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# **BENEFICIAL IMPACT ON CARDIOVASCULAR RISK PROFILE OF WATER BUFFALO MEAT CONSUMPTION**

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## ABSTRACT

**BACKGROUND/OBJECTIVES :** Meat is a good source of proteins and irons, yet its consumption has been associated with unfavorable cardiovascular effects. Whether this applies to all types of meat is unclear. We thus aimed to appraise the impact of water buffalo meat consumption on cardiovascular risk profile with an observational longitudinal study.

**METHODS:** Several key cardiovascular risk features were appraised at baseline and at 12-month follow-up in 300 adult subjects divided in groups: recent consumers of water buffalo meat vs. subjects who had never consumed water buffalo meat. In addition, long-standing consumers of water buffalo meat were evaluated.

**RESULTS:** Age, gender, height, body weight and the remaining diet (with the exception of cow meat consumption) were similar across groups 1 and 2. From baseline to follow-up, recent consumers of water buffalo meat change their intake of water buffalo meat from none to  $600\pm 107$  g per week ( $p<0.001$ ), with ensuing reductions in cow meat consumption from  $504\pm 104$  to  $4\pm 28$  ( $p<0.001$ ). At study end, recent consumers of water buffalo meat showed a significant decrease in total cholesterol and triglycerides levels, lower pulse wave velocity, as well as a more blunted response to oxidative stress from baseline to follow-up in comparison to subjects who had never consumed water buffalo meat (all  $p<0.05$ ).

**CONCLUSIONS:** Consumption of buffalo meat appears associated with several beneficial effects on cardiovascular risk profile. Awaiting further randomized clinical trials, this study suggest that a larger consumption of water buffalo meat could confer significant cardiovascular benefits, while continuing to provide a substantial proportion of the recommended daily allowance of protein.

## KEY-WORDS

Cardiovascular disease; Coronary artery disease; Diet; Domestic water buffalo; Meat

## INTRODUCTION

The role of meat in human nutrition has been the focus of intense debate in the last decades. Common wisdom, several pathophysiologic data and observational studies provide an apparently strong mechanistic link between increased meat consumption and adverse health and economic effects,(Keys, 1980; Barnard *et al.*, 1995; Brunner *et al.*, 2008; Trichopoulou *et al.*, 2009) including higher prevalence of adverse cardiovascular risk factors and ensuing atherosclerotic disease. However, given our evolution as hunter-gatherers used to a meat-based, yet non-atherogenic diet,(Cordain *et al.*, 2002) it can be easily explained that moderate amounts of selected meat types and cuts are remarkably safe and may even improve the serum lipid profile.(Watts *et al.*, 1988) Accordingly, several authorities recommend a return to a diet and lifestyle more in line with our paleolithic genome, thus becoming a 21<sup>st</sup> century hunter-gatherer, avidly consuming low-fat meat.(O’Keefe & Cordain, 2004) Conclusions which are also shared by a comprehensive systematic review appraising the evidence in support of a causal link between dietary factors and coronary heart disease, which poignantly concludes that insufficient evidence of association with coronary heart disease is currently present for meat, eggs, and milk. Indeed, not all types and cuts of meats are born equal, as huge variations in composition of meat are well established, and this heterogeneity might by itself explain such uncertainty.(United Nations Food and Agriculture Organization, 2000)

Water buffalo (i.e. domestic Asian water buffalo,) a large bovine animal that pertains to a specific cattle species (*Bubalus bubalis*), is frequently used as a draft, meat and dairy animal livestock in Southern Europe, Northern Africa, Asia, and South America.(Verkaar *et al.*, 2002) Water buffalo milk, in comparison to cow milk, has a much higher concentration of protein (4.5 g vs. 3.2 g every 100 g) and lipid (8.0 g vs. 3.5 g), especially the healthier mono-unsaturated (1.7 g vs. 1.1 g) and poly-unsaturated lipids (0.2 g vs. 0.1 g). Conversely, water buffalo meat, in comparison to ground cow meat, has a lower energy yield (131 Kcal vs. 289 Kcal every 100 g of cooked meat) and a higher concentration of protein (26.8 vs. 24.1 g), but a lower concentration of lipid (1.8 g vs. 20.7

g), especially saturated fatty acids (0.6 g vs. 8.1 g), despite rather similar iron content (2.1 mg vs. 2.4 mg).

Despite such favorable composition in terms of cardiovascular profile, no study has so far appraised the impact of consuming water buffalo meat on cardiovascular risk or events, as established by a dedicated PubMed search strategy updated on June 2009 (“water AND buffalo AND meat AND cardiovascular disease”), and yielding no hits. We hypothesized that water buffalo meat could confer significant benefits in terms of cardiovascular risk profile in comparison to a standard diet. Thus, we designed and conducted a cross-sectional study focusing on cardiovascular risk features, enrolling apparently healthy individuals, and **comparing recent consumers of water buffalo meat vs. subjects who had never consumed water buffalo meat.**

## **METHODS**

### **Study design**

The study was designed as an observational longitudinal study.

### **Participants**

Adult men and women aged 40-69 years, without smoking history, familiar dyslipidemias, or diabetes mellitus and living in the Campania region were included. Two hundred subjects were randomly selected by phone interview, and then invited to participate in the study. **Specifically, they were divided into 2 groups: the first was offered water buffalo meat on a weekly basis for 12 months (1 kg/person\*week; group 1) plus recommendations on life-style measures to decrease cardiovascular risk, whereas the other group received only recommendations on life-style measures to decrease cardiovascular risk (group 2).** In addition, 100 family members of water buffalo farmers were appraised, provided that they were long-standing (>5 years) consumers of water buffalo meat with contact details provided by the Water Buffalo Meat Consortium, Naples, Italy. These subjects

were offered recommendations on life-style measures to decrease cardiovascular risk and then simply followed.

All subjects were screened by an experienced physician by means of clinical history and physical examination to exclude ongoing cardiovascular disease, thus enabling the inclusion also of patients with previous cardiovascular events but without major symptomatic or functional impairment (New York Heart Association class >2). All participants provided written informed consent.

### **Procedures and definitions**

Dietary patterns, including type and quantity of meat in the diet, as well as consumption of butter, olive oil and wine, were assessed with dedicated forms at study entry. Glucose, total serum cholesterol, high-density lipoprotein and triglycerides levels were measured after an overnight fast at study entry and at the end of the study. Saturated fatty acid intake was estimated based on dietary patterns, and divided in 3 categories: low, moderate, and high. Treadmill stress testing with ECG monitoring was performed according to a standard Bruce protocol at study entry, distinguishing test results as normal, abnormal but not showing signs of myocardial ischemia (e.g., if associated with abnormal blood pressure changes), or positive (i.e., showing diagnostic ST-segment changes). Oxidative stress test was conducted at study entry and at the end of the study with a photometric method, with results reported in Fort units (Callegari Formplus). (Cesarone *et al.*, 1999) Common carotid artery intima-media thickness and carotid atherosclerotic plaques were measured at study entry with bidimensional ultrasound with a 10-7 MHz probe (Vivid 7, General Electric) of the right carotid bifurcation, and distinguished as thinner or thicker than 2 mm. Pulse wave velocity was appraised by comparing velocities of posterior tibial artery and homolateral common carotid artery (Callegari Photoplethysmograph). Left ventricular ejection fraction was appraised at study entry and at the end of the study with echocardiogram by means of the Simpsons formula using 4-2 MHz probes (ATL 5000). All tests were performed by experienced laboratory technicians or clinicians unaware of the subject group or dietary habits.

Participants were then followed for an average of  $12\pm 1$  months after enrolment for re-appraisal of changes in cardiovascular risk profile features or incident cardiovascular disease. Hypertension was defined as systolic blood pressure  $>140$  mm Hg on at least 2 measurements at least 24 hours apart or as diastolic blood pressure  $>90$  mm Hg on at least 2 measurements at least 24 hours apart. Myocardial infarction was defined according to the World Health Organization definitions. Stroke was defined as any cerebrovascular neurologic deficit lasting more than 24 hours, and transient ischemic attack as any cerebrovascular neurologic deficit lasting less than 24 hours.

### **Statistical analysis**

Given the lack of previous data on this topic, a formal sample size computation was waived. Nonetheless, 100 subjects per group (total 200) were deemed sufficient to achieve standard errors and 95% confidence intervals of acceptable size. **Continuous variables are reported as mean $\pm$ standard deviation, and were compared with unpaired Gosset t test for between-group comparisons, and with paired Gosset t test for within-group comparisons. Categorical variables are reported as %, and were compared with chi-squared test for both between- and within-group comparisons. Statistical significance was set at the 2-tailed 0.05 level.**

### **RESULTS**

Baseline participants' characteristics are reported in Table 1. Specifically, age, gender, height, body weight were similar across the groups. **Dietary patterns at baseline and follow-up for group 1 and group 2 were also largely similar, including intake of pork, poultry, fish, butter, olive oil, saturated fatty acids, and wine (all  $p>0.05$ ).**

**However, from baseline to follow-up, subjects in group 1 changed their intake of water buffalo meat from none to  $600\pm 107$  g per week ( $p<0.001$ ), with corresponding reductions in cow meat consumption from  $504\pm 104$  to  $4\pm 28$  ( $p<0.001$ ). Appraisal of cardiovascular risk profile (Table 2)**

showed that recent consumers showed from baseline to follow-up a significant decrease in total cholesterol and triglycerides levels, lower pulse wave velocity, as well as a more blunted response to oxidative stress in comparison to ever consumers of water buffalo meat (all  $p < 0.05$ ).

Prevalence of cardiovascular disease (i.e. hypertension, myocardial infarction, or transient ischemic attack/stroke) during the study follow-up trended to increase progressively from long-standing to recent and ever consumers, with the latter group showing the highest, albeit statistically non-significant, rates at follow-up (Table 3).

Subjects with long-standing intake of water buffalo meat had a cardiovascular risk profile similar to those of recent consumers of water buffalo meat, thus suggesting that the benefits associated with this type of meat can persist even during several years of continuous assumption.

## **DISCUSSION**

This cross-sectional study, appraising the impact on cardiovascular risk profile of water buffalo meat consumption, has the following implications: a) a significant shift from cow meat consumption to water buffalo meat consumption appears associated after a few weeks with several benefits of cardiovascular risk markers, including a more favorable blood lipid profile, lower carotid atherosclerotic burden and decreased susceptibility to oxidative stress; b) similar benefits are evident in subjects who have been long-standing consumers of water buffalo meat; c) these findings suggest that water buffalo meat could be recommended as a safer and healthier alternative to cow meat, while continuing to provide a substantial proportion of the recommended daily allowance of protein.

### **Current research context**

Despite the use of meat in human diet since the earliest ages, the precise risk-benefit balance of meat in human nutrition is still unclear. Observational data suggest that a prudent diet rich in



vegetables, fruit, legumes, fish, poultry and whole grains is much safer than a typically Western diet rich in red meat, processed meat, refined grains, French fries, and sweets/desserts.(Heidemann *et al*, 2008). Moreover, the association in this non-experimental studies between increased usage of red meat and unfavorable clinical outcomes, including but not limited to increased total mortality, cancer mortality, and cardiovascular disease mortality, as well as increased healthcare expenditure is apparently strong.(Keys, 1980; Barnard *et al.*, 1995; Brunner *et al.*, 2008; Trichopoulou *et al.*, 2009; Iqbal *et al.*, 2008; Sinha *et al.*, 2009) Specifically, red meat consumption seems associated with unfavorable lipid profile, increased production of free oxygen radicals, and increased blood pressure.(Masala *et al.*, 2008) Thus, worldwide authorities recommend diets mainly based on nonhydrogenated unsaturated fats, whole grains, and an abundance of fruits and vegetables, leaving only a niche role to meat and other animal products.(Hu & Willett, 2002)

The dogma of meat unhealthy role of has however more recently challenged by innovative research focusing on our evolution as hunter-gatherers often consuming meat.(Cordain *et al.*, 2002; O'Keefe & Cordain, 2004) Indeed, our ancestors were often eaten game, yet they disclose limited evidence of atherosclerosis, thus suggesting that even meat-based diets can be non-atherogenic diet.(Cordain *et al.*, 2002) This can be substantiated also by the fact that selected quantities, types and cuts of meat can be remarkably safe and even beneficial in terms of serum cholesterol and triglycerides.(Watts *et al.*, 1988) In addition, extent of meat consumption (e.g. meat vs. pork vs. poultry) has not been repeatedly associated with changes in cardiovascular risk profile, including serum lipids, and thus consumption of moderate amounts of lean meat with appropriately healthier changes in other foods, might appear necessary to meet current dietary recommendations.(Nicklas *et al.*, 1995) This holds even truer in selected population subsets which are at higher risk of developing nutrient deficiencies such as iron status in women of childbearing age with small iron stores.(Tetens *et al.*, 2007) The most comprehensive work to date on the association between coronary heart disease and diet further emphasizes this stance, showing that, whether some

evidence in support of the detrimental role of meat, eggs, and milk can be found in the literature, this appears insufficient to draw definitive conclusions.(Mente *et al.*, 2009)

Meat is a very generic term to identify diet components derived from animals, as nutritional characteristics of meat appear to vary substantially depending on species, cut, and preparation. (United Nations Food and Agriculture Organization, 2000) Water buffalos (*Bubalus bubalis*) are large bovines diffusely used in Europe, Africa, Asia, and America.(Verkaar *et al.*, 2002) Its unique features include differences in milk and meat composition, in comparison to cow. Specifically, water buffalo meat has less fats and is richer in proteins. Yet, before our study, no data were available on the impact of water buffalo meat on cardiovascular risk profile.

### **Implications of the present study**

In the present cross-sectional investigation, we found that subjects who have been consumed routinely since several months or years water buffalo meat show a much healthier and more favorable blood lipid profile, including lower levels of total cholesterol, higher high-density lipoprotein cholesterol, and lower triglycerides. In addition these adults showed a more favorable oxidative-reductive homeostasis, and vascular parameters associated with cardiovascular events, such as pulse wave velocity, carotid intima-media thickness, and carotid atherosclerotic plaques, were all more favorable. Recent consumers of water buffalo meat, despite not showing a similar low-risk cardiovascular profile, proved to be at a much lower risk of adverse clinical events in comparison to those who had never consumed water buffalo meat. Specifically, recent consumers displayed significant reductions in total cholesterol and triglycerides levels, lower pulse wave velocities, and a healthier oxidative-reductive balance.

Our results thus suggest that water buffalo meat could provide a safer and healthier alternative to cow meat. Given the ongoing favor of consumers for specific dairy products originating from water buffalo (e.g. the *mozzarella di bufala* cheese), making this livestock rather available in Europe, as well as its ubiquitous presence in Asia, an increased consumption of water buffalo meat could be

feasible and beneficial. In addition, this change in dietary pattern could lead to a more resource-conscious use of currently available livestock, in keeping with worldwide recommendations.(Walker *et al.*, 2005)

### **Limitations of the present study**

This study has several limitations, including the observational and cross-sectional design.(Biondi-Zoccai *et al.*, 2003) However, these drawbacks should be viewed in light of the novelty of the study subject, and thus the lack of previous data able to inform and guide study design. In addition, all imaging and laboratory studies were performed by technicians and clinicians unaware of dietary patterns of participants. Finally, a major limitation of our work is the reliance on markers of cardiovascular risk profile, i.e. surrogate end-points which have been consistently proved associated with future adverse events, but which are not *per se* adverse cardiovascular events. **Indeed, further follow-up with thorough adjudication of clinically relevant end-points would be a major plus, but was beyond the scope of our work. Moreover, only a randomized clinical trial with adequate power for hard end-points would definitely quantify the role of increasing the consumption of water buffalo meat.**

### **Conclusions**

Consumption of meat of the water buffalo appears associated with several beneficial effects on cardiovascular risk profile, including lower carotid atherosclerotic burden and susceptibility to oxidative stress.

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**FUNDING**

None.

**CONFLICTS OF INTEREST**

None.

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## TABLES

**Table 1.** Baseline and dietary characteristics.

	<b>Long-standing consumers of water buffalo meat (N=100)</b>	<b>Recent consumers of water buffalo meat (N=100)</b>	<b>Ever consumers of water buffalo meat (N=100)</b>	<b>P comparing recent vs ever consumers</b>
Age (years)	55±8	55±9	56±10	>0.05
Female gender	50%	53%	54%	>0.05
Baseline buffalo meat consumption (g/week)	488±108	0*	0	>0.05
Follow-up buffalo meat consumption (g/week)	488±107	600±107*	0	<0.001
Baseline cow meat consumption (g/week)	6±34	504±104*	502±112	>0.05
Follow-up cow meat consumption (g/week)	6±34	4±28*	502±112	<0.001
Baseline pork meat consumption (g/week)	80±8	79±8	72±7	>0.05
Follow-up pork meat consumption (g/week)	40±80	32±79	34±76	>0.05
Baseline poultry meat consumption (g/week)	24±71	44±83	26±67	>0.05
Follow-up poultry meat consumption (g/week)	24±71	44±83	26±68	>0.05
Baseline fish consumption	4%	12%	10%	>0.05
Follow-up fish consumption	4%	12%	10%	>0.05
Butter consumption	0	0	1%	>0.05
Olive oil consumption	100%	100%	100%	>0.05
<b>Baseline saturated fatty acid intake</b>				>0.05
Low	20%	19%	18%	
Moderate	67%	70%	69%	
High	13%	11%	13%	
<b>Follow-up saturated fatty acid intake</b>				>0.05
Low	29%	32%	30%	
Moderate	60%	61%	63%	
High	11%	7%	7%	
Wine consumption	96%	94%	92%	>0.05

\*p<0.05 at paired t test comparing baseline vs. follow-up within the same group



**Table 2.** Cardiovascular risk profile.

	<b>Long-standing consumers of water buffalo meat (N=100)</b>	<b>Recent consumers of water buffalo meat (N=100)</b>	<b>Ever consumers of water buffalo meat (N=100)</b>	<b>P comparing recent vs ever consumers</b>
Baseline body weight (kg)	74±9	76±9	79±12	>0.05
Follow-up body weight (kg)	74±9	76±9	79±12	>0.05
Baseline hypertension	17%	19%	20%	>0.05
Follow-up hypertension	17%	19%	26%	>0.05
Baseline cholesterol (mg/dL)	167.3±16.9	223.4±27.3*	229.0±31.0	<0.001
Follow-up cholesterol (mg/dL)	168.8±15.8	193.9±18.5*	230.9±29.6	<0.001
Baseline HDL (mg/dL)	48.6±5.6	33.9±5.0	34.5±5.9	<0.001
Follow-up HDL (mg/dL)	49.0±6.4	33.9±5.0	34.5±5.9	<0.001
Baseline triglycerides (mg/dL)	129.2±29.4	181.0±38.0*	189.5±46.9	<0.001
Follow-up triglycerides (mg/dL)	130.8±29.2	156.2±34.8*	189.5±46.9	<0.001
Baseline glucose (mg/dL)	84.7±11.8	90.4±10.6	89.4±11.1	0.017
Follow-up glucose (mg/dL)	86.7±11.8	87.2±13.3	87.8±13.6	>0.05
Baseline carotid IMT (mm <sup>2</sup> )	8.0±1.3	9.7±1.6	9.7±1.7*	<0.001
Follow-up carotid IMT (mm <sup>2</sup> )	8.0±1.3	9.7±1.6	10.2±1.5*	0.017
Baseline carotid plaques <2 mm				<0.001
0	97%	67%	65%	
1	3%	24%	26%	
≥2	0	9%	9%	
Baseline carotid plaques >2 mm				0.003
0	99%	86%	80%	
1	1%	10%	12%	
≥2	0	4%	8%	
Follow-up carotid plaques <2 mm				<0.001
0	97%	67%	53%	
1	3%	24%	33%	
≥2	0	9%	14%	
Follow-up carotid plaques >2 mm				0.003
0	98%	85%	76%	
1	2%	10%	16%	
≥2	0	5%	8%	
Baseline pulse wave velocity (cm/sec)	899.5±67.5*	1114.5±157.9*	1098.5±165.1*	<0.001

Follow-up pulse wave velocity (cm/sec)	927.8±66.9*	1059.4±109.2*	1149.4±169.5*	<0.001
Baseline oxidative stress test (Fort)	273.0±24.9	354.3±38.5*	350.7±39.5	<0.001
Follow-up oxidative stress test (Fort)	275.0±27.9	278.7±28.3*	360.3±43.0	<0.001
Baseline LVEF (%)	57.5±4.4	56.9±4.2	57.0±4.2	>0.05
Follow-up LVEF (%)	57.6±4.3	57.1±4.0	57.3±4.3	>0.05
ECG stress test				>0.05
Normal	97%	96%	96%	
Abnormal, but not ischemic	3%	2%	2%	
Ischemic	0	2%	2%	

\*p<0.05 at paired t test comparing baseline vs. follow-up within the same group

HDL=high-density lipoprotein; IMT=intima-media thickness; LVEF=left ventricular ejection

fraction

**Table 3.** Cardiovascular events.

	<b>Long-standing consumers of water buffalo meat (N=100)</b>	<b>Recent consumers of water buffalo meat (N=100)</b>	<b>Ever consumers of water buffalo meat (N=100)</b>	<b>P</b>
Baseline acute myocardial infarction	0	1%	1%	>0.05
Follow-up acute myocardial infarction	0	1%	2%	>0.05
Baseline stroke or transient ischemic attack	1%	1%	1%	>0.05
Follow-up stroke or transient ischemic attack	1%	1%	4%	>0.05

## **FIGURES**

**Figure 1.** Total serum cholesterol levels according to water buffalo meat consumption, at baseline and follow-up.

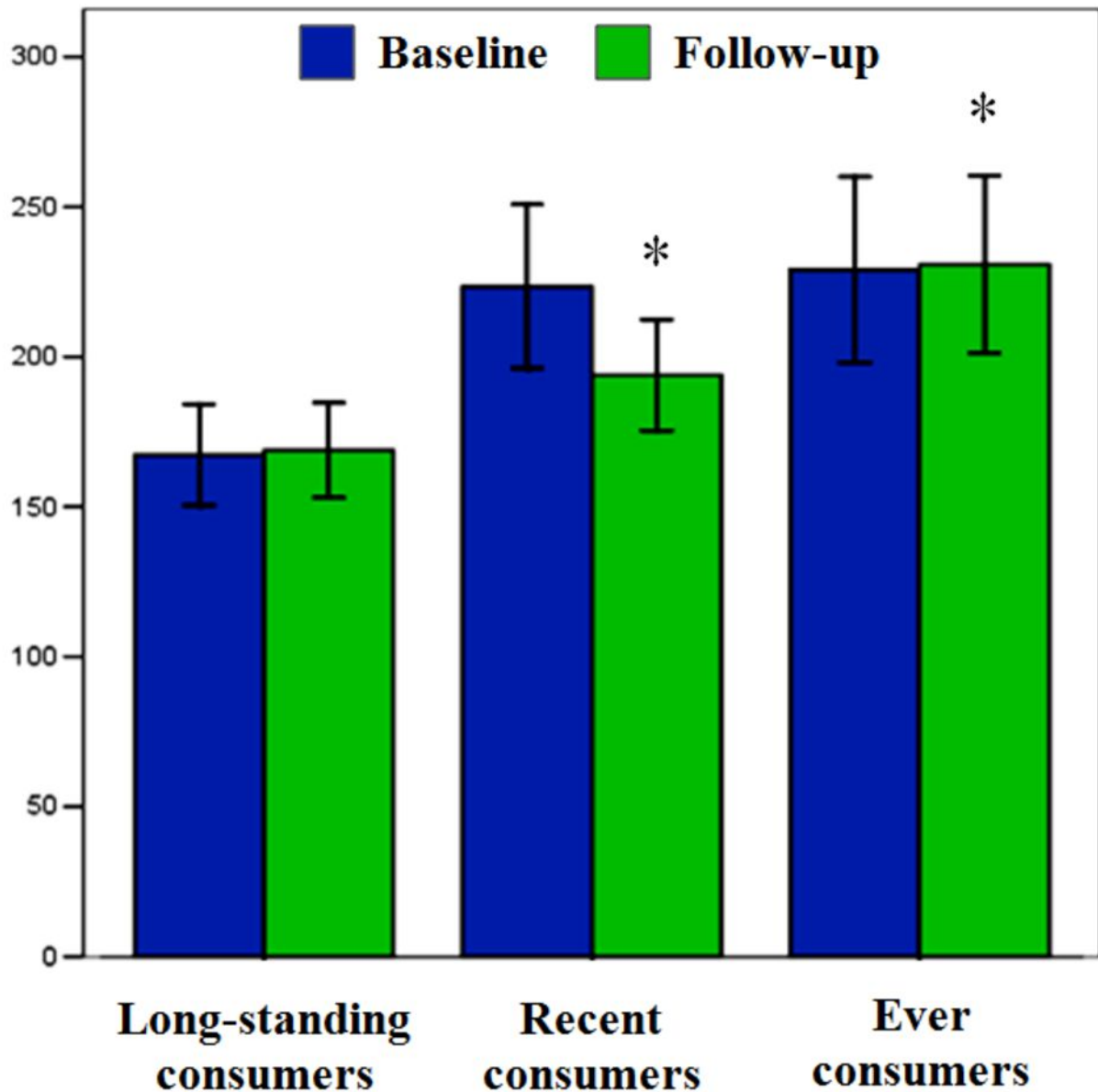
**Figure 2.** Ultrasound measurement of intima-media thickness in the right common carotid artery according to water buffalo meat consumption, at baseline and follow-up.

**Figure 3.** Doppler ultrasound measurement of pulse wave velocity according to water buffalo meat consumption, at baseline and follow-up.

**Figure 4.** Response to oxidative stress test according to water buffalo meat consumption, at baseline and follow-up.

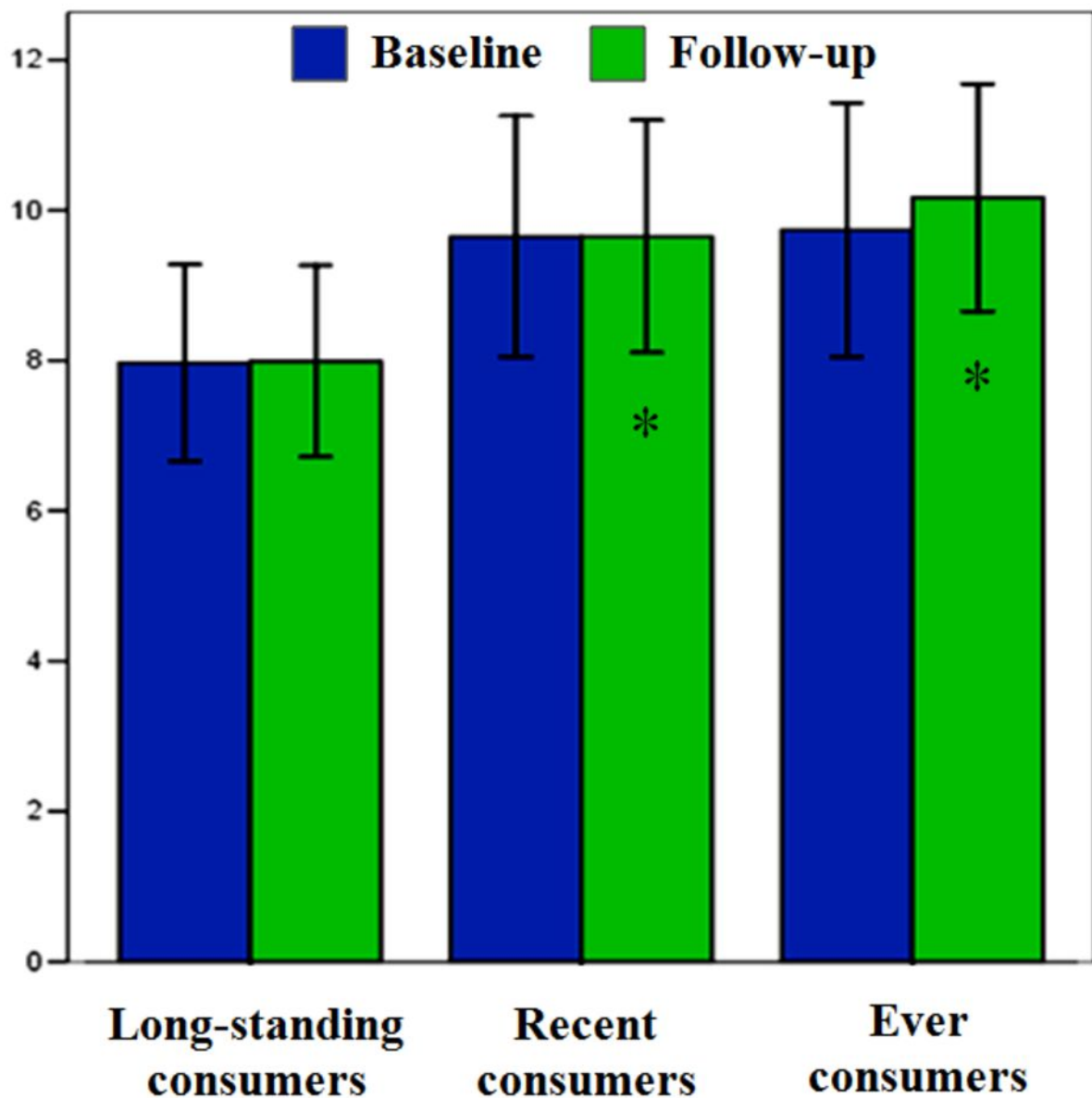
**Figure 5.** Prevalence of cardiovascular disease according to water buffalo meat consumption ( $p>0.05$ ).

Total cholesterol (mg/dL)



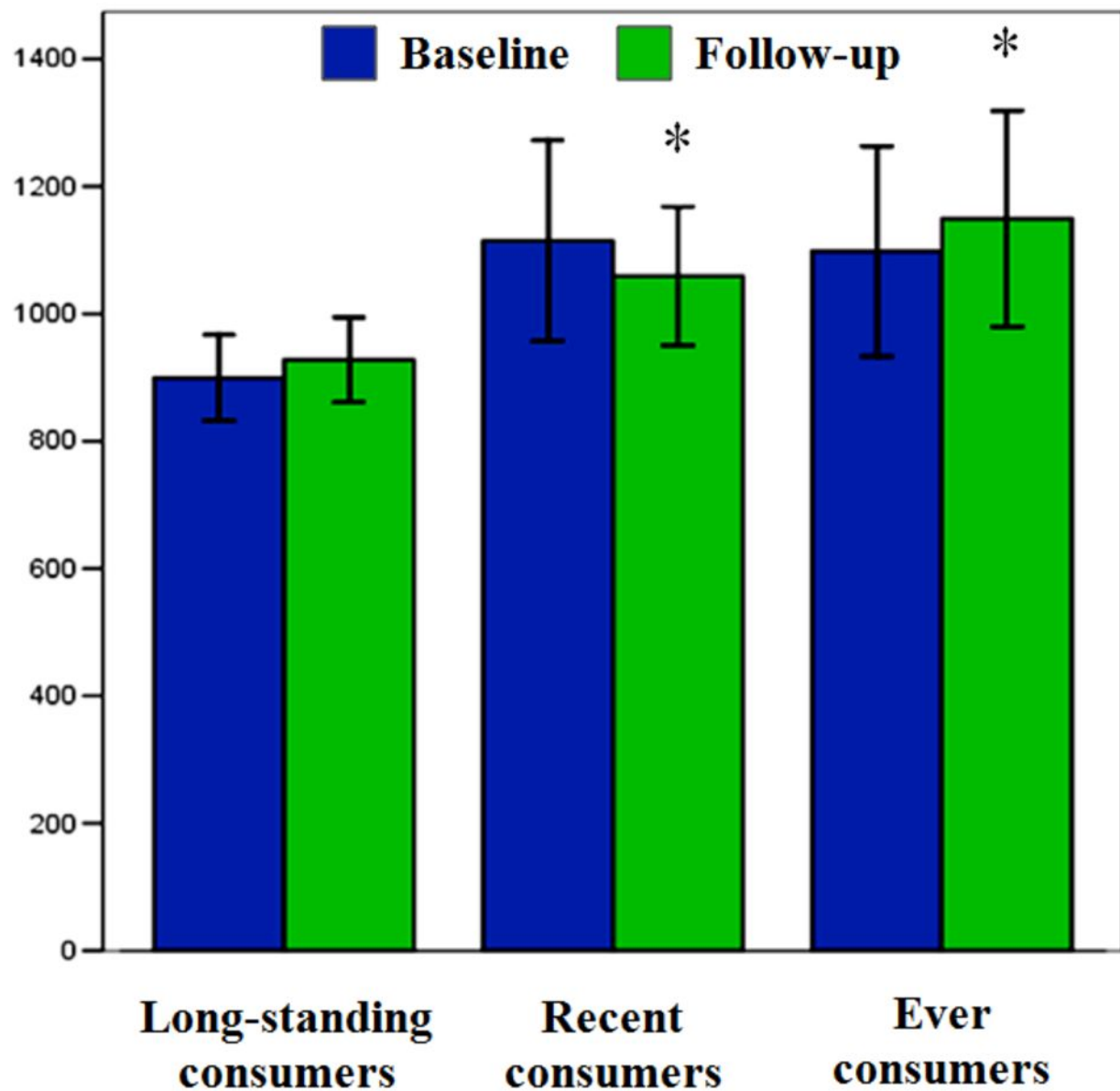
\* $p < 0.001$  comparing recent vs. ever consumers at follow-up

**Right common carotid  
intima-media thickness**



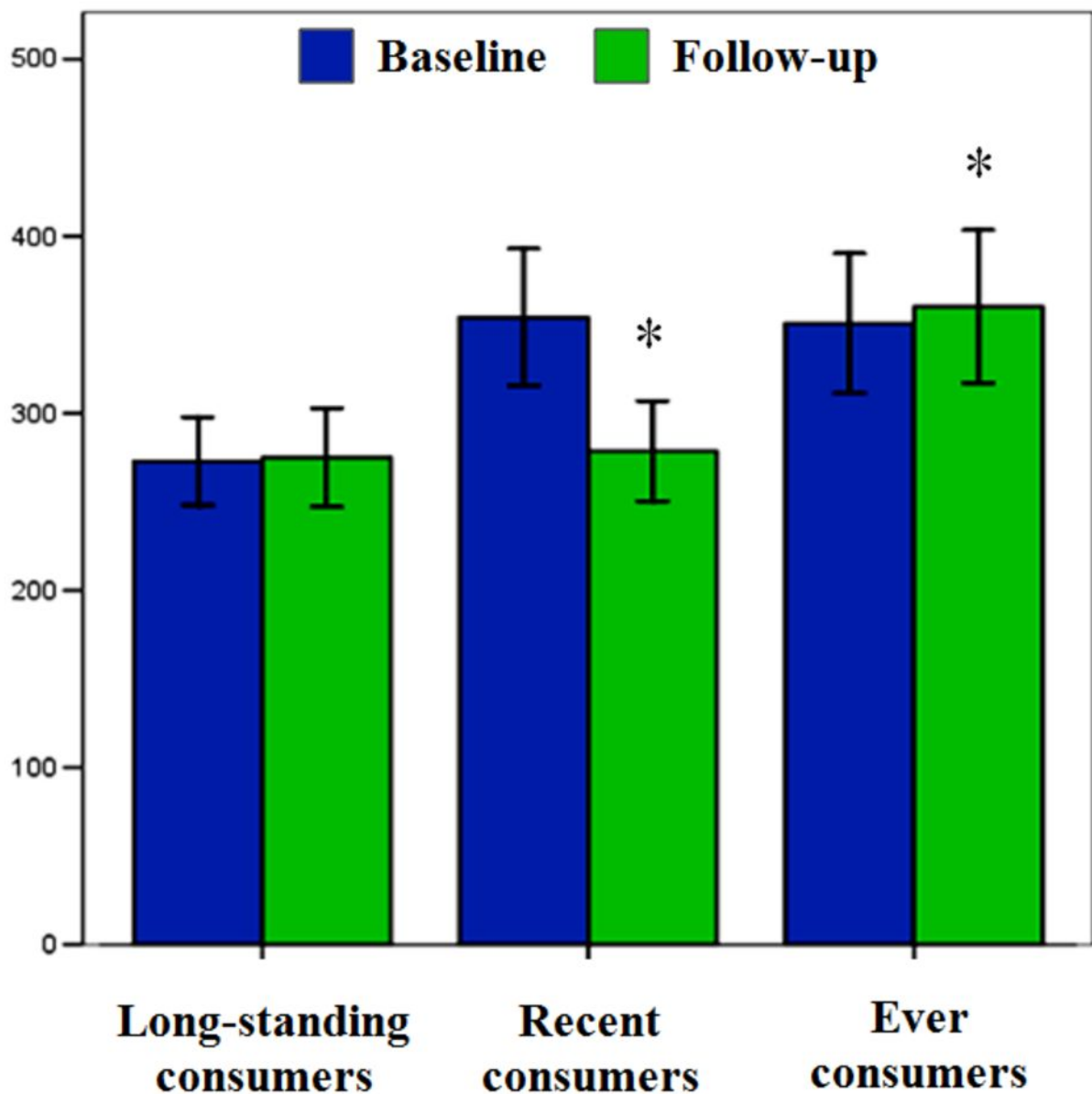
\*p=0.017 comparing recent vs. ever consumers at follow-up

**Pulse wave velocity**



\*p<0.001 comparing recent vs. ever consumers at follow-up

# Oxidative stress test



\*p<0.001 comparing recent vs. ever consumers at follow-up



