Paper IV

Dose-dependent coronary artery intimal thickening after local delivery of the anti-oxidant tetradecylthioacetic acid from stents

Karel K.J. Kuiper[1], Mohamed Salem[1], Oddrun Anita Gudbrandsen[2], Ziad A. Muna[2], Rolf K. Berge[2] and Jan Erik Nordrehaug[1]

Short title: anti-oxidant increases intimal thickening

[1]Department of Heart Disease, Haukeland University Hospital, Bergen, Norway.

[2]Institute of Medicine, Section of Medical Biochemistry, University of Bergen, Haukeland University Hospital, Bergen, Norway.

Adress for correspondance:

Karel K.J. Kuiper, MD

Department of Heart Disease

Haukeland University Hospital

N-5021 Bergen, Norway

Phone: +4755972220, fax: +4755975150

E-mail: karel.kuiper@helse-bergen.no

Subject code: Restenosis, Animal models of human disease, Smooth muscle proliferation and differentiation, Lipid and lipoprotein metabolism

Abstract

The in vitro uptake and elution of the anti-oxidant tetradecylthioacetic acid (TTA) from phosphorylcholine (PC)-coated stents, and the in vivo uptake, retention, inflammatory response and histomorphometric changes after overstretch injury of the porcine coronary artery were examined. PC-coated stents were loaded in one of 3 different concentrations of TTA (87, 174 and 347 mmol/L, i.e. 25, 50 and 100 mg/mL) and randomized vs PC-coated stents to the right coronary or left circumflex artery (18 pigs). Uptake of TTA into the coronary wall from the 347 mmol/L concentration was measured after 3 and 24 hours, 7, 14 and 28 days (2 pigs at each time point). In vitro, TTA was successfully loaded onto the stents and elution was nearly complete after 48 hours. In vivo, TTA could be demonstrated in the vessel wall for up to 4 weeks. Percent area stenosis was significantly higher in the TTA group, 35.2±20.9 vs 27.5±17.0 %, p=0.03. Dose-related comparison showed increased intimal thickness, 0.66±0.53 vs 0.29±0.26 mm, p=0.008 and intimal area, 2.83±1.61 vs 1.58±0.91 mm^2, p=0.004 for the 347 mmol/L TTA vs controls. There was a significantly positive relationship between the TTA loading dose and both intimal area (B=0.69, p=0.01) and maximal intimal thickness (B=0.17, p=0.02). The pro-inflammatory precursor arachidonic acid increased 4-fold in the arterial wall of the TTA group, while the anti-inflammatory fatty acid index (AIFAI), calculated as (docosapentaenoic acid + docosahexaenoic acid + dihomo-linolenic acid)/arachidonic acid, was suppressed to 0.65±0.27 compared to 1.13±0.23 in control vessels (p<0.001). We conclude that TTA caused a dose-dependent intimal thickening and reduced anti-inflammatory index contrary to expectations. Anti-oxidants may change property to pro-oxidants when used as stent coatings.

Key-words: eluting-stent, restenosis, anti-oxidant, porcine

Introduction

The development of in-stent restenosis (ISR) is a current limitation to the clinical success of coronary metal stents. While systemic administration of a number of drugs has been unsuccessful to counteract intima hyperplasia, local application using drug eluting stent technology has significantly reduced restenosis rates [1;2]. Some problems remain with paclitaxel and rapamycin such as a 6-9% restenosis rate in subgroups of patients [3;4], 8% MACE rate [3], malapposition over time [5], late stent thrombosis [6] and the issue of coating drugs directly onto metal stents or to a basis polymer. Stent coatings acting as drug-reservoirs have the advantage of sustained elution of drugs compared to the application of drugs to bare stents [7;8]. Stent coatings should be biocompatible and inert, not provoking inflammatory reactions [9]. Phosphorylcholine (PC) does not cause increased intimal hyperplasia in animal models [10] and has the ability to contain and release agents over time.

Reactive oxygen species (ROS) are initiated during angioplasty, and may act as vascular smooth muscle cell mitogens. Anti-oxidants, like probucol have been shown to be effective in preventing restenosis after balloon angioplasty in humans [11-13], mainly by reducing negative remodeling. Combined treatment with probucol and cilastozol was reported to reduce restenosis after stent implantation [14], while probucol alone did not suppress in-stent restenosis [15]. Carvedilol used as stent coating reduced vascular smooth muscle cell proliferation and vessel wall thickness [16].

Tetradecylthioacetic acid (TTA) {CH_3-$(CH_2)_{13}$-S-CH_2-COOH} is a potent anti-oxidant both in vitro [17] and in vivo [18] with anti-proliferative properties in vitro [19]. In vivo, TTA reduces negative remodeling after balloon angioplasty injury in a rabbit iliac model, given as peroral supplements [19]. Identical effects were seen when TTA was delivered locally to balloon injured porcine coronary arteries [20].

In this study we examined the vessel wall reaction after implantation of a PC-coated Bio*divYsio*® stent onto which TTA was loaded and compared this to a bare PC-coated Bio*divYsio*® stent in a porcine coronary artery model. Secondly, we examined the uptake and retention of TTA from this stent into the vessel wall and surrounding tissue.

Materials and methods

Three different studies were performed. 1. An in vitro study was performed to determine the maximal loading dose of TTA (molecular weight 288) to the PC coating and the pharmacokinetics of 3 different TTA stent-loading doses. 2. Two sets of in vivo studies were performed. In the first set of 8 pigs (*Sus scrofa*, weight 44.3±7.3 kg), the uptake and retention of TTA from TTA-loaded Bio*divYsio*® stents was studied. In the second set of 18 pigs (*Sus scrofa*, weight 38.5±7.2 kg), the arterial wall response to TTA-loaded Bio*divYsio*® stents or controls implanted in RCA and LCx was examined. Bio*divYsio*® stents are coated with phosphorylcholine, designed to absorb pharmaceuticals of molecular weight $\leq$ 1200.

1. In vitro assessment of TTA-loaded Bio*divYsio*® stents.

Eighteen mm long Bio*divYsio*® DD stents were immersed in solutions of different concentrations of TTA in ethanol for 30 minutes (35, 87, 174 and 347 mmol/L, i.e. 10, 25, 50 and 100 mg TTA /mL, 4 stents for each concentration). Loading concentrations of TTA greater than 374 mmol/L resulted in flocculation and lumping of TTA on the stent strut surface in scanning electron microscopy. The TTA content per stent was determined by gas chromatography. Thereafter, 18 mm Bio*divYsio*® stents were loaded with TTA by immersing the stents in a concentration of 174 mmol/L (50 mg TTA /mL) and placed in a dissolution bath filled with phosphate buffered saline and perfused for varying lengths of time (4 stents for each time interval). To quantify the amount of TTA on the stents, the stents were

immersed in acetone in an ultrasound bath for ten minutes (two washings). The acetone was evaporated and TTA was measured as previously described [21;22].

2. In vivo studies.

All pigs were pretreated with acetylsalicylic acid 330 mg perorally the night before stent implantation. No additional anti-thrombotics were given during follow-up. The animals were on a Standard pig feed®, without cholesterol supplementation and kept under controlled environmental conditions. The study protocol was approved by the local ethical committee for animal care and use.

Anesthesia was induced as described earlier [10]. A 6F coronary guiding catheter (Multipurpose Hockeystick to the left, Judgkins Right to the right) was advanced to the coronary ostia via the right femoral artery. A bolus of 100 IU/kg of heparin was administered intraarterially. After nitroglycerine 100 µg/mL intracoronarily, angiography was performed using ioxaglate (Hexabrix®) as contrast medium. During the procedure ECG and temperature were monitored continuously.

Post-procedure the femoral artery was repaired to restore blood flow, the subcutis and skin were closed with separate ligatures. Intramuscular diazepam (1 mg/kg) and ketorolac (Toradol®) (30 mg/mL) 2mL i.v. were given before conclusion of the procedure. Ketorolac (Toradol®) was administered for the first days if pain, penicillin/streptomycin combination for 3 to 4 days to prevent post-procedure infections.

All stents were deployed according to randomization. Angiography after implantation showed open arteries without obvious dissections.

In vivo uptake and retention of TTA. TTA was loaded onto 18 mm premounted Bio*divYsio*® stent in the lab prior to implantation by immersing the stents into a TTA solution

(347 mmol/L) for 15 minutes. The stents were then allowed to dry for 10 minutes. A TTA loaded stent was implanted in the proximal part of the RCA and LCx. The animals were anesthetized and sacrificed by an overdose of potassium chloride intraarterially after 3 or 24 hours, 7, 14 or 28 days (2 animals at each time point). The stented parts of the arteries were excised for biochemical analysis. The stents were then cut longitudinally and the tissue on both sides of the stent was removed and prepared for further analysis. All samples were immediately frozen in liquid nitrogen and stored at –80 ^{o}C. Sections from untreated LAD served as controls.

TTA versus controls: Three different TTA concentrations (87, 174, 347 mmol/L) were used to load TTA onto Bio*divYsio* stents in series of 6 pigs each. An 18 mm TTA-loaded Bio*divYsio* stent and an 18 mm Bio*divYsio* stent were implanted in either RCA or LCx according to randomization. All stents were premounted on a 3.5 mm balloon catheter and dilated at a final pressure of 8 atmospheres over 30 seconds, assuring a stent:artery ratio$\geq$1.1 by visual assessment, causing a moderate overstretch injury. Angiography was repeated to assess vessel patency using identical views.

After 4 weeks a follow-up angiography was performed using an identical protocol as for the first procedure. The pigs were killed by an overdose of potassium chloride intraarterially. The heart was excised and the coronary vasculature perfusion fixed at 100 mmHg for 15 minutes with 4% formalin after flushing with heparinized prewarmed (37 ^{o}C) saline. The stented parts of the arteries with a 5 to 10 mm margin on each side of the stent were excised, immersed in 4% formalin overnight and prepared for histomorphometry by microscopical examination.

TTA preparation: TTA was synthesized as previously described [23].

Determination of fatty acid composition: Total lipids were extracted from the vessel wall and myocardium as described by Bligh and Dyer [24]. The fatty acid composition was analyzed as previously described [21;22].

Histomorphometry: The stented artery segments were dehydrated in acetone. The stents were embedded in the resin Technovit 8100 [25]. The blocks were cut into 100 µm cross-sections using a diamond-tipped rotary saw (Isomet 4000, Buehler) and glued to slides. Thereafter the sections were ground to 30 µm with the Metaserv 2000 grinder and grounded further to 10 µm and polished with the Biothin grinder (Buehler) [25]. Histomorphometry was performed blinded to the randomization after digital transition using computer-assisted planimetry (analySIS vs 3.2, Soft Imaging System). Sections of the proximal, middle as well as distal part of the stents were analyzed. Areas surrounded by lumen, the stent struts and by the external elastic lamina were traced. Neointima was defined as the area between the lumen and the stent struts. Vessel area was defined as the area within the external elastic area. The extent of arterial injury at each stent strut was determined according to the score proposed by Schwartz et al. [26]. Morphologic area stenosis was calculated as 100 x (1-Stenotic lumen area/Original lumen area). Stenotic lumen area was defined as the lumen vessel area, original lumen area as the area within the internal elastic lamina.

Statistical analysis: All data are presented as mean ± standard deviation (SD). Student T-test was used to compare histological measurements between the TTA-eluting stent group and the non-eluting stent group. Differences between the fatty acid composition in the arterial wall and control vessel were tested by the Mann-Whitney test. Linear regression analysis was performed to detect relation between the TTA-loading concentration and difference in intimal area and difference in maximal intimal thickness for the TTA-eluting stents and the non-eluting stents. A p-value <0.05 was considered significant. Statistical analysis was performed using the statistical computing program SPSS® for Windows™ version 13.0.

Results

TTA loading and elution in vitro

Gas chromatography confirmed a relatively linear relationship between the loading concentrations and the TTA content per stent (Fig. 1A), ranging from 19±6 μg per stent using a solution of 10 mg TTA /mL concentration to 173±17 μg per stent using a 100 mg TTA /mL concentration. The release of TTA from the stents into a dissolution bath, determined as TTA remaining on the stent, was fast during the first hours. The total amount left on the stent was reduced from 100 μg/stent to 40 μg/stent after 1 hour, to 6 μg/stent after 8 hours, whereas nearly all of the loaded TTA was eluted after 48 hours (Fig. 1B).

TTA loaded stents in vivo

Safety: During follow-up there were no acute deaths or subacute stent thrombosis. None of the animals showed signs of discomfort and body weight had increased from 38.5±7.2 kg at baseline to 51.4±6.1 kg at follow-up, $p < 0.001$. All animals were restudied at the scheduled time points, none of the pigs experienced increase of body temperature. Angiography during follow-up studies did not detect any total occlusions.

Histopathology: The IEL area, EEL area and the injury score were similar for the total TTA-loaded stent group and the non-loaded stent group (Table 1). There were no signs of thrombosis or neoangiogenesis. All stent struts were well apposed to the arterial wall and no aneurysm was observed.

Histomorphometric analysis. Analysis for the total group is shown in Table 1. There was a trend towards increased intimal hyperplasia and medial area in the TTA-loaded stents vs non-loaded stents. Percent area stenosis was 35.2±20.9 vs 27.5±17.0 for the TTA-loaded stent group and the non-loaded stent group, respectively ($p=0.03$), representing in a 22% increase

in area stenosis in the TTA group (Table 1). Dose-related comparison showed a significantly increased intimal thickness (0.66±0.53 vs 0.29±0.26 mm, p=0.008) and intimal area (2.83±1.61 versus 1.58±0.91 mm^2, p=0.004) for the 347 mmol/L TTA-eluting stent group compared to the non-eluting stent group. In addition, there was a significant positive relationship between TTA-loading dose and both the intimal area (B=0.69, p=0.01) and maximal intimal thickness (B=0.17, p=0.02) (Fig. 2).

The fatty acid composition and anti-inflammatory fatty acid index (AIFAI) in the arterial wall. TTA was present in the arterial wall after 3 hours and up to 4 weeks after implantation of the stents (Fig. 3). TTA was not detected in the surrounding perivascular fat or myocard. TTA is previously shown to affect the lipid metabolism in tissues of several animal models [27], and it was therefore plausible that the TTA-loaded stent could affect the fatty acid composition of the arterial wall. No change was seen in the total level of saturated fatty acids (SFA) in the arterial wall surrounding the TTA-loaded stent during the experiment, and the levels were comparable to those of the control vessels (Fig. 4A) as well as the levels found in the perivascular fat and the arterial wall proximally and distally for the implanted stent (Table 2). It is worth noting that the total amounts of the longest SFA (20:0 and 22:0) were significantly higher in the arterial wall surrounding the TTA-loaded stents than in the arterial wall of the control vessels (Fig 4B). The level of monounsaturated fatty acids (MUFA) was significantly lower in the arterial wall of the TTA-eluting stent compared to the arterial wall of control vessel (Fig. 4C) and of perivascular fat (Table 2). The reduced level of MUFA is mostly due to the lower level of oleic acid (18:1n-9) (Table 2), resulting in a reduced Δ9 desaturase index in the arterial wall (Fig. 5A). The levels of omega-3 and omega-6 polyunsaturated fatty acids (PUFA) in the arterial wall surrounding the TTA-eluting stent were not changed during the course of the experiment, and the levels were not significantly different from that of the control vessel, perivascular fat or from the arterial wall proximally

and distally for the implanted stent (Table 2). The ratio of arachidonic acid (20:4n-6) to linoleic acid (18:2n-6) tended to increase from 3 hours to 28 days in the artery around the TTA-loaded stent, and the ratio was significantly higher compared to the arterial wall of the control vessel at the conclusion of the experiment (Fig. 4D). The high arachidonic acid/linoleic acid ratio implied that the synthesis of arachidonic acid, the precursor for pro-inflammatory prostaglandins, was increased in the vicinity of the TTA-eluting stents. Indeed, the level of arachidonic acid increased in these arteries compared to the control vessels, the perivascular fat and the arterial wall proximally and distally for the implanted stent (Table 2). However, the indirect index of Δ6 desaturase, an important enzyme in the production of arachidonic acid, was not changed in the arterial walls during the experiment, regardless of the loading on the stents (Fig. 5B). The indirect indexes of the other enzymes involved in biosynthesis of arachidonic acid, i.e. elongase (Fig. 5C) and Δ5 desaturase (Fig. 5D) were higher in the arterial wall of the TTA-loaded stents compared to the control vessels.

The anti-inflammatory fatty acid index (AIFAI), calculated as (docosapentaenoic acid + docosahexaenoic acid + dihomo-linolenic acid)/arachidonic acid [28], decreased after 3 hours to 2 weeks and then increased after 4 weeks both in the arterial wall surrounding the TTA-loaded stents and in the arterial wall of the control vessels (Fig. 6). At all times, the AIFAI was lower in the arterial wall surrounding the TTA-loaded stents compared to the arterial wall of the control vessels (Fig. 6, Table 2).

Discussion

TTA has been shown to have anti-oxidant effects in vitro [17] and in vivo [18], and we have recently demonstrated that TTA also have immunomodulatory properties in human peripheral blood mononuclear cells of healthy donors [29] and in HIV-infected patients [30]. TTA has the ability to attenuate tumor necrosis factor-α mediated endothelial cell activation [31], further supporting anti-inflammatory effects of this modified fatty acid.

In this study we tested the in vitro and in vivo uptake, elution and vessel wall retention as well as the safety and efficacy of a TTA-eluting stent coated with phosphorylcholine in a porcine coronary model. We established that TTA could be easily loaded onto the phosphorylcholine coating and was released into the vessel wall. However, contrary to expectations [19;20] TTA increased intimal thickening in a progressive dose dependent manner compared to the phosphorylcholine coating alone.

We have previously shown that phosphorylcholine coated metal stents are biocompatible and well tolerated in porcine coronary arteries compared to controls [10]. The coating can act as a drug reservoir which allows uptake, retention and release of bioactive agents mainly from the outer surface of the stent. This surface comes in direct contact with the vessel wall immediately during stent deployment. Drug loading on the phosphorylcholine coating has been reported for a variety of substances such as dexamethasone, angiopeptin [32], estradiol [33] and methylprednisolone [34]. The release curves for these compounds were largely terminated before 40 minutes. In this respect the anti-oxidant probucol [16] seems to be different by showing a slower release. TTA obtained a high concentration with distinct differences according to the dip-solutions. About 60% of the release was completed within 60 minutes, but some TTA remained on the stents for up to 48 hours. The lipophilic properties of TTA are probably responsible for this, if diffusion was the only mechanism the release would have been expected by far to be more rapid. A unique feature with TTA is its ability to bind to the phospholipids in the cell membranes in the vessel wall for up to 4 weeks even after a single bolus injection via a local drug delivery angioplasty coronary balloon [35].

The doses used in the present study were based on previous data showing strong anti-oxidant and anti-inflammatory properties for TTA [20;27]. Furthermore, TTA in a concentration of 100 μM has been shown to inhibit proliferation of aortic smooth muscle cells in vitro [19] and this was linked to its anti-oxidant and anti-inflammatory effects.

It is possible that a high uptake and local release of TTA from the stents could change its property from anti-oxidative to pro-oxidative, as has been reported after high oral doses of anti-oxidant vitamins [36]. However, this is not likely since the doses in the present study were based on experience from previous studies [20]. We have demonstrated that TTA altered the fatty acid composition of different tissues, and this is partly due to increased mitochondrial and peroxisomal fatty acid β-oxidation [27]. It is generally believed that β-oxidation in the peroxisomes mainly function as a chain-shortening system for long-chain fatty acids, before they are further β-oxidized in the mitochondria. In the present study there was a higher amount of long-chain SFAs (i.e. 20:0 and 22:0) in the arterial wall surrounding the TTA-eluting stents, indicating that rate of the peroxisomal fatty acid β-oxidation could be down-regulated. A lower capacity for peroxisomal β-oxidation in the arterial walls in the vicinity of TTA-eluting stents may also explain the almost 4-fold increase in the level of arachidonic acid, a precursor of proinflammatory prostaglandins.

The synthesis of prostaglandins depends on the availability of 20 carbon PUFAs, either arriving via the circulation or arising from local production catalyzed by Δ6 and Δ5 desaturases. We have recently shown that TTA upregulates the hepatic gene expression of both Δ6 and Δ5 desaturases, making it likely that an increased biosynthesis of arachidonic acid locally could contribute to an inflammation in the artery after the highest TTA stent loading. The markedly higher level of arachidonic acid in the arterial wall surrounding the TTA-eluting stent led to a lower AIFAI when compared to the control vessels, strongly implying that TTA acts as a pro-oxidant under the present conditions.

The decreased level of oleic acid in the arterial wall surrounding the TTA-loaded stent could be caused by a reduced Δ9 desaturation of stearic acid (18:0) locally or due to replacement of oleic acid by PUFAs in the sn-2 position of the phospholipids in the arterial wall. This is of great interest, as a reduced level of oleic acid has also been found in liver of

transgenic hTNFα-mice, which resembles chronic inflammation, suggesting that the decreased amount of oleic acid could contribute to a pro-inflammatory effect of the TTA-loaded stents.

Oxidative stress stimulates type I gene expression and thereby increases collagen production in human fibroblasts [37;38]. Several previous studies have suggested that TTA inhibits coronary stenosis formation [19 20]. We have also previously shown that TTA prevented the hypertension-induced development of fibrosis in kidneys [39]. Thus, TTA seems to have a favorable biological action on the vessel wall reaction to injury. A positive remodeling with increased vessel lumen has been suggested in the previous studies. The most likely explanation for this is an inhibitory effect of TTA on collagen which deserves further investigation in vessels that have not been stented, because stents effectively inhibit remodeling.

Acknowledgements

This study was supported by a grant from the Bergen Heart Foundation, Bergen, Norway.

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Figure Legend

Figure 1

A. TTA content on phosphorylcholine-coated stents in solutions with different TTA-concentrations (4 stents per solution).

B. The elution of TTA-loaded stents (174 mmol/L) in a dissolution bath, measured as the TTA remaining on the stents at different time points (4 stents at each time point).

Figure 2

Difference in maximal intimal diameter (—●—), in medial area (—▼—) and in intimal area (—○—) at three loading concentrations of TTA, calculated as the difference between the respective measurements for TTA-eluting stents and non-eluting stents. A positive correlation was found between TTA-loading concentration and difference in intimal area (* B= 0.69, p=0.01) and difference in maximal intimal thickness ($^{\#}$ B=0.17, p=0.02).

Figure 3

The amount of TTA demonstrated in the arterial wall at different time points after implantation of TTA-eluting stents.

Figure 4

Fatty acid composition in the arterial wall of TTA-eluting stents (—●—) and of control vessels (—○—) at several time points after implantation of the TTA-eluting stents.

A: Saturated fatty acids. **B:** Sum of 20:0 and 22:0 fatty acids. **C:** Mono saturated fatty acids. **D:** Ratio of arachidonic acid and linoleic acid. *p=0.004 for the mean of TTA group vs

controls. [#]p=0.004 for the mean of TTA group vs controls. [£]p<0.001 for the mean of TTA group vs controls.

Figure 5

Indirect indexes of enzymes involved in the biosynthesis of fatty acids in the in the arterial wall of TTA-eluting stents and in control vessels. Bars indicate mean of measurements at several time-points after implantation of TTA-eluting stents.

A: Delta 9 desaturase index. **B:** Delta 6 desaturase index. **C:** Elongase index. **D:** Delta 5 desaturase index. *p=0.004 for the TTA group vs controls. [#]p=0.001 for the TTA group vs controls. [£]p<0.001 for the TTA group vs controls.

Figure 6

The anti-inflammatory fatty acid index (AIFAI) in the arterial wall of TTA-eluting stents (—●—) and in control vessels (—○—) at several time points after implantation of the TTA-eluting stents. *p<0.001 for the differences between both groups for the mean of the measurements.

Table 1 Histomorphometric analysis of stented segments (n=18)

	Non-eluting stent	**TTA-eluting stent**
Maximal neointimal thickness (mm)	0.36±0.33	0.47±0.40
Lumen area (mm^2)	5.18±1.49	4.68±1.88
Intimal area (mm^2)	2.05±1.57	2.42±1.35
Medial area (mm^2)	1.47±0.46	1.63±0.56
Intimal/ Medial area	1.39±0.98	1.48 ±0.67
Intimal/ Luminal area	0.50±0.56	1.05±1.92*
Area stenosis (%)	27.5±17.0	35.2±20.9#
IEL area (mm^2)	7.23±1.50	7.10±1.44
EEL area (mm^2)	8.69±1.79	8.70±1.66
Injury score	1.02±0.43	1.14±0.46

PC: phosphorylcholine. TTA: tetradecylthioacetic acid. IEL area: area within the internal elastic lamina. EEL area: area within the external elastic lamina. Area stenosis was calculated as 100 x (1-lumen area/IEL area). *p=0.04 for the TTA-eluting stent group vs the non-eluting stent group. #p=0.03for the TTA-eluting stent group vs the non-eluting stent group.

Table 2 Fatty acid composition in the arterial wall

	Control vessel	TTA-eluting stent	Perivascular fat	Segments outside stent
SFA	35.46±1.20	41.98±14.04	37.90±2.58	42.23±11.10
MUFA	42.74±2.74	34.60±10.00*	41.48±5.51#	37.40±7.63
Omega-3 PUFA	2.18±0.28	2.68±1.45	1.94±0.56	1.95±0.44
Omega-6 PUFA	19.58±2.91	20.65±6.65	18.66±6.35	18.37±4.04
Oleic acid	37.32±2.17	29.72±8.81*	35,84±4,54#	32,66± 6,96
Arachidonic acid	1.13±0.18	4.44±3.90*	1.58±0.21#	1.82±0.75£
AIFAI	1.13±0.23	0.65±0.27*	1.41±0.72#	0.74±0.15

SFA: saturated fatty acids. MUFA: mono unsaturated fatty acids. PUFA: poly unsaturated fatty acids. AIFAI: anti-inflammatory fatty acid index, calculated as (docosapentaenoic acid + docosahexaenoic acid + dihomo-linolenic acid)/arachidonic acid.

*$p<0.05$ for control vessel versus TTA-eluting stent. #$p<0.05$ for perivascular fat versus the TTA-eluting stent. £$p<0.05$ for arterial wall proximally and distally for the TTA-eluting stent versus the TTA-eluting stent.

Figure 1

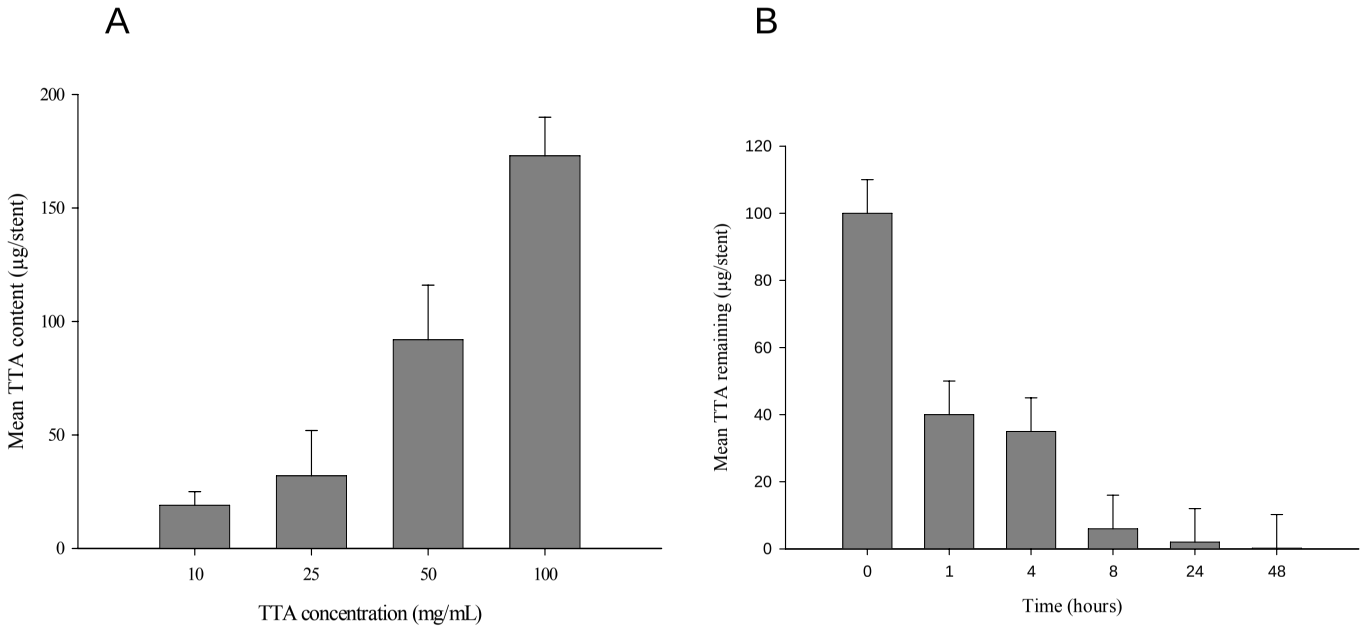

A
Mean TTA content (µg/stent)
200
150
100
50
0
10
25
50
100
TTA concentration (mg/mL)
B
Mean TTA remaining (µg/stent)
120
100
80
60
40
20
0
0
1
4
8
24
48
Time (hours)

Figure 2

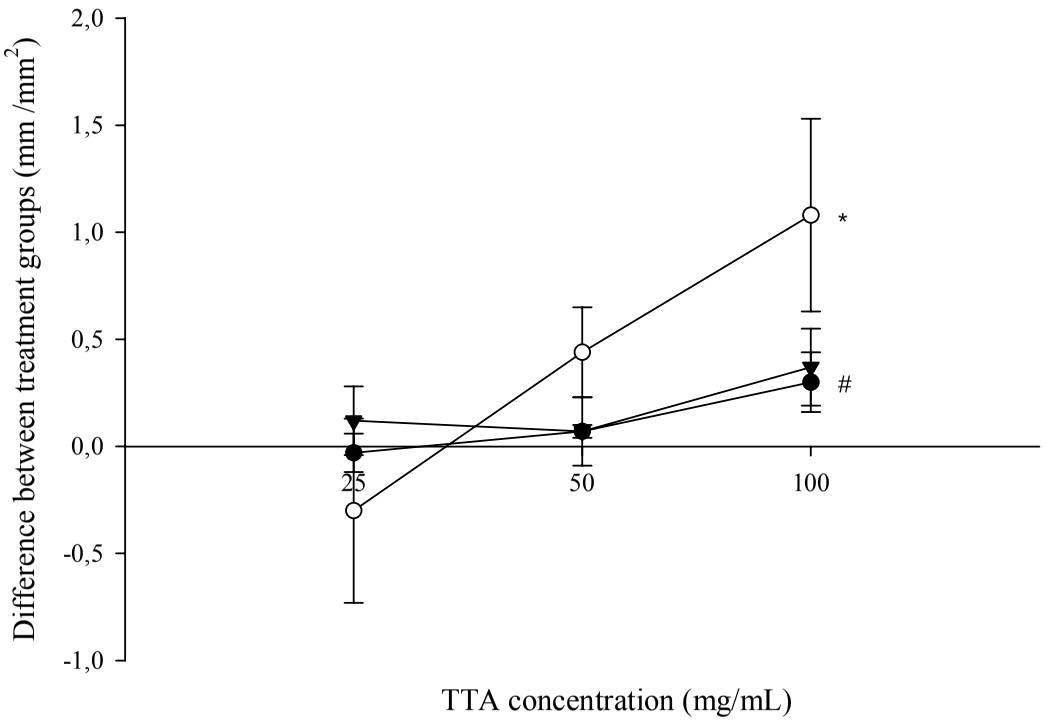

2,0
1,5
1,0
0,5
0,0
-0,5
-1,0
Difference between treatment groups (mm /mm2)
25
50
100
*
#
TTA concentration (mg/mL)

Figure 3

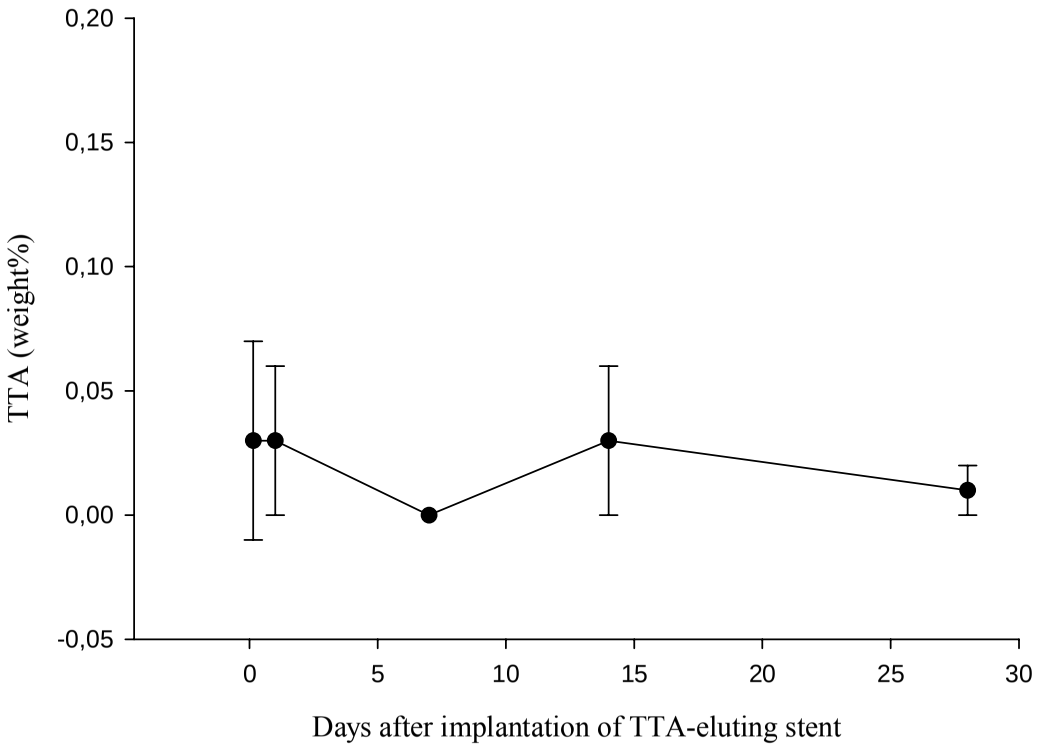

0,20
0,15
0,10
0,05
0,00
-0,05
TTA (weight%)
0
5
10
15
20
25
30
Days after implantation of TTA-eluting stent

Figure 4

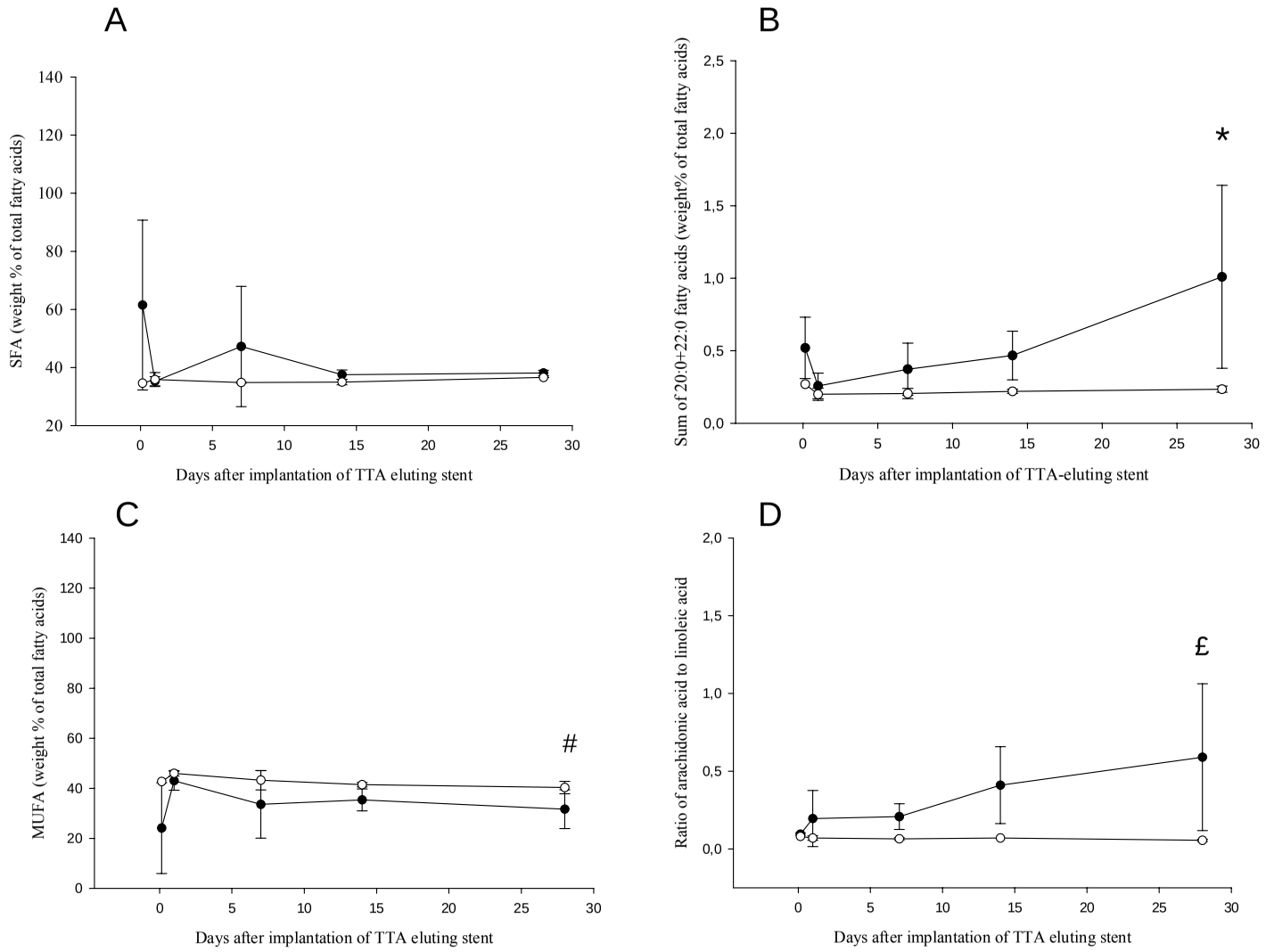

A
SFA (weight % of total fatty acids)
Days after implantation of TTA eluting stent
B
Sum of 20:0+22:0 fatty acids (weight% of total fatty acids)
*
Days after implantation of TTA-eluting stent
C
MUFA (weight % of total fatty acids)
#
Days after implantation of TTA eluting stent
D
Ratio of arachidonic acid to linoleic acid
£
Days after implantation of TTA eluting stent

Figure 5

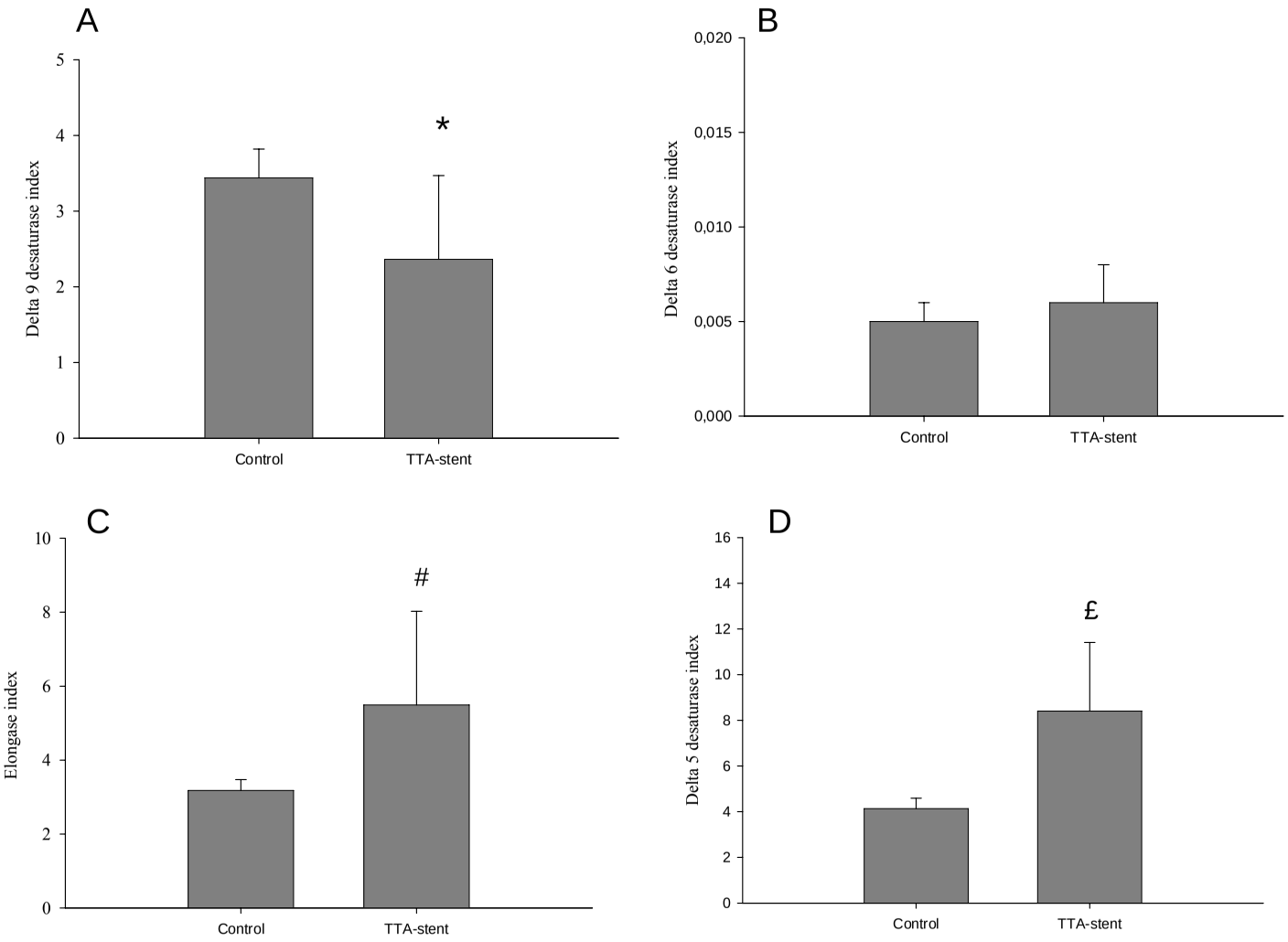
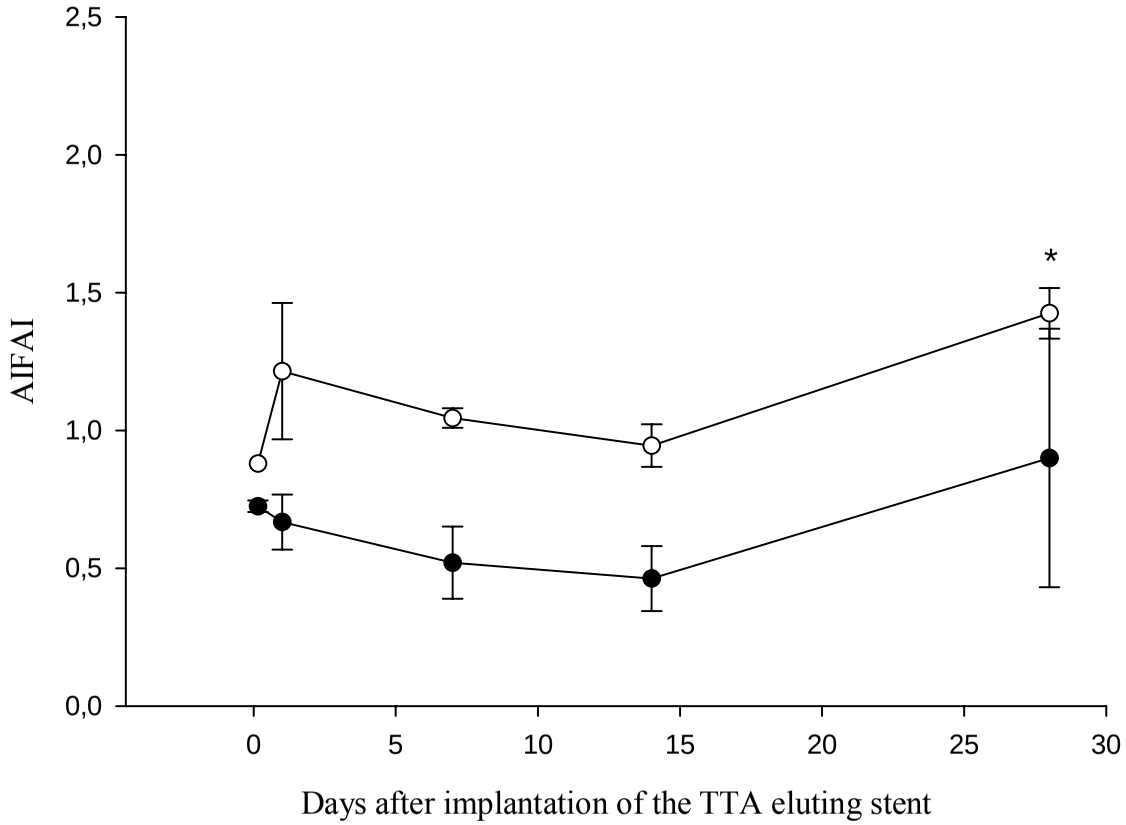

A
Delta 9 desaturase index
0
1
2
3
4
5
*
Control
TTA-stent
B
Delta 6 desaturase index
0,000
0,005
0,010
0,015
0,020
Control
TTA-stent
C
Elongase index
0
2
4
6
8
10
#
Control
TTA-stent
D
Delta 5 desaturase index
0
2
4
6
8
10
12
14
16
£
Control
TTA-stent

Figure 6


2,5
2,0
1,5
1,0
0,5
0,0
AIFAI
*
0
5
10
15
20
25
30
Days after implantation of the TTA eluting stent