were randomly assigned into two groups of 26 subjects each: a test group (HM) that replaced two portions of red meat (beef and pork) with two portions of 175 g/week of horse meat, and a control group (C) in which volunteers abstained from the consumption of horse meat maintaining their eating meat habits (in particular beef and pork). Throughout the 90 days period, subjects kept a daily dietary record and a short interview was randomly conducted in order to check diet compliance. From dietary records we observed an energy intake of 2450 kcal, and a consumption of 90 g of proteins and 93 g of fats. The difference within the subjects was about 20% for the energy, proteins and fats in according to Turrini et al, (2008). Every 2 weeks, volunteers came to the laboratory to deliver the dietary records and to receive four portions of horse meat, under vacuum skin-pack, that were consumed at home; barbecued and roast cooking were not permitted. During the experimental period none of the volunteers has deserted the study or changed eating habits. The HM group introduced about 5 mg of iron and 500 mg of PUFA per week more than C group. At the beginning and after 90 days, venous blood samples were collected for the analysis.

Proximate analysis

Proximate analysis was conducted using AOAC official method (AOAC 942.05 and AOAC 934.01, 2006).

Iron determination in food

Iron (Fe^{2+}) was measured by ICP-MS (Varian 820 ICP-MS) as ^{56}Fe. Typical interferences (like ArO^{+}) were removed by using CRI with an H_2 flow of 70 mL/min^{-1} and Fe^{2+} was determined in quadruplicate (Chen *et al.*, 2005).

Cholesterol determination in food

Meat samples (0.5 g) were placed into a 125 mL flask followed by the addition of 500 μL of 6-ketocholestanol as internal standard (IS 1mg/mL), 4 mL of absolute ethanol and 1 mL of 60% potassium hydroxide in water. The mixtures were vortexed and treated at 50°C for 1 h till the complete digestion of the samples. Samples were then cooled to room temperature (20°C) prior to the addition of 5 mL of water and 5 mL of hexane for the first extraction. Then, at least four extractions with hexane were carried out and the supernatants were brought to a final volume of 20 mL. An aliquot (3 mL) of sample was evaporated under nitrogen and dissolved with 200 μL of the mobile phase for the HPLC injection (20 μL). The chromatographic system consisted of an Alliance mod. 2695 (Waters, Milford, MA, US) coupled to a photodiode array detector mod. 2998 (Waters Milford, MA, USA). A 3μm C18 Luna HST column (100x2 mm, Phenomenex, Torrence, CA, USA) was used for the separation at a flow-rate of 0.35 mL/min. The column was maintained at 50°C and the separation was performed in isocratic mode. The mobile phase was a mixture of acetonitrile:methanol (50:50 v/v). For peak identification, chromatograms were acquired in the range of 195-320 nm and integrated at 205 nm. The primary stock solutions of cholesterol and IS (1 mg/mL) were prepared in methanol and diluted to give working solutions in the range of 10-500 μg/mL.

Red blood cells separation

Blood samples were collected into a vacutainer containing silicon, and plasma was separated by centrifugation at 1400xg for 15 min at 4°C. The buffy layer of white blood cells was removed by using a pipette and the red blood cells were washed twice with an equal volume of a physiologic solution (0.9 % NaCl, w/v). An aliquot (0.5 g) of red blood cells was stored at -80°C until analysis.

Iron status and lipid profile in humans

Iron status and lipid profile were determined in serum following standard laboratory techniques. Hemoglobin was measured by using a CELLTAC MEK-6400 analyser (Nihon Kohden Corporation, Tokyo, Japan). Ferritin was determined by using an AIA-600 analyser (Tosoh Bioscience, San Francisco CA-USA). Sideremia, transferrin, triglycerides, total cholesterol and HDL cholesterol were measured by using an ILAB-600 analyser (Instrumentation Laboratory, Lexington, MA-USA). Transferrin Saturation was calculated by the formula (Serum Iron / Total Iron Binding Capacity) x 100%; LDL cholesterol was calculated using the Friedewald's method.

Analysis of fatty acid composition of meats and phospholipids of red blood cells

Fatty acids extraction from red blood cells (RBC) and meats was performed in accordance to the method previously described (Simonetti *et al.*, 2002).

The GC analysis was performed as described by Ackman (1989). Fatty acid retention times were obtained by injecting the Omegawax test mix as standard.

Statistical analysis

Sample size was calculated to include 20 subjects per group in order to detect significant differences in the fatty acid composition of red blood cells and iron status with a power of 80%. STATISTICA software (Statsoft Inc, Tulsa, OK) was used for statistical analysis of data. One-way ANOVA was

applied to compare the nutritional composition of horse, beef and pork meat. Two-way ANOVA was applied to compare the effect of the diet (HM vs C group) and time (time 0 *vs* time 90 days) on serum levels of triglycerides, cholesterol, iron status and fatty acid composition of red blood cells. Differences were considered significant at $P \leq 0.05$; post-hoc analysis of differences between treatments was assessed by the Least Significant Difference (LSD) test with $P \leq 0.05$ as level of statistical significance. All data are presented as mean and standard deviation (SD).

Results

Proximate composition and fatty acid profile of horse, pork and beef meat

In **Table 1** the proximate and nutritional composition, for 100 g of product, of three different cuts of meat (rump from horse and beef and loin from pork) is presented. Data are reported as mean ± SD. Horse meat is characteristically high in iron (3.81 ± 0.46 mg/100g) and low in cholesterol (53.3 ± 1.20 mg/100g) with respect to beef and pork. The content of lipids (2.2 ± 0.00 g/100g) is similar to beef and pork. Horse meat is high in PUFAs (655.8 ± 3.2 mg/100g) that represent 30% of the total lipid content with respect to beef (505.6 ± 68.9 mg/100g; 19%) and pork (348.9 ± 5.9 mg/100g; 13%). The content of n-3 is significantly higher ($P \leq 0.05$) in the horse meat (217.4 ± 5.7 mg/100g) with respect to beef (61.3 ± 14.1 mg/100g) and pork (21.5 ± 0.3 mg/100g). Among the n-3 fatty acids, the content of alpha-linolenic acid (18:3n3) and docosapentaenoic acid (22:5n3) in the horse meat (167.8 ± 5.2 mg/100g and 31.2 ± 0.2 mg/100g, respectively) is considerably higher ($P \leq 0.05$) with respect to beef and pork. A significant difference ($P \leq 0.05$) was also observed for the ratio n-3/n-6 that in horse meat is much higher (0.49 ± 0.03) with respect to beef and pork (0.13 ± 0.03 and 0.07 ± 0.04, respectively).

Effect of horse meat consumption on serum iron status

The concentrations of sideremia, transferrin, transferrin saturation and ferritin are reported in **Table 2**. At the beginning of the study, the serum iron status did not differ significantly between HM and C groups. Although during the experimental period the biochemical parameters were within the normal range, the consumption of horse meat significantly ($P < 0.001$) reduced the serum levels of transferrin with respect to baseline and C group at the onset and at the end of the study. Sideremia and transferrin saturation tended to increase in the HM group with respect to C group.

Effect of horse meat consumption on serum lipid profile

The levels of total cholesterol, HDL and LDL cholesterol, triglycerides, before and after 90 days are reported in **Table 3**. At the beginning of the study, the serum lipid profile did not differ significantly between diet groups. During the experimental period all the biochemical parameters were within the normal range and were identical in the HM and C groups. However, compared to baseline the serum concentrations of total cholesterol, LDL-cholesterol and the ratio of total cholesterol/HDL-cholesterol significantly declined after the intervention study in the HM group compared to C group. In particular, total cholesterol concentration was 0.29 mmol/L (6.2%) lower ($P < 0.02$); LDL-cholesterol concentration was 0.26 mmol/L (9.1%) lower ($P < 0.01$); the ratio total cholesterol/HDL-cholesterol was 9.5% lower after the intervention. No significant effect was detected on triglyceride and HDL cholesterol levels.

Effect of horse meat consumption on fatty acids composition of red blood cells

The mean percent of fatty acid composition of red blood cells in the HM and C groups after 90 days is presented in **Table 4**.

At the end of the study, the consumption of horse meat significantly changed the fatty acids composition of red blood cells. In particular, the content of PUFA and n-6 was significantly reduced (P

221 < 0.01) in the HM group by about 4.4% and 7.2% respectively, with respect to baseline and C group.
222 Among n-6 fatty acids, linoleic acid (18:2n6), dihomo-gamma-linolenic acid (20:3n6) and arachidonic
223 acid (20:4n6) significantly decreased ($P < 0.01$) after horse meat consumption by about 7.8%, 8.4% and
224 6.2% respectively. On the contrary, SFA and n-3 significantly increased ($P < 0.05$) in the HM group by
225 about 3.0% and 7.8% respectively after 90 days of horse meat consumption, while no significant
226 changes occurred in the C group. Docosahexaenoic acid showed a significant increase (from $4.92 \pm$
227 1.04% to $5.46 \pm 0.89\%$; $P < 0.05$) in the HM group after 90 days consumption of horse meat. When
228 considering the long chain PUFA (LCPUFA; C≥20, double bonds ≥3), a significant modulation was
229 observed only for the content of LCPUFA-n3, which increased significantly ($P < 0.05$) in the HM
230 group by about 8% with respect to C group. Horse meat consumption induced a statistically significant
231 increase in the n-3/n-6 ratio (15.3%; $P < 0.01$) and the omega-3 index (defined as the summation of
232 EPA and DHA content) (7.5%; $P < 0.05$). No difference was observed for the ratio LC n-3/n-6.
233
234 **Discussion**
235 Cardiovascular diseases are one of the most leading causes of morbidity and mortality in the Western
236 world (Mottillo *et al.,* 2010). Modifiable risk factors include: smoke, alcohol consumption, physical
237 activity and diet (Yusuf *et al.*, 2004). Evidence shows that the Mediterranean diet is the best way to
238 prevent many diseases. It is characterized by high consumption of fruits, vegetables, a moderate
239 consumption of white meat (poultry, rabbit, etc.) and a limited consumption of red meat to a few times
240 per month because of the high content in cholesterol and saturated fats (Sparling & Anderson, 2009).
241 In recent years, red meat is leaner and lower in fat content than that produced in the past (Martuzzi *et*
242 *al.,* 2001). Among red meats, horse meat is very interesting from a nutritional point of view because it
243 is low in cholesterol, rich in iron and PUFA such as α-linolenic (18:3 n3). For these reasons the

consumption of horse meat could have beneficial effects on health than that of other red meats (Badiani *et al.,* 1997; Lee *et al.,* 2007).

The objective of the present study was to investigate whether the replacement of red meat with two portions of 175 g/week of horse meat for 90 days, can improve iron status, lipid profile and fatty acid composition of red blood cells in free-living healthy male subjects.

The consumption of two portions per week of horse meat provided 3 g of SFA, 2 g of MUFA, 186 mg of cholesterol. In addition horse meat provided a greater amount of PUFA (2.3 g) and iron (13.34 mg) with respect to beef (1.8 g of PUFA and 8.1 mg of iron) and pork (1.2 g of PUFA and 6.1 mg of iron) while the energy intake was comparable between meats (372.4 kcal for horse meat, 388.2 kcal for beef and 424.4 kcal for pork). The results of the study have shown that the consumption of horse meat can significantly affect iron status, in particular the levels of transferrin. Transferrin is an iron- binding blood glycoprotein that controls the level of free iron in the blood; when the amount of iron is high, the body reduces the production of transferrin so that less of the iron is taken up and transported (Geissler & Singh, 2011). Two portions per week of horse meat significantly reduced ($P < 0.001$) the serum levels of transferrin in the HM group (- 4.5%) with respect to C group. No modulation was observed for the concentration of hemoglobin and serum ferritin (intracellular protein that binds and sequesters intracellular iron not utilized in the functional iron pool) while an improvement, even if not significant, was observed for the sideremia (the amount of circulating iron that is bound to transferrin) and the percentage of transferrin saturation (the percentage of total iron binding capacity that is occupied by serum iron). These results are in accordance with data reported in literature, in which the consumption of meat (beef, pork, poultry) has been associated with a positive effect on iron status (Worthington-Roberts *et al.,* 1988; Leggett *et al.,* 1990; Brussaard *et al.,* 1997; Ortega *et al.,* 1998). However, very few dietary intervention studies evidenced an improvement of the iron status in healthy/unhealthy subjects (Navas-Carretero *et al.,* 2009; Tetens *et al.,* 2007). Most of the evidence reported, involves

anemic individuals in which iron was supplemented with tablets or fortified food (Navas-Carretero *et al.,* 2009; Szymlek-Gay *et al.,* 2009; Zhu & Haas, 1998).

Several studies suggest that the fat content of red meat might be an important risk factor for the development of CVDs. However, it is extremely important to evaluate not only the contribution of fat intake from meat consumption but also from the total diet. To our knowledge there is not enough evidence able to demonstrate that consuming lean red meat, low in total fat and cholesterol can increase the risk factors for CVDs. In fact, no negative effect on blood lipids was observed in different human intervention studies after the consumption of lean red meat (Hodgson *et al.,* 2007; Beauchesne-Rondeau *et al.,* 2003; O'Dea *et al.,* 1990). In our experimental conditions, the replacement of two portions of red meat with horse meat, seems to improve the cholesterol profile of healthy human volunteers. The consumption of horse meat reduced the serum concentrations of total cholesterol (-6.2%), LDL-cholesterol (-9.1%) and improved the ratio of total cholesterol/HDL-cholesterol (-9.5%) in the HM group with respect to C group. Similar results have been observed by other authors that have demonstrated a reduction in total and LDL-cholesterol in hypercholesterolomic men after a 26 day intervention study with lean beef, lean fish or poultry (Beauchesne-Rondeau *et al.,* 2003).

Long-chain (LC) n-3 PUFA play also an important role in the modulation of lipid and HDL-cholesterol profile. However, most of the beneficial evidence on HDL-cholesterol and lipid profiles has been observed after administration of high doses of LC n-3 PUFA (>450 mg/day) and fish related to their high content of EPA and DHA. Milte *et al.,* (2008) reported that 3g tuna fish oil per day (providing 780 mg DHA and 180 mg EPA) reduced the levels of triglycerides (-23%) and increased the level of HDL-cholesterol (+4.4%) in healthy subjects after a 12-week intervention study. Lindqvist *et al.,* (2009) demonstrated that 6-weeks of a herring-rich diet significantly reduced the plasma levels of triglycerides (-22%) and improved the level of HDL-cholesterol (+4%) in healthy but overweight men. Similar results were also obtained after regular consumption for 12 weeks, of n-3 fatty acid-enriched

292 pork. Coates *et al.,* (2009) demonstrated that the weekly consumption of 200g of pork meat (providing
293 1.3g PUFA per week) significantly reduced the serum levels of triglycerides. In our experimental
294 conditions, no significant difference was observed in serum concentration of triglycerides and HDL-
295 cholesterol. The lack of the effect after a 12 week horse meat intervention may be attributed to different
296 factors such as: physiologic concentration of the levels of lipids and cholesterol of the volunteers or
297 their healthy status.

298 Several studies reported that balance of omega-3 fatty acids in the plasma are a good indicator
299 of cardiac health. Different studies have shown that n-3 enriched food can increase the percentage of n-
300 3 PUFA incorporated into red blood cell membranes. Coates *et al.,* (2009) demonstrated that a regular
301 consumption of n-3 fatty acid-enriched pork increased the erythrocyte DHA levels (+15%) in healthy
302 adult volunteers. McAfee *et al.,* (2011) shown that consumption of three portions (200g) per week of
303 grass-fed animals (beef and lamb), may provide valuable amount of LC n-3 PUFA and modulate the
304 fatty acid composition of plasma and platelets by improving LC n-3 PUFA status. In the present study,
305 the consumption for 12 weeks of two portions/week of horse meat (providing 2.3 g per week of n-3
306 PUFA) was able to increase significantly ($P < 0.05$) the content of n-3 fatty acids (9.6%), and in
307 particular the content of DHA acid (+13.6%) with respect to linoleic acid and n-6 fatty acids. This
308 could be attributable to a preferential uptake of n-3 PUFA or a different metabolism that convert PUFA
309 to longer and more unsaturated species. These processing activities seem particularly higher for n-3
310 than n-6 PUFA (Di Nunzio *et al.,* 2010). This evidence suggests that the low content of EPA and DHA
311 into erythrocytes is an important but independent risk factor for the development of CVDs. In this
312 regard Harris & von Schacky, (2004) have defined an Omega-3 index as a sum of EPA + DHA
313 contained within phospholipids of erythrocyte membranes; a low index (≤ 4%) is associated with a
314 high risk of mortality (von Schacky, 2010). In the present study the consumption of horse meat
315 significantly ($P < 0.05$) increased the Omega-3 index by about 7.5%. ~~In addition, an improvement of~~

~~the ratio n-3/n-6 (+15.3%) and a reduction of the total content of n-6 PUFA(-6.7%) was observed in HM with respect to C group.~~

Conclusions

The present study shows that the horse meat is an important source of omega 3 and iron. With respect to other meats, it is very low of SFA but rich of PUFA. A regular consumption of horse meat may contribute to reduce total and LDL cholesterol, and improve omega 3 index, docosahexaneoic acid (22:3n6) and iron status. Therefore, a regular consumption of horse meat with respect to other read meats, may improve the fatty acid profile of red blood cells and in particular modulate PUFA, the most useful to maintain nutritional status and the protective effects.

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References

Ackman RG, (1986). Wcot (capillary) gas-liquid chromatography. In R. J. Hamilton & J. B. Rossel (Eds.), Analysis of Oils and Fats (pp 137-206). London: U.K. Elsevier Applied Science Publishers.

AOAC method 942.05, Ash of Animal Feed. Official Methods of Analysis, 16th ed. AOAC International, Gaithersburg, MD, 1995.

AOAC method 934.01, Loss on Drying (Moisture) at 95–100°C for Feeds/Dry Matter on Oven Drying at 95–100°C for Feeds. Official Methods of Analysis, 16th ed. AOAC International, Gaithersburg, MD, 1995.

Badiani A, Nanni N, Gatta PP, Tolomelli B, Manfredini M. (1997). Nutrient profile of horse meat. J Food Compost Anal 10:254-269.

Beauchesne-Rondeau E, Gascon A, Bergeron J, Jacques H. (2003). Plasma lipids and lipoproteins in hypercholesterolaemic men fed a lipid-lowering diet containing lean beef, lean fish, or poultry. Am J Clin Nutr 77: 587-593.

Brussaard JH, Brants HA, Bouman M, Lowik MR. (1997). Iron intake and iron status among adults in the Netherlands. Eur J Clin Nutr 51: S51-S58.

Chen Z, Griffin IJ, Plumlee LM, Abrams SA. (2005). High resolution inductively coupled plasma mass spectrometry allows rapid assessment of iron absorption in infants and children. J Nutr 135: 1790-1795.

Coates AM, Sioutis S, Buckley JD, Howe PRC. (2009). Regular consumption of n-3 fatty acid-enriched pork modifies cardiovascular risk factors. Br J Nutr 101: 592-597.

Cross AJ, Leitzmann MF, Gail MH, Hollenbeck AR, Schatzkin A, Sinha R. (2007). A prospective study of red and processed meat intake in relation to cancer risk. PLoS Med 4: e325.

Di Nunzio M, van Deursen D, Verhoeven AJM, Bordoni A (2010). n-3 and n-6 Polyunsaturated fatty acids suppress sterol regulatory element binding protein activity and increase flow of non-esterified cholesterol in HepG2 cells. Br J Nutr 103: 161-167.

Geissler C & Singh M. (2011). Iron, meat and health. Nutrients 3: 283-316.

Harris WS, & von Schacky C. (2004). The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev Med 39: 212-220.

Hodgson J, Wards NC, Burke V, Beilin LJ, Puddey IB. (2007). Increased lean red meat intake does not elevate markers of oxidative stress and inflammation in humans. J Nutr 137: 363-367.

Iqbal R, Anand S, Ounpuu S, Islam S, Zhang X, Rangarajan S, Chifamba J, Al-Hinai A, Keltai M, Yusuf S, INTERHEART Study Investigators (2008). Dietary patterns and the risk of acute myocardial infarction in 52 countries: Results of the INTERHEART study. Circulation 118: 1929-1937.

Kelemen LE, Kushi LH, Jacobs DR Jr, Cerhan JR. (2005). Associations of dietary protein with disease and mortality in a prospective study of postmenopausal women. Am J Epidemiol 161:239-249.

Kontogianni MD, Panagiotakos DB, Pitsavos C, Chrysohoou C, Stefanadis C. (2008). Relationship between meat intake and the development of acute coronary syndromes: The CARDIO2000 case-control study. Eur J Clin Nutr 62: 171-177.

Lanza M, Landi C, Scerra M, Galofaro V, Pennisi P. (2009). Meat quality and intramuscular fatty acid composition of Sanfratellano and Hafliger foals. Meat Sci 81:142-147.

Lee CE, Seong PN, Oh WY, Ko MS, Kim KI, Jeong JH. (2007). Nutritional characteristics of horsemeat in comparison with those of beef and pork. Nutr Res Pract 1:70-73.

Legget BA, Brown NN, Bryant SJ, Duplock L, Powell LW, Halliday JW. (1990). Factors affecting the concentrations of ferritin in serum in a healthy Australian population. Clin Chem 36:1350-1355.

Lindqvist HM, Langkilde AM, Underland I, Sanderberg AS. (2009). Herring (*Clupea harengus*) intake influences lipoproteins but not inflammatory and oxidation markers in overweight men. Br J Nutr 101: 383-390.

Martuzzi F, Catalano AL, Sussi C. (2001). Characteristics of horse meat consumption and production in Italy. Annali della Facoltà Medicina Veterinaria (Vol. 21). Italy: University of Parma. 213-223.

McAfee AJ, McSorley EM, Cuskelly GJ, Fearon AM, Moss BW, Beattie JAM, Wallace JMW, Bonham MP, Strain JJ. (2011). Red meat from animals offered a grass diet increases plasma and platelet n-3 PUFA in healthy consumers. Br J Nutr 105:80-89.

Milte CM, Coates AM, Buckley JD, Hill AM, Howe PRC. (2008). Dose-dependent effects of docosahexanoic acid-rich fish oil on erythrocyte docosahexanoic acid and blood lipid levels. Br J Nutr 99:1083-1088.

Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P. Rinfret S, Schiffrin EL, Eisenberg MJ. (2010). The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol 56:1113-1132.

Navas-Carratero S, Pérez-Granados AM, Sarria B, Vaquero MP. (2009). Iron absorption from meat pate fortified with ferric pyrophosphate in iron-deficient women. Nutrition 25: 20-24.

Navas-Carratero S, Pérez-Granados AM, Schoppen S, Sarria B, Carbajal A, Vaquero MP. (2009). Iron status biomarkers in iron deficient women consuming oily fish versus red meat diet. J Physiol Biochem 65:165-174.

O'Dea K, Traianedes K, Chisholm K, Leyden D, Sinclair AJ. (1990). Cholesterol-lowering effect of a low-fat diet containing lean beef is reversed by the addition of beef fat. Am J Clin Nutr *52:*491-494.

Ortega RM, Lopez-Sobaler AM, Requejo AM, Quintas ME, Gaspar MJ, Andrés P, Navia B. (1998). The influence of meat consumption on dietary data, iron status and serum lipid parameters in young women. Int J Vitam Nutr Res 68:255-262.

Simonetti P, Ciappellano S, Gardana C, Bramati L, Pietta P. (2002). Procyanidins from Vitis vinifera seeds: in vivo effects on oxidative stress. J Agric Food Chem 50:6217-6221.

Sparling MC, Anderson JJB. (2009). The Mediterranean diet and cardiovascular diseases. Nutr Today 44:124-133.

Szymlek-Gay EA, Ferguson EL, Heath ALM, Gray AR, Gibson RS. (2009). Food-based strategies improve iron status in toddlers: a randomized controlled trial. Am J Clin Nutr 90:1541-1551.

Tetens I, Bendtsen KM, Henriksen M, Ersbøll AK, Milman N. (2007). The impact of a meat- versus a vegetable- based diet on iron status in women of childbearing age with small iron stores. Eur J Nutr 46:439-445.

Turrini A, Leclercq C, Arcella D, Bevilacqua N, Capriotti M, Catasta G, D'Addezio L, Di Lena F, Le Donne C, Martines S, Pettinelli A, Piccinelli R, Roccaldo R, Sette S, Spadoni F, Toti E (2008): Osservatorio dei consumi alimentari. In Progetto Strategico Qualità Alimentare, pp.687-724. Roma: INRAN. http://www.inran.it/pubblicazioni_divulgative/SCAI.pdf.

Von Schacky, C. (2010). Omega-3 index and sudden cardiac death. Nutrients 2:375-388.

Wei EK, Giovannucci E, Wu K, Rosner B, Fuchs CS, Willet WC, Colditz GA. (2004). Comparison of risk factors for colon and rectal cancer. Int J Cancer Suppl 108:433-442.

Worthington-Roberts BS, Breskin MW, Monsen ER. (1988). Iron status of premenopausal women in a university community and its relationship to habitual dietary sources of protein. Am J Clin Nutr 47:275-279.

Yusuf S, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L, INTERHEART Study Investigators. (2004). Effects of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. The Lancet, 364:937-952.

Zhu YI, Haas JD. (1998). Response of serum transferring receptor to iron supplementation in iron-depleted, nonanemic women. Am J Clin Nutr 67:271-275.

Zyriax B, Algenstaedt P, Hess UF, Schoffauer M, Bamberger C, Boeing H, Windler E. (2008). Factors contributing to the risk of cardiovascular disease reflected by plasma adiponectin. Data from the coronary risk factors for atherosclerosis in women (CORA) study. Atherosclerosis 200:403-409.

451 **Table 1- Nutritional composition of horse, pork and beef meat.**

	Horse	Beef	Pork
Water, %	72.70 ± 0.10	74.85 ± 0.07	71.55 ± 0.07
Ash, %	1.20 ± 0.00	1.10 ± 0.00	1.15 ± 0.07
Protein, %	21.71 ± 0.10	21.65 ± 0.35	24.35 ± 0.07
Lipids, %	2.18 ± 0.00	2.70 ± 0.10	2.65 ± 0.07
Cholesterol, mg/100g	53.30 ± 1.20^{b}	65.50 ± 2.00^{a}	67.30 ± 2.10^{a}
Iron, mg/100g	3.81 ± 0.46^{a}	2.32 ± 0.31^{b}	1.73 ± 0.43^{b}
Fatty acids, mg/100g			
14:0	45.2 ±1.2^{a}	53.5 ± 10.7^{a}	34.6 ± 1.8^{b}
16:0	576.2 ± 2.1	673.8 ± 55.5	611.4 ± 10.4
18:0	274.6 ± 14.5^{b}	621.4 ± 26.8^{a}	452.4 ± 73.9^{c}
20:0	6.8 ± 0.4	9.0 ± 2.7	7.0 ± 0.4
22:0	2.9 ± 1.0^{b}	4.6 ± 2.1^{a}	2.2 ± 0.0^{b}
24:0	3.7 ± 0.1^{a}	2.8 ± 1.2^{a}	1.3 ± 0.2^{b}
16:1n9	7.4 ± 0.5	6.4 ± 0.8	7.3 ± 0.4
16:1n7	77.8 ± 0.9	59.5 ± 2.1	69.6 ± 1.8
18:1n9	478.9 ± 18.4^{b}	693.5 ± 37.7^{b}	1016.4 ± 46.4^{a}
18:1n7	39.1 ± 1.6^{c}	263.5 ± 0.4^{a}	82.9 ± 9.1^{b}
20:1n9	8.6 ± 0.5^{c}	4.1 ± 1.0^{b}	14.4 ± 1.1^{a}
24:1n9	3.0 ± 0.5	2.4 ± 2.2	1.5 ± 0.2
18:2n6	371.8 ± 5.3^{a}	319.8 ± 31.2^{b}	255.2 ± 2.1^{b}
18:3n6	0.6 ± 0.2	1.7 ± 0.7	1.8 ± 0.2
20:2n6	6.8 ± 0.0^{a}	7.1 ± 0.8^{b}	9.9 ± 0.1^{b}
20:3n6	9.8 ± 0.8^{b}	17.7 ± 2.9^{c}	6.7 ± 0.4^{a}
20:4n6	47.9 ± 2.7^{a}	88.6 ± 16.8^{b}	47.3 ± 4.3^{a}
22:4n6	1.6 ± 0.2^{a}	9.4 ± 2.4^{c}	6.4 ± 0.4^{b}
18:3n3	167.8 ± 5.2^{a}	19.8 ± 0.5^{b}	7.5 ± 0.0^{b}
20:4n3	1.9 ± 0.0	1.9 ± 0.9	0.7 ± 0.2
20:5n3	11.0 ± 0.5^{a}	12.4 ± 3.6^{a}	5.3 ± 0.4^{b}
22:5n3	31.2 ± 0.2^{a}	23.0 ± 7.2^{b}	6.0 ± 0.6^{c}
22:6n3	5.5 ± 0.2^{a}	4.3 ± 1.8^{a}	2.0 ± 0.2^{b}
Total SFA	909.4 ± 16.2^{b}	1365.0 ± 33.4^{a}	1109.0 ± 62.3^{b}
Total MUFA	614.8 ± 19.4^{b}	829.4 ± 35.5^{b}	1192.1 ± 56.4^{a}
Total PUFA	655.8 ± 3.2^{a}	505.6 ± 68.9^{b}	348.9 ± 5.9^{b}
n -3	217.4 ± 5.7^{a}	61.3 ± 14.1^{b}	21.5 ± 0.3^{c}
n -6	438.4 ± 8.8^{b}	444.3 ± 54.9^{a}	327.3 ± 6.3^{c}
n3/n6	0.49 ± 0.03^{a}	0.13 ± 0.03^{b}	0.07 ± 0.04^{b}

SFA = Saturated fatty acid, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, n-6= Omega 6 fatty acids, n-3= Omega 3 fatty acids. Data are reported as mean ± SD. Data with different letters are significantly different, $P \leq 0.05$

Table 2- Change in serum iron status between horse meat and control groups after 90 days of consumption of horse or control meat.

Iron status	HM group baseline	HM group 90 days	C group baseline	C group 90 days
Hemoglobin, g/dL	15.1±0.8	14.8±1.1	14.9±0.9	14.7±0.8
Sideremia, μg/ dL	99.1±29.5	104.2±32.9	113.2±32.0	107.0±27.4
Transferrin, mg/ dL	262.6±31.0[a]	250.5±30.0[b]	265.0±33.0[a]	260.0±31.7[a]
Transferrin saturation, %	27.2±9.6	29.7±9.9	30.6±10.0	29.4±8.6
Ferritin, ng/mL	113.2±79.9	112.5±72.2	119.6±68.4	117.8±73.8

HM group = horse meat group
C group = control group
Data are reported as mean ± SD. Data with different letters are significantly different.
[b] Different from HM group at baseline and C group (before and after 90 days), $P \leq 0.001$

Table 3- Change in serum lipid profile between horse meat and control groups after 90 days of consumption of horse or control meat.

Lipid profile	HM group baseline	HM group 90 days	C group baseline	C group 90 days
Triglycerides, mmol/L	0.94±0.40	0.92±0.43	0.97±0.40	0.96±0.40
Total cholesterol, mmol/L	4.74±0.92[a]	4.45±0.82[b]	4.66±0.65[a]	4.53±0.49[a]
HDL-cholesterol, mmol/L	1.28±0.29	1.32±0.23	1.32±0.32	1.35±0.27
LDL-cholesterol, mmol/L	2.88±0.73[a]	2.62±0.66[b]	2.78±0.59[a]	2.64±0.47[a]
Total Cholesterol/HDL	3.80±0.85[c]	3.44±0.70[d]	3.64±0.73[c]	3.46±0.66[c]

HM group = horse meat group
C group = control group
Data are reported as mean ± SD. Data with different letters are significantly different.
[b] Different from HM group at baseline, $P \leq 0.05$
[d] Different from HM group at baseline, $P \leq 0.001$

Table 4- Change in fatty acid composition of red blood cells between horse meat and control groups after 90 days of consumption of horse or control meat.

%	HM group baseline	HM group 90 days	C group baseline	C group 90 days
Total SFA,	47.22±1.19[b]	48.61±1.88[a]	47.40±1.29[b]	47.58±3.18[b]
Total MUFA	19.32±1.27	19.43±1.33	19.63±1.35	19.47±1.66
Total PUFA	33.01±1.71[c]	31.57±1.51[d]	32.49±1.74[c]	32.56±2.44[c]
SFA/PUFA	1.44±0.10[d]	1.55±0.13[c]	1.46±0.11[d]	1.47±0.20[d]
18:2n6	11.45±1.17[c]	10.56±1.43[d]	11.24±1.38[c]	10.88±1.84[c]
20:3n6	2.02±0.42[c]	1.85±0.42[d]	1.79±0.34[d]	1.73±0.30[d]
20:4n6	11.04±1.62[a]	10.36±1.12[b]	10.73±1.27[a]	11.02±1.93[a]
22:4n6	1.81±0.45	1.66±0.37	1.81±0.44	1.94±0.78
Total n-6	26.31±2.40[c]	24.42±1.77[d]	25.68±2.28[c]	25.57±2.94[c]
18:3n3	0.06±0.02	0.06±0.03	0.06±0.03	0.04±0.03
20:5n3	0.39±0.12	0.39±0.14	0.43±0.24	0.43±0.17
22:5n3	1.32±0.18	1.25±0.16	1.32±0.24	1.33±0.26
22:6n3	4.92±1.04[d]	5.46±0.89[c]	5.08±0.90[d]	5.18±0.96[d]
Total n-3	6.63±1.11[b]	7.15±0.96[a]	6.90±1.16[b]	6.99±1.04[b]
n-3/ n-6	0.26±0.06[d]	0.30±0.06[c]	0.27±0.07[c]	0.28±0.07[c]
n-3 index	6.24±1.04[a]	6.71±0.96[b]	6.41±1.00[a]	6.51±0.95[a]
LCPUFA n-6	14.86±1.91	14.33±1.74	13.86±1.29	14.70±2.60
LCPUFA n -3	6.56±1.13[a]	7.09±0.97[b]	6.86±1.13[a]	6.94±1.04[a]
LC n-3/n-6	0.45±0.13	0.50±0.09	0.50±0.10	0.49±0.12

SFA = Saturated fatty acid, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, LCPUFA= Long Chain Polyunsaturated fatty acids (C≥20, double bonds ≥3). Data are reported as means ± SD. Data with different letters are significantly different.