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Effect of dietary fatty acids on calcified aortic root of mice with metabolic syndrome

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Running title: Fatty acids on aortic calcification in MetS

ABSTRACT

Metabolic syndrome (MetS) is associated with obesity, dyslipidemia, type 2 diabetes, and chronic low-grade inflammation. The aim of this study was to determine the role of high-fat low-cholesterol diets (HFLCDs) rich in SFAs (HFLCD-SFAs), MUFAs (HFLCD-MUFAs) or MUFAs plus omega-3 long-chain PUFAs (HFLCD-PUFAs) on vascular calcification by the modulation of RANKL/RANK/OPG system in aortic roots from $Lep^{ob/ob}LDLR^{-/-}$ mice. Animals fed with HFLCD-SFAs had increased weight and a greater atheroma plaque size, calcification, and RANKL/CATHK expression in aortic root than mice on MUFA-enriched diets, the latters increasing OPG expression in aortic roots. Our study demonstrates that compared to dietary SFAs, MUFAs from olive oil protect against atherosclerosis by interfering on vascular calcification via the RANKL/RANK/OPG system in the setting of MetS. These findings open opportunities for developing novel nutritional strategies with olive oil as the most important dietary source of MUFAs (notably oleic acid) to prevent cardiovascular complications in the MetS.

Keywords: olive oil, RANKL, osteoprotegerin, metabolic syndrome, vascular calcification, atherosclerosis.

INTRODUCTION

Dyslipidemia, insulin resistance, and obesity, the defining components of the metabolic syndrome (MetS), are well-known risk factors implicated in the aetiology and pathogenesis of certain cardiovascular diseases (CVDs) such as atherosclerosis, the main cause of CVD death in developed and some developing countries.[1] Recently, increasing interest has focused on understanding how atherosclerotic pathology is related to a common plaque constituent: calcium mineral deposits. Important studies in the last decade have now spawned a model wherein calcification in atherosclerotic plaque is viewed as an active, complex, and presumably regulated process that exhibits intriguing similarities to bone remodelling.[2] Atherosclerotic calcification appears to result from induction of osteogenic differentiation in subpopulations of vascular cells by inflammatory factors such as receptor activator for nuclear factor-κB (RANK) ligand (RANKL) and cathepsin K (CATHK).[3] In contrast, osteoprotegerin (OPG) is a glycoprotein member of the TNF receptor superfamily that functions as a soluble decoy substrate for RANK and competes with RANKL, inhibiting RANK-RANKL interactions for osteoclast proliferation and differentiation, and bone degradation.[4] Interestingly, it has been reported that OPG-deficient mice develop both osteoporosis and arterial calcification[5] and that administration of soluble OPG or transgenic OPG overexpression may retain the development of atherosclerosis.[6]

Previous studies carried out by our group have reported that the type of dietary fat in the meals may have a greater impact on osteoclast induction and maturation via RANKL/RANK/OPG system.[7] Dietary saturated

fatty acids (SFAs) as compared to monounsaturated fatty acids (MUFAs) and omega-3 long-chain polyunsaturated fatty acids (PUFAs) have been found to be the most potent inducer of *in vitro* osteoclastogenesis. However, it is unknown whether a dietary fat rich in MUFAs without (olive oil) or with omega-3 long chain PUFAs (olive oil + EPA and DHA) compared to a dietary fat rich in SFAs (cow´s milk cream) may have benefits in atherosclerosis and vascular calcification by the modulation of the RANKL/RANK/OPG system. Taken together, the global aim of this paper is to assess the influence of diets enriched in SFAs, MUFAs or MUFAs + omega-3 long-chain PUFAs on calcified aortic roots from mice with MetS ($Lep^{ob/ob}LDLR^{-/-}$).

MATERIALS AND METHODS

Fatty acid composition of dietary fats

The fatty acid composition of dietary fats [cow's milk cream, rich in SFAs; refined olive oil, rich in MUFAs; and refined olive oil plus eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), rich in MUFAs and omega-3 long-chain PUFAs] was determined by the method described in EEC/796/2002,[8] using a gas chromatography system (HP-5890, Hewlett-Packard, Palo-Alto, USA) equipped with flame ionization detector and a SP-2380 capillary column (Supelco, Bellefonte, USA, 30 m x 0.32 mm) packed with cyanopropyl siloxane (0.25 μm). The initial column temperature was 165 ºC, which was held for 10 min, then programmed from 165 ºC to 200 ºC at 1.5 ºC/min. Injector and detector temperature were 250 ºC, with the carrier gas H_2. The fatty acid composition of different dietary fats is detailed in **Table 1**.

Animal diets and experimental design

Male Lep$^{ob/ob}$LDLR$^{-/-}$ mice bred onto a C57BL/6J background (B6.Cg-Lepob Ldlrtm1Her/J, The Jackson Laboratory, Bar Harbor, ME, USA) was used for the study. These mice are obese and develop plasma lipid alterations that closely reflect MetS-related hyperlipidaemia.[9] All diets were prepared by Panlab Laboratoires (SAFE, Augy, France) and presented as pellets to the animals. Mice received one of the following diets for 8 weeks: a standard normal-fat diet (low-fat low-cholesterol diet, LFLCD) containing 3% energy as fat, used as control, or high-fat low-cholesterol diets (HFLCDs), which contained 24% energy as fat. All the diets were based on the standard rodent diet A04-10, containing 0.01% cholesterol, 20 mg/kg BHT, and 3% binder. Three different HFLCDs were prepared by replacing the fat source from A04-10 diet by cow's milk cream (21% energy) (HFLCD-SFAs), refined olive oil (21% energy) (HFLCD-MUFAs) or refined olive oil (20% energy) plus EPA+DHA in the form of ethyl esters (1% energy) (HFLCD-PUFAs). The cow's milk cream provided an additional amount of 0.006% cholesterol by weight. All the diets contained equal proportion of protein (19.5% energy) and carbohydrate was used to adjust the total energy content.

After weaning, mice were randomly allocated into 4 groups (n = 10 per group) as follows: (1) group that received LFLCD; (2) group that received HFLCD-SFAs; (3) group that received HFLCD-MUFAs; and (4) group that received HFLCD-PUFAs. Body weight, food, and water intake were daily evaluated (data not shown). Sacrifice of all animals was carried out within animal facilities (Instituto de Biomedicina de Sevilla, IBiS) at the beginning of the light cycle and after 10 h of food deprivation. Animals were

euthanized with an overdose of pentobarbital (1:10 in PBS, 150 mg/kg body weight). Heart samples were collected upon sacrifice. All animal protocols received appropriate institutional approval (Animal Care and Use Committee of the University of Seville) and were performed according to the official rules formulated in the Spanish law on the care and use of experimental animals (UE Directive of 2010: 2010/63/UE; RD 53/2013).

Immunohistochemistry

Mouse heart was dissected, fixed in 1% paraformaldehyde, and embedded in paraffin. Size of atherosclerotic lesions was determined as previously described.[10] Serial sections (6 μm) of the aortic root were cut and stained with haematoxylin-eosin (HE) for morphometric analysis and routine qualitative examination of collagen content, necrosis, and amount of inflammatory B (B220) and C (CD3) cells. Alizarin Red (AR) staining was performed to detect vascular calcification. Corresponding sections on separate slides were stained with antibodies against RANKL, OPG, and CATHK (AbCAM, Cambridge, UK). Subsequently, slides were incubated with an avidin-biotin-complex (Elite vector-stain ABC, PK-6100) and stained with AEC (Vector, peroxidase substrate kit, SK-4200). Three different slides from each animal were used for the quantification of either histological and immunohistochemical staining. Samples were captured at 20× magnifications by a Leica DM3000 light microscope (Leica, Wetzlar, Germany) and an independent operator, in a blinder manner, performed histological analyses using Quantimet with Qwin3 quantification software (Leica).

RNA isolation and RT-qPCR

Total RNA was extracted by using Trisure Reagent (Bioline). RNA quality was assessed by A_{260}/A_{280} ratio in a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). Briefly, RNA (250 ng) was subjected to reverse transcription (iScript, Bio-Rad, Madrid, Spain). An amount of 20 ng of the resulting cDNA was used as a template for real-time PCR amplifications. The mRNA levels for specific genes were determined in a CFX96 system (Bio-Rad). For each PCR reaction, cDNA template was added to Brilliant SYBR green QPCR Supermix (Bio-Rad) containing the primer pairs for either gene or for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a housekeeping gene (**Table 2**). All amplification reactions were performed in triplicate and average threshold cycle (Ct) numbers of the triplicates were used to calculate the relative mRNA expression of candidate genes.[11] The magnitude of change of mRNA expression for candidate genes was calculated by using the standard $2^{-(\Delta\Delta Ct)}$ method. All data were normalized to endogenous reference (GAPDH) gene content and expressed as relative fold-change of control.

Statistical analysis

All values in the figures and text are expressed as the arithmetic mean ± SD. Data were evaluated with Graph Pad Prism Version 5.01 software. The statistical significance of any difference in each parameter among the groups was evaluated by one-way analysis of variance (ANOVA), using Tukey's test for multiple comparison analysis. *P* values of <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The literature often refers to “Mediterranean diet” as protective due to its content in oleic acid (MUFA) and minor constituents from olive oil, which play a role in the prevention of many pathological conditions such as cardiovascular and rheumatic diseases by improving classical risk factors and by promoting intense anti-inflammatory effects.[12-16] This study is the first to address the effects of predominant fatty acids in dietary fats on vascular calcification in mice with MetS. We used male mice homozygous for the *ob/ob* and *Ldlr*^{tm1Her} targeted mutations lacking the hormone leptin and LDL receptor onto a C57BL/6J background. When subjected to a HFLCD rich in SFAs, these animals become obese, hyperinsulinemic, and develop severe dyslipidaemia (**Table 3**), which are pathological manifestations analogous to those characteristics of human MetS.[17] Atherosclerotic lesion development (**Fig. 1A**) and calcification (**Fig. 1B**) were assessed in the aortic root of Lep$^{ob/ob}$LDLR$^{-/-}$ mice after 8 weeks feeding on LFLCD (control) and HFLCDs (HFLCD-SFAs, HFLCD-MUFAs or HFLCD-PUFAs). As depicted in **Fig. 1C**, the animals fed with the HFLCDs (HFLCD-SFAs > HFLCD-MUFAs = HFLCD-PUFAs) had an increased plaque size by means of HE staining when compared to animals fed with the LFLCD. Similar pattern for calcification by means of AR staining was observed in plaque areas (**Fig. 1D**).

Calcification is a hallmark of atherosclerosis but its role in plaque rupture has long been controversial.[18] It has been proposed that atherosclerotic plaque proceeds through progressive stages where instability and rupture can be followed by calcification, perhaps to provide stability to an unstable lesion.[19] Current experimental models indicate that vascular calcification likely involves signalling mediators, such as OPG, RANKL, and

CATHK, traditionally associated with bone remodelling.[20] Vascular calcification was also assessed in plaque areas of aortic root of $Lep^{ob/ob}LDLR^{-/-}$ mice after 8 weeks feeding on LFLCD (control) or HFLCDs (HFLCD-SFAs, HFLCD-MUFAs or HFLCD-PUFAs) by immunohistochemical staining of RANKL (**Fig. 2A**), OPG (**Fig. 2B**), and CATHK (**Fig. 2C**). Immunohistochemical quantification revealed that only animals fed with HFLCD-SFAs increased RANKL (**Fig. 2D**) and CATHK (**Fig. 2F**) positive cells in plaque areas**.** However, it was noteworthy to observe a markedly decreased accumulation of OPG positive cells in plaque areas of animals fed with HFLCD-SFAs when compared to other dietary groups (**Fig. 2E**). HFLCD-SFAs also increased RANK (**Fig. 3A**) and decreased OPG (**Fig. 3B**) gene expression, in addition to an up-regulation of CATHK gene (**Fig. 3C**) in the aortic roots of animals. Our findings are in line with previous evidence of the effects of high intakes of SFAs on plaque instability and inflammation (including a rise in TNFα serum levels), and of MUFAs and PUFAs on cardiovascular protection.[9,21,22] Furthermore, the osteoclastogenic potency of dietary fatty acids (SFAs >>> MUFAs = PUFAs) has recently been associated with an unbalance of pro-osteoclastogenic (RANKL) and anti-osteoclastogenic (OPG) cytokines.[7] Taken together, these observations demonstrate that fatty acids in pro-obesity/obesogenic diets are pivotal in terms of regulating vascular calcification in the context of atherosclerosis in the setting of MetS.

Our study demonstrates that compared to dietary SFAs, MUFAs from olive oil prevent against atherosclerosis by interfering on vascular calcification via RANKL/RANK/OPG system and CATHK in the setting of MetS.

Abbreviations

AR: alizarin red, **CATHK:** cathepsin K, **CVD:** cardiovascular disease, **DHA:** docosahexaenoic acid, **EPA:** eicosapentaenoic acid, **HE:** haematoxylin-eosin, **HFLCD:** high-fat low-cholesterol diet, **LDLR:** low-density lipoprotein receptor, **LFLCD:** low-fat low-cholesterol diet, **MetS:** metabolic syndrome, **MUFA:** monounsaturated fatty acid, **OPG:** osteoprotegerin, **PUFA:** polyunsaturated fatty acid, **RANK:** receptor activator of nuclear factor κB, **RANKL:** receptor activator of nuclear factor κB ligand, **SFA:** saturated fatty acid.

Conflicts of interest

The authors state no conflict of interest.

Acknowledgements

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References

1. A. Vishnu, M. J. Gurka and M. D. DeBoer, The severity of the metabolic syndrome increases over time within individuals, independent of baseline metabolic syndrome status and medication use: The Atherosclerosis Risk in Communities Study. *Atherosclerosis*, 2015, 243, 278-285.

2. R. Nicoll, J. McLaren Howard and M. Y. Henein, A Review of the effect of diet on cardiovascular calcification. *Int. J. Mol. Sci.,* 2015, 16, 8861-8883.

3. N. Schmidt, C. Brandsch, A. Schutkowski, F. Hirche and G. I. Stangl, Dietary vitamin D inadequacy accelerates calcification and osteoblast-like cell formation in the vascular system of LDL receptor knockout and wild-type mice. *J. Nutr.,* 2014, 144, 638-646.

4. C. Perez de Ciriza, M. Moreno, P. Restituto, G. Bastarrika, I. Simon, I. Colina, et al, Circulating osteoprotegerin is increased in the metabolic syndrome and associates with subclinical atherosclerosis and coronary arterial calcification. *Clin. Biochem.,* 2014, 47, 272–278.

5. B. J. Bennett, M. Scatena, E. A. Kirk, M. Rattazzi, R. M. Varon, M. Averill, et al. Atherosclerosis and lipoproteins. *Arterioscler. Thromb. Vasc. Biol.,* 2006, 26, 2117-2124.

6. A. Gluba-Brzozka, M. Michalska-Kasiczak, B. Franczyk, M. Nocun, P. Toth, M. Banach, et al, Markers of increased atherosclerotic risk in patients with chronic kidney disease: a preliminary study. *Lipids Health Dis.,* 2016, 15, 22.

7. M. C. Naranjo, I. Garcia, B. Bermudez, S. Lopez, R. Abia, F. J. G. Muriana, et al., Acute effects of dietary fatty acids on osteoclastogenesis via RANKL/RANK/OPG system. *Mol. Nutr. Food Res.,* 2016, 60, 2505-2513.

8. S. Montserrat-de la Paz, M. C. Naranjo, B. Bermudez, S. Lopez, W. Moreda, R. Abia, et al, Postprandial dietary fatty acids exert divergent inflammatory responses in retinal-pigmented epithelium cells. *Food Funct.,* 2016, 7, 1345-1353.

9. S. Montserrat-de la Paz, M. C. Naranjo, S. Lopez, R. Abia, F. J. G. Muriana and B. Bermudez, Niacin and olive oil promote skewing to the M2 phenotype in bone marrow-derived macrophages of mice with metabolic syndrome. *Food Funct.,* 2016, 7, 2233-2238.

10. M. J. Gijbels, M. van der Cammen, L. J. van der Laan, J. J. Emeis, L. M. Havekes, M. H. Hofker, et al, Progression and regression of atherosclerosis in apoe3-leiden transgenic mice: An immunohistochemical study. *Atherosclerosis* 1999, 143, 15-25.

11. S. Montserrat-de la Paz, M. C. Naranjo, S. Lopez, R. Abia, F. J. G. Muriana and B. Bermudez, Niacin and its metabolites as master regulators of macrophage activation. *J. Nutr. Biochem.,* 2017, 39, 40-47.

12. M. Guasch-Ferre, F. Hu, M. A. Martinez-Gonzalez, M. Fito, M. Bullo, R. Estruch, et al, Olive oil intake and risk of cardiovascular disease and mortality in the PREDIMED Study. *BMC Med.,* 2014, 12*,* 78.

13. C. Sales, F. Oliviero and R. Spinella, The mediterranean diet model in inflammatory rheumatic diseases. *Reumatismo,* 2009, 61, 10-14.

14. S. Montserrat-de la Paz, B. Bermudez, S. Lopez, M. C. Naranjo, Y. Romero, M. J. Bando-Hidalgo, et al, Exogenous fatty acids and niacin on acute prostaglandin D2 production in human myeloid cells. *J. Nutr. Biochem.,* 2017, 39, 22-31.

15. S. Lopez, B. Bermudez, S. Montserrat-de la Paz, S. Jaramillo, R. Abia and F. J. G. Muriana, Virgin olive oil in hypertension. *Curr. Vasc. Pharmacol.,* 2016, 14, 323-329.

16. S. Montserrat-de la Paz, B. Bermudez, M. P. Cardelo, S. Lopez, R. Abia and F. J. G. Muriana, Olive oil and postprandial hyperlipidemia: Implications for atherosclerosis and metabolic syndrome. *Food Funct.,* 2016, 7, 4734-4744.

17. A. J. Kennedy, K. L. J. Ellacott, V. L. King and A. H. Hasty, Mouse models of the metabolic syndrome. *Dis. Model Mech.,* 2010, 3, 156-166.

18. N. Alexopoulos, P. Raggi, Calcification in atherosclerosis. *Nat. Rev. Cardiol.,* 2009, 6, 681-688.

19. T. M. Doherthy, K. Asotra, L. A. Fitzpatrick, J. H. Qiao, D. J. Wilkin, R. C. Detrano, et al, Calcification in atherosclerosis: Bone biology and chronic inflammation at the arterial crossroads. *Proc. Natl. Acad. Sci. USA,* 2003, 100, 11201-11206.

20. E. Harper, H. Forde, C. Davenport, K. D. Rochfort, D. Smith, P. M. Cummins, et al, Vascular calcification in type-2 diabetes and cardiovascular disease: integrative roles for OPG, RANKL and TRAIL. *Vasc. Pharmacol.,* 2016, 82, 30-40.

21. S. Sudheendran, C. C. Chang and R. J. Deckelbaum, N-3 vs. Saturated fatty acid: Effects on the arterial wall. *Prostaglandins Leukot. Essent. Fatty Acids,* 2010, 82, 205-209.

22. S. Montserrat-de la Paz, M. C. Naranjo, S. Lopez, R. Abia, F. J. G. Muriana and B. Bermudez, Olive oil, compared to a saturated dietary fat, has a protective role on atherosclerosis in niacin-treated mice with metabolic syndrome. *J. Funct. Foods,* 2016, 26, 557-564.

Figure Legends

Figure 1. Representative pictures of (**A**) HE and (**B**) AR stained sections (original magnification ×40) and quantification of (**C**) aortic root plaque area and (**D**) percentage of calcification in plaque area in $Lep^{ob/ob}LDLR^{-/-}$ mice fed with LFLCD, HFLCD-SFAs, HFLCD-MUFAs, and HFLCD-PUFAs during 8 weeks. Values are presented as the mean ± SD (n = 10) and those marked with different lowercase letter are statistically different ($P < 0.05$).

Figure 2. Representative pictures of immunohistochemically stained sections (original magnification ×40) for (**A**) RANKL, (**B**) OPG, and (**C**) CATHK positive cells and their quantification (**D**, **E**, and **F**, respectively) in aortic root plaque area of $Lep^{ob/ob}LDLR^{-/-}$ mice fed with LFLCD, HFLCD-SFAs, HFLCD-MUFAs, and HFLCD-PUFAs during 8 weeks. Values are presented as the mean ± SD (n = 10) and those marked with different lowercase letter are statistically different ($P < 0.05$).

Figure 3. Gene expression analysis of (**A**) RANK, (**B**) OPG, and (**C**) CATHK in aortic root plaque area of $Lep^{ob/ob}LDLR^{-/-}$ mice fed with LFLCD, HFLCD-SFAs, HFLCD-MUFAs, and HFLCD-PUFAs during 8 weeks. Values are presented as the mean ± SD (n = 10) and those marked with different lowercase letter are statistically different ($P < 0.05$).

Table 1. Fatty acid composition of dietary fats.

	Cow's milk cream	Refined olive oil	Refined olive oil plus EPA + DHA
Fatty acid	**g/100 g of fatty acid**		
10:0, capric	2.5 ± 0.1	-	-
12:0, lauric	3.1 ± 0.4	-	-
14:0, myristic	10.9 ± 0.9	-	-
16:0, palmitic	35.5 ± 0.8	20.4 ± 0.9	20.5 ± 0.6
16:1(n-7), palmitoleic	3.6 ± 0.3	1.0 ± 0.2	0.8 ± 0.1
18:0, stearic	11.5 ± 0.8	5.7 ± 0.1	4.5 ± 0.4
18:1(n-9), oleic	25.3 ± 0.7	61.9 ± 1.2	61.5 ± 1.0
18:2(n-6), linoleic	4.3 ± 0.8	8.0 ± 0.7	8.0 ± 0.5
18:3(n-3), α-linolenic	0.4 ± 0.1	1.0 ± 0.1	0.9 ± 0.0
20:5(n-3), eicosapentaenoic	-	-	0.9 ± 0.1
22:6(n-3), docosahexaenoic	-	-	0.7 ± 0.1
Others	3.0 ± 1.7	2.1 ± 1.1	2.0 ± 0.9
SFAs	63.5 ± 1.9[a]	26.1 ± 1.0[b]	25.0 ± 0.9[b]
MUFAs	28.9 ± 0.8[b]	62.8 ± 1.4[a]	62.4 ± 1.0[a]
PUFAs	4.7 ± 0.8[c]	9.0 ± 0.7[b]	10.6 ± 0.7[a]

Values are expressed as the mean ± SD (n = 3) and those marked with different lowercase letter in the same row are statistically different (P < 0.05).

Table 2. Detailed information about primers' sequences used in this study.

Target	GenBank accession Number	Direction	Sequence (5´→3´)
CATK	NM_000396	Forward	TTCTGCTGCTACCTGTGGTG
		Reverse	CCAGGTGGTTCATAGCCAGT
GAPDH	NM_001289746	Forward	CACATGGCCTCCAAGGAGTAAG
		Reverse	CCAGCAGTGAGGGGTCTCTCT
OPG	NM_002546	Forward	GGCAACACAGCTCACAAGAA
		Reverse	CTGGGTTTGCATGCCTTTAT
RANK	NM_001270949	Forward	GGTGCAGCCTCTAACTCCTG
		Reverse	TTGAGACCAGGCTGGGTAAC

Table 3. Food intake, final body and relative weights, and biochemical analysis.

	Control	SFAs	MUFAs	PUFAs
Food intake (g/wk/animal)	26.17 ± 4.43[a]	30.07 ± 5.21[a]	29.01 ± 7.72[a]	30.49 ± 5.53[a]
Final body weight (g)	28.62 ± 2.71[c]	35.19 ± 2.32[a]	30.93 ± 1.11[b]	30.67 ± 1.49[b]
Body weight gain (g)	8.97 ± 0.70[c]	16.13 ± 1.88[a]	10.32 ± 0.45[b]	10.41 ± 0.57[b]
Serum parameters				
TC (mmol/L)	3.61 ± 0.22[b]	4.12 ± 0.18[a]	3.97 ± 0.19[a]	4.01 ± 0.14[a]
TG (mmol/L)	0.37 ± 0.08[c]	0.92 ± 0.13[a]	0.58 ± 0.09[b]	0.59 ± 0.07[b]

Values are expressed as the mean ± SD (n = 10) and those marked with different lowercase letter in the same row are statistically different ($P < 0.05$).

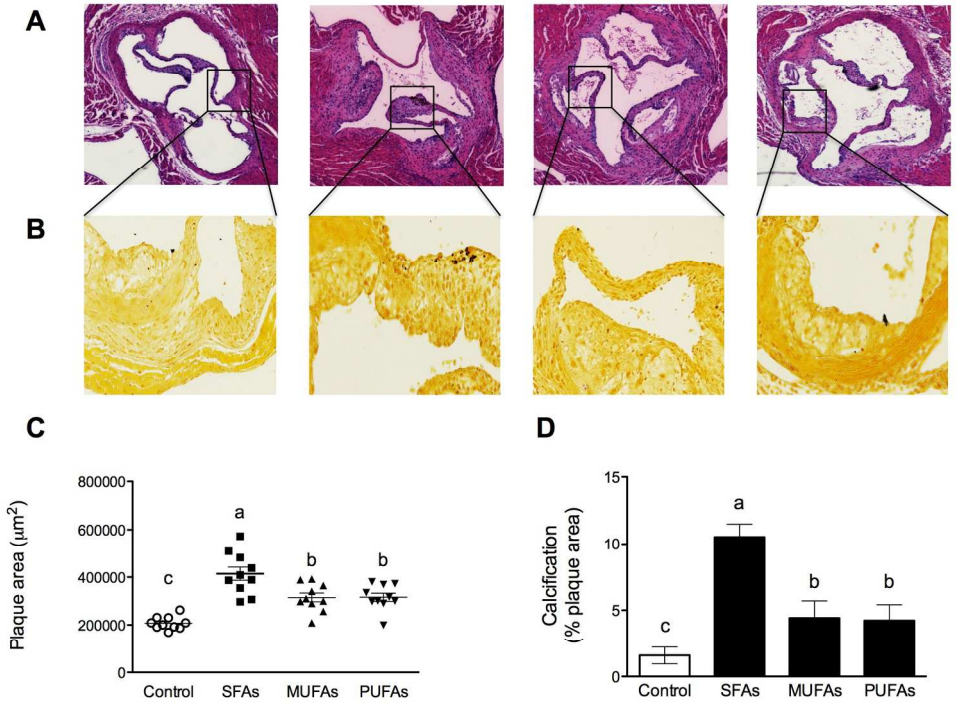

Figure 1

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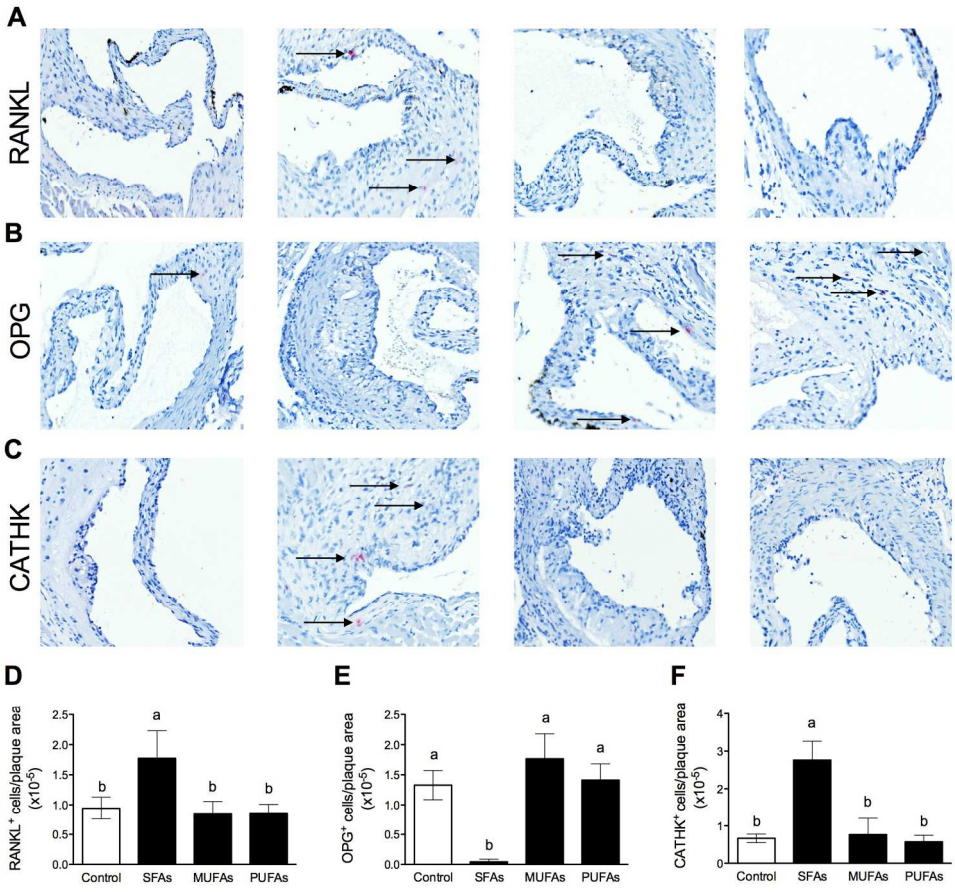

Figure 2

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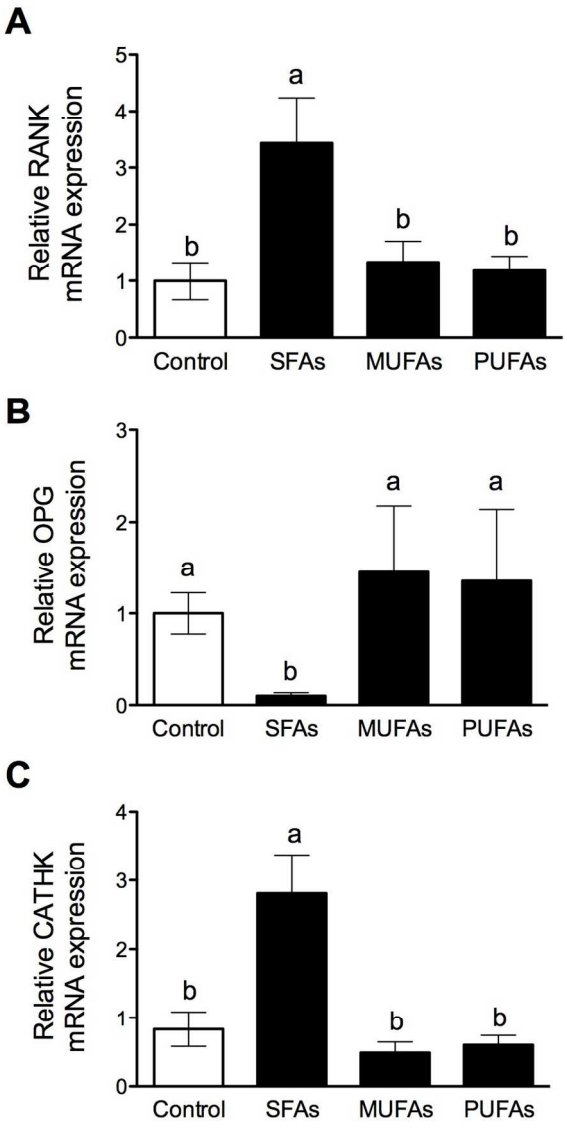

Figure 3

85x163mm (300 x 300 DPI)