Polish-Norwegian Research Fund, University of Bergen, 21-22 April 2010

Task 4 and 5: Analysis of the effects of humanin peptides (HN),TTA and L-arginine in different animal models.

Hypothesis:

Administration of humanin bioactive peptides, TTA and L-arginine ameliorates inflammation mediated by the cytokine TNFα (and/or NFKβ).

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Institute of Medicine
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Background:

- Maladaptive lipid metabolism is a condition which is harmful to several organs mainly due to inflammatory responses and known to be affected in several CNS and cardiovascular disorders like Alzheimers and Parkinsons's disease, and atherosclerosis.

- The pro-inflammatory cytokine TNFα and secretory phospholipase A2 (PLA2) and lipoprotein-PLA2 are implicated in vascular inflammation leading to the formation of atherosclerotic plaque.

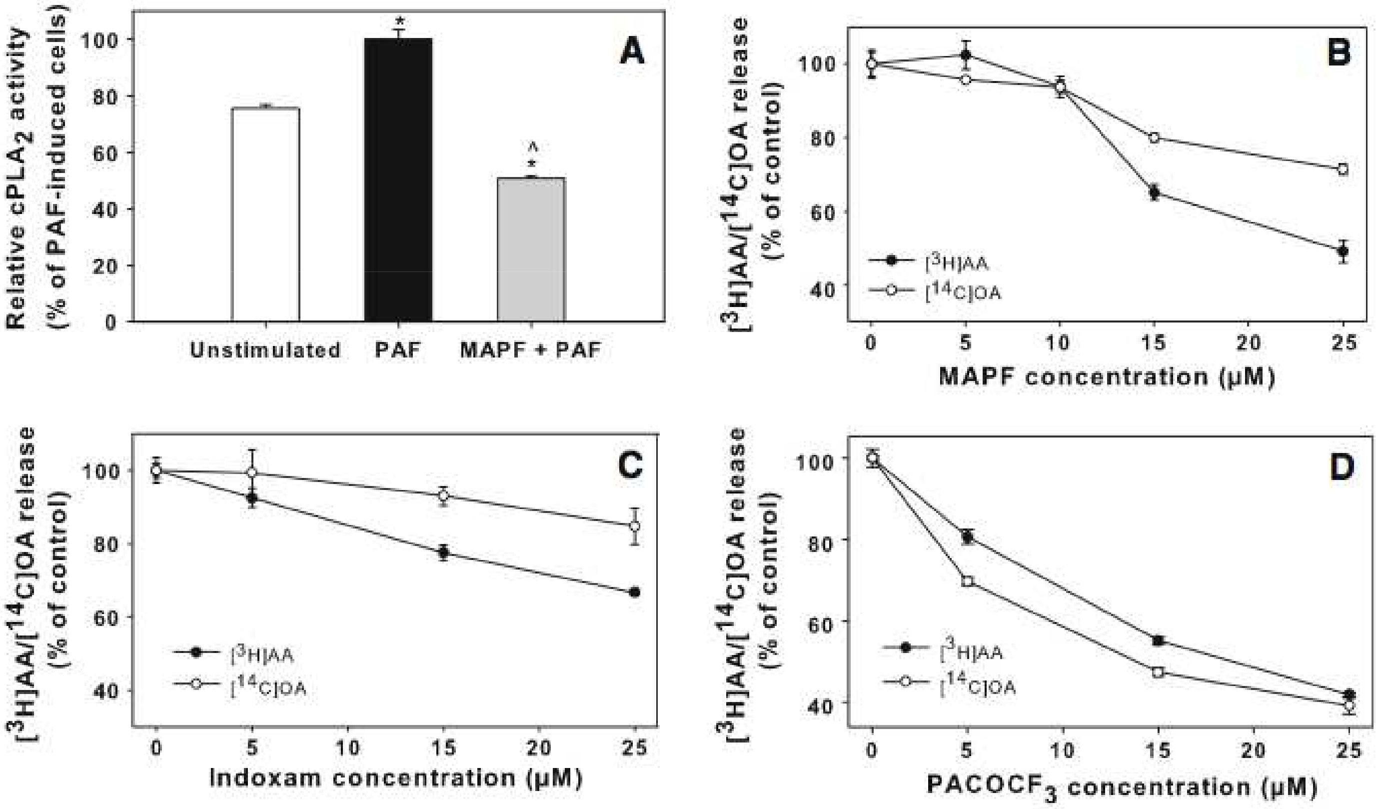
- Several neurological disorders is associated with TNFα mediated alteration of lipid metabolism reducing the level of cholesterols and thereby neurosteroids as well as increasing eicosanoids, ceramide and reactive oxygen species whereas lipid peroxidation by PLA2 is associated with Parkinson's disease.

PLA_2 enzymes are central mediators of inflammatory response

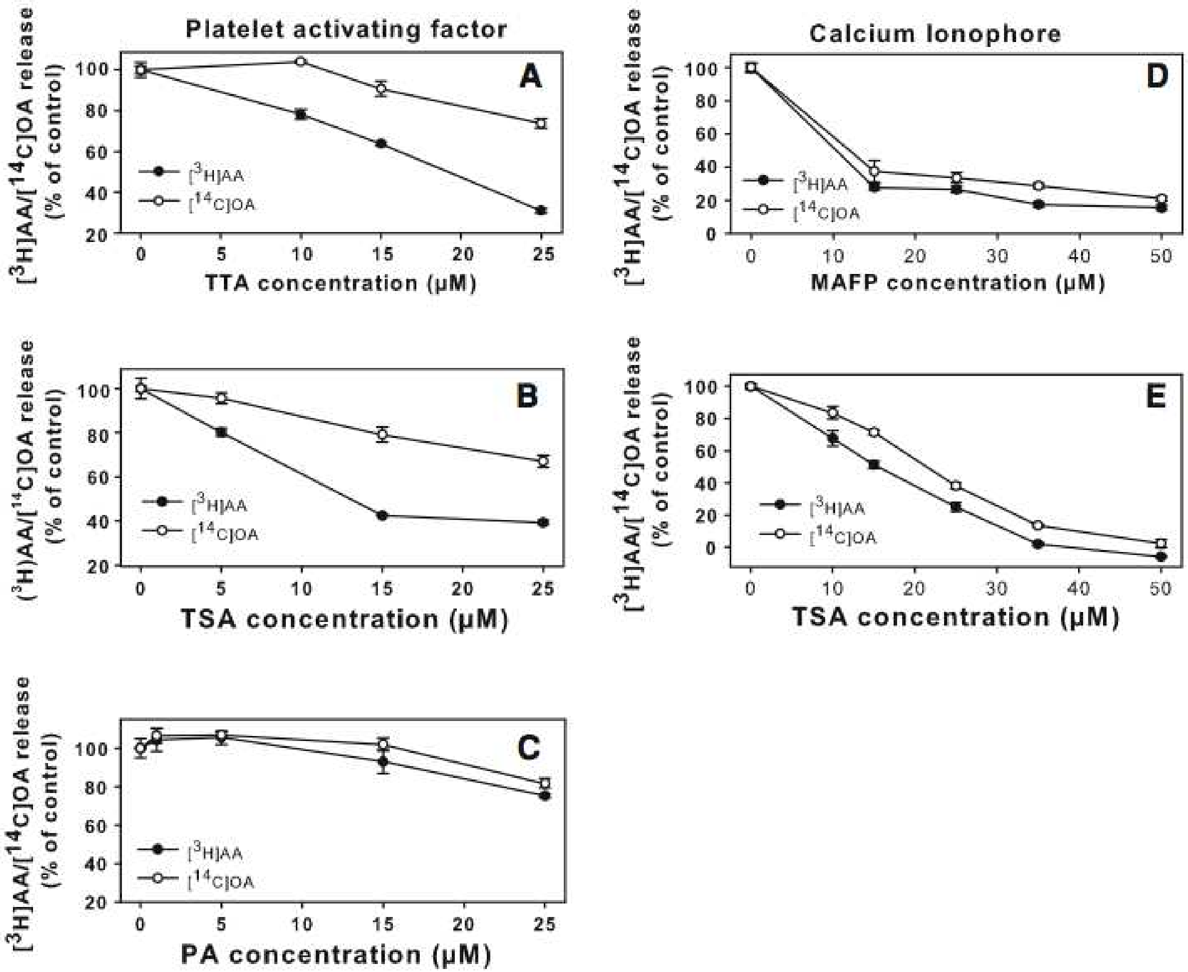
- Extracellular secretory PLA_2 ($sPLA_2$)
- Cytosolic calcium-dependent PLA_2 ($cPLA_2$)
- Cytosolic calcium-independent PLA_2 ($iPLA_2$)

All classes of PLA_2 enzymes may participate in agonist-induced arachidonic acid (AA) release

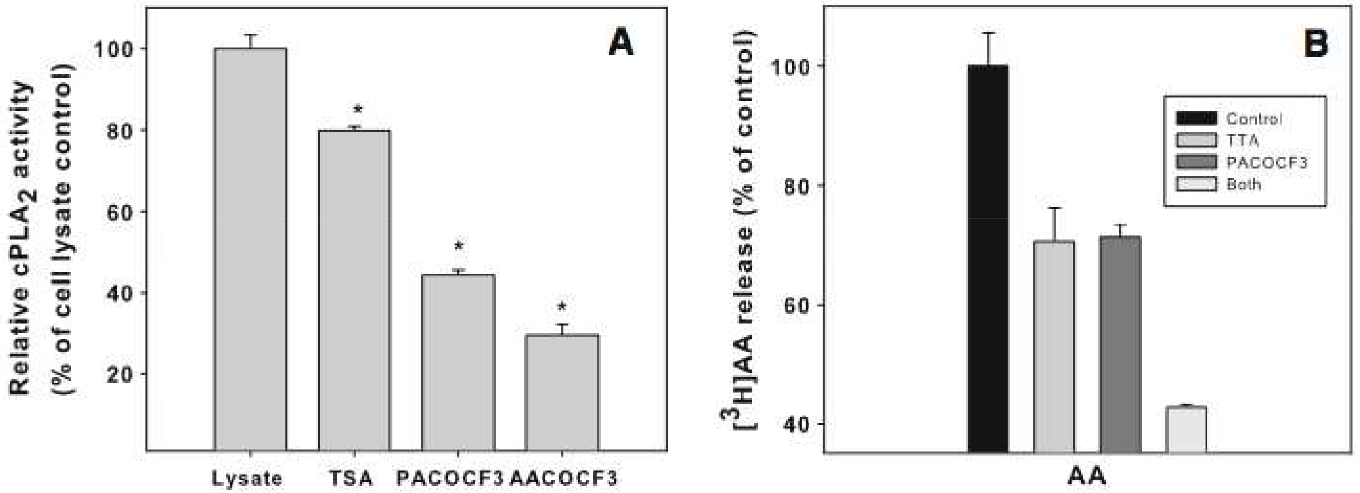
Different PLA_2 inhibitors reduce platelet activating factor (PAF)-induced AA and OA release



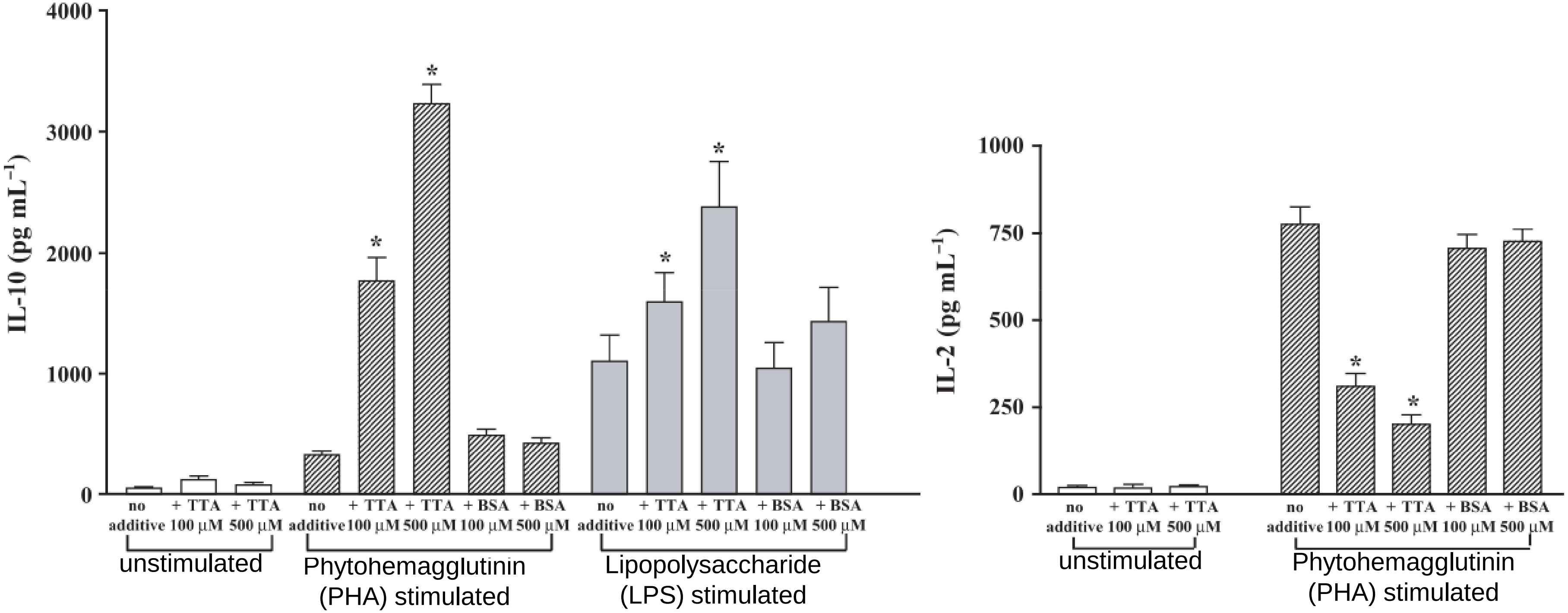
Palmitic acid (PA)-derived lipids TTA and TSA inhibit AA release



TTA and TSA point of action in AA-release is upstream of group IV PLA_2

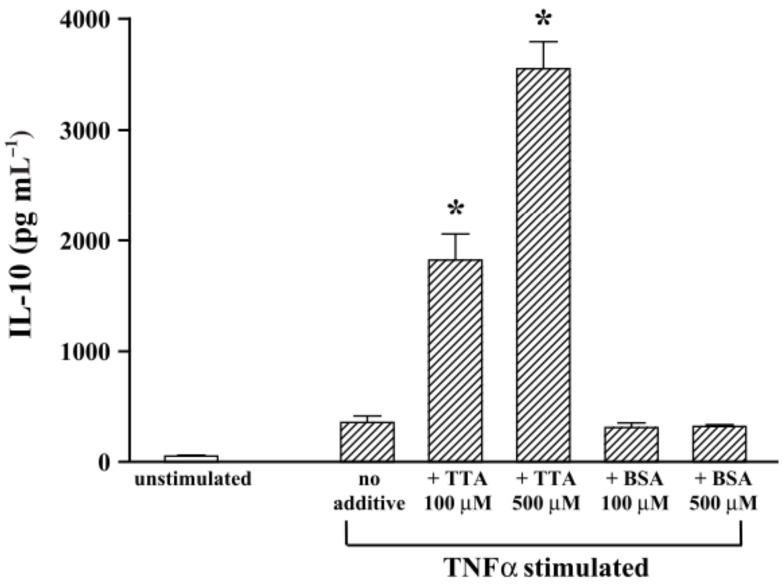


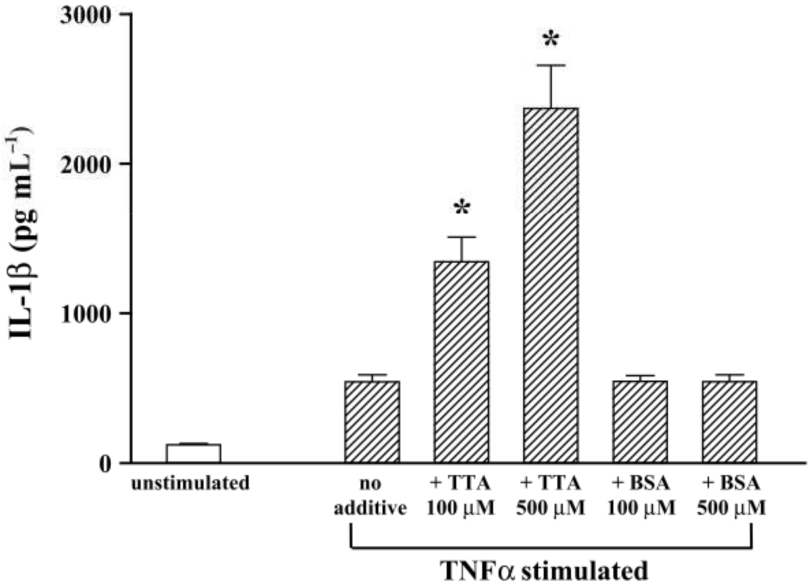
Effect of TTA on cytokine release from activated peripheral mononuclear cells (PBMC)



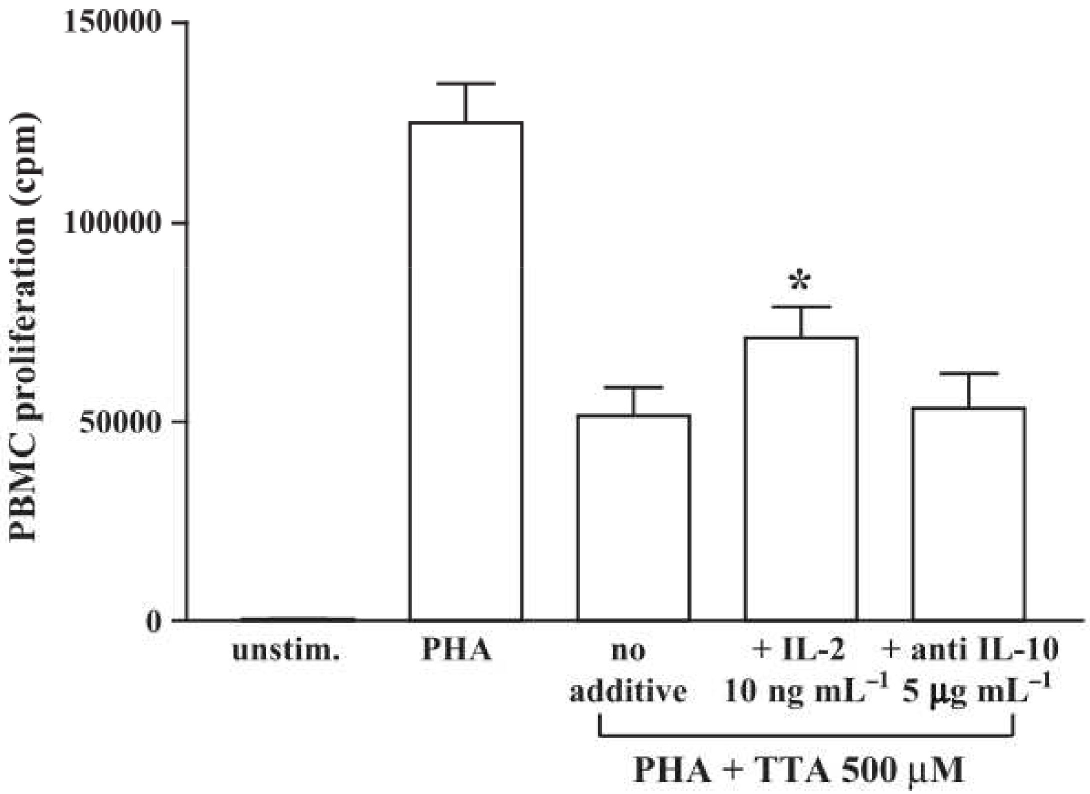
These cytokine modifying effects were found both in T cells and monocytes, when cultured separately

Effect of TNFα alone or in combination with TTA on the release of IL-10 and IL-1β

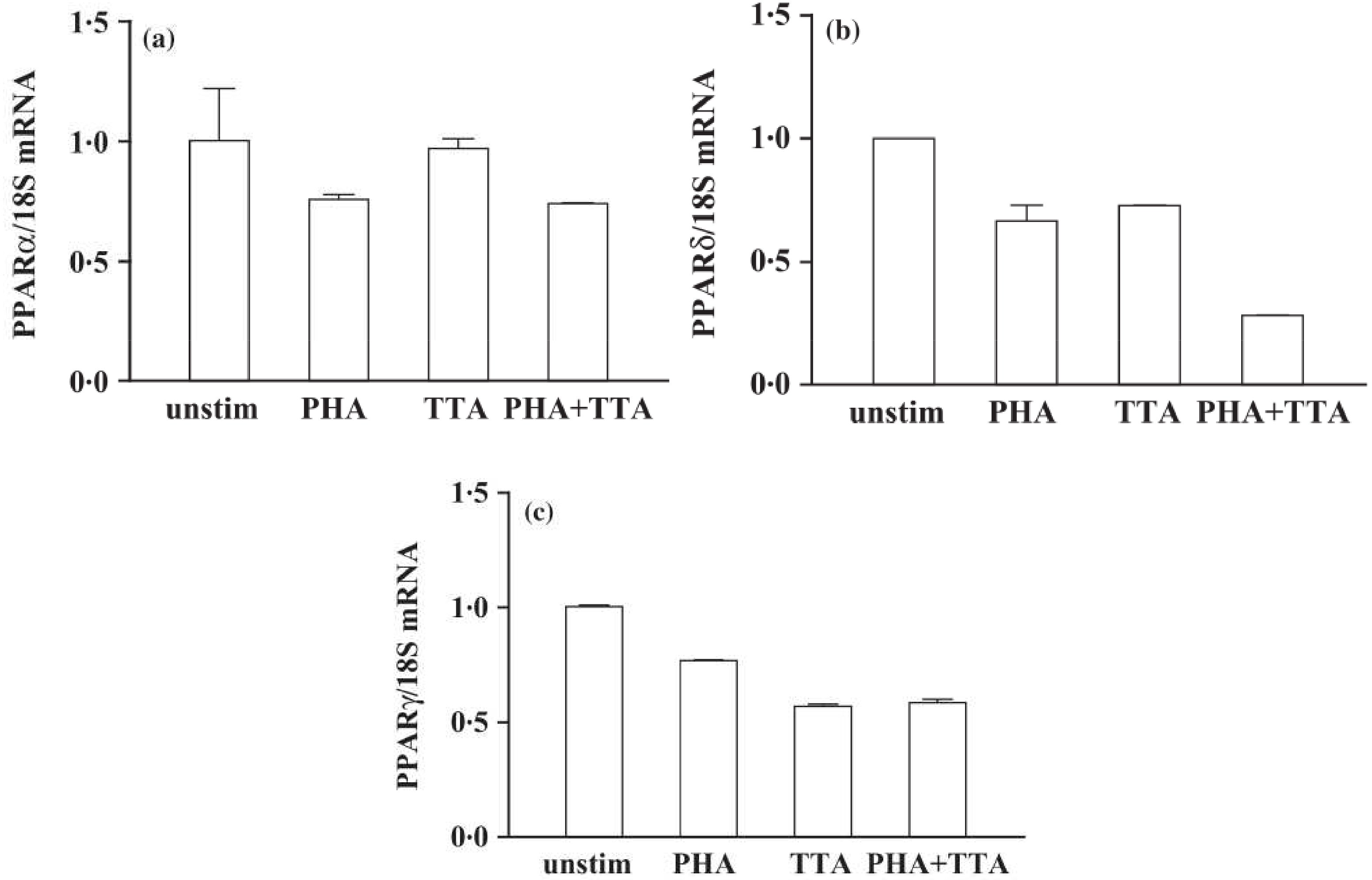




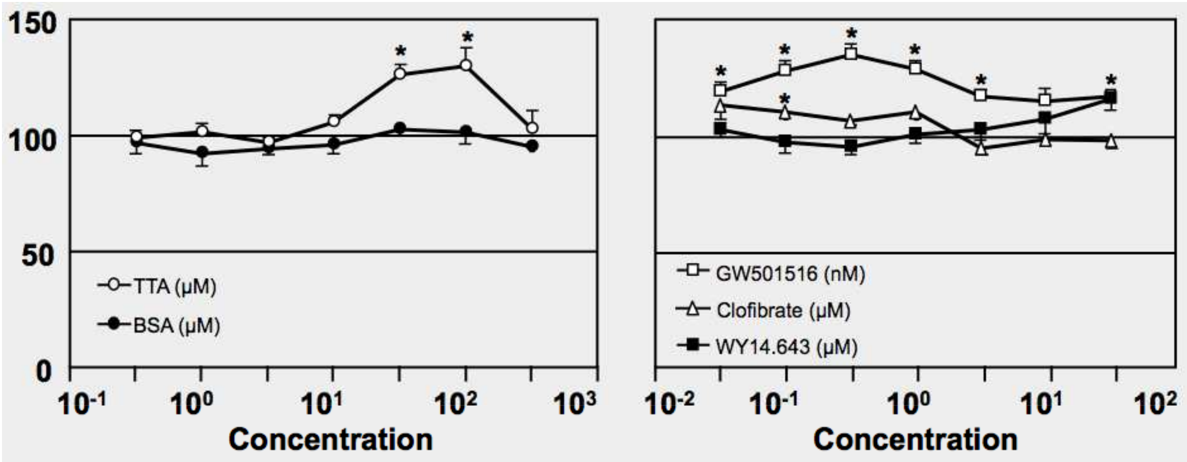
TTA suppresses PBMC proliferation, but the anti-proliferative property does not involve enhanced apoptosis or necrosis

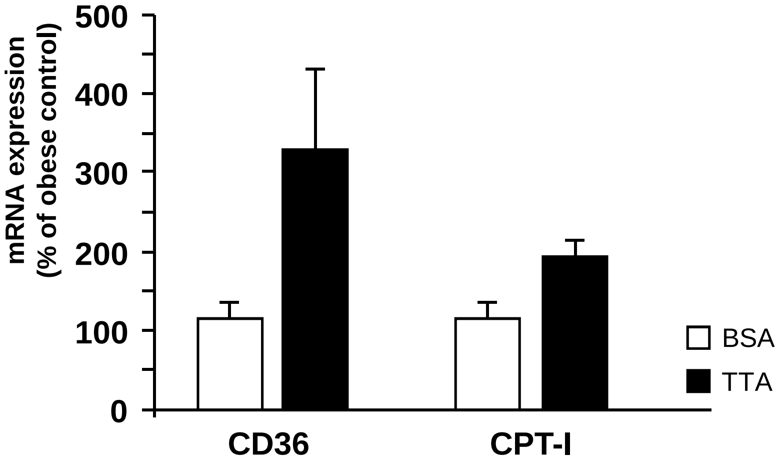


mRNA expression of PPARs in PBMCs in the presence of TTA



TTA and the PPARδ ligand work in human muscle cells





TTA suppresses smooth muscle cell proliferation

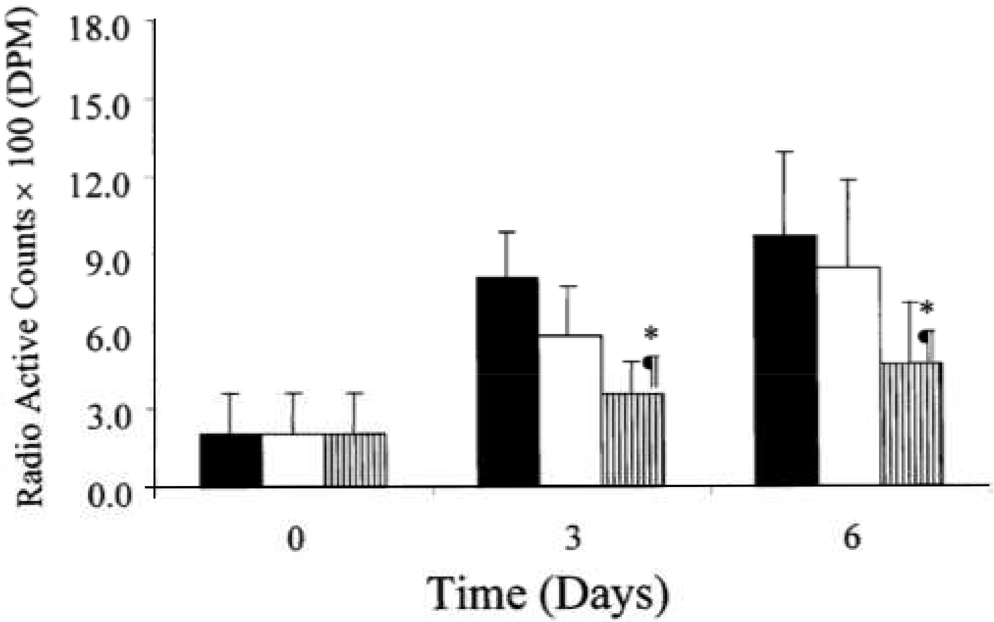
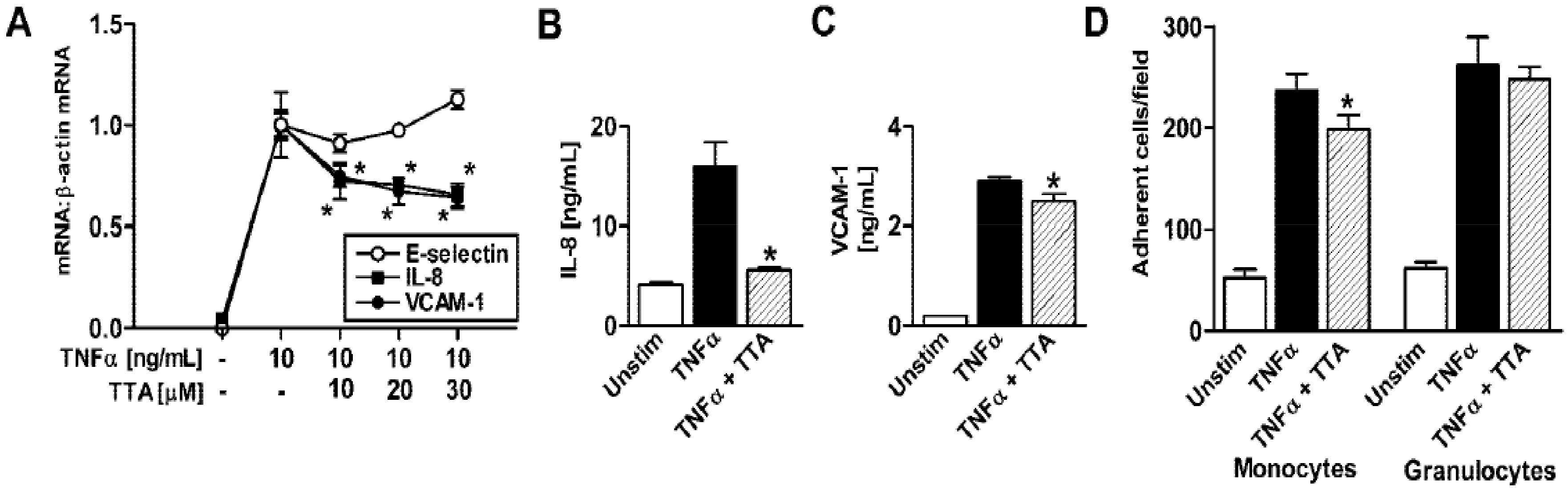
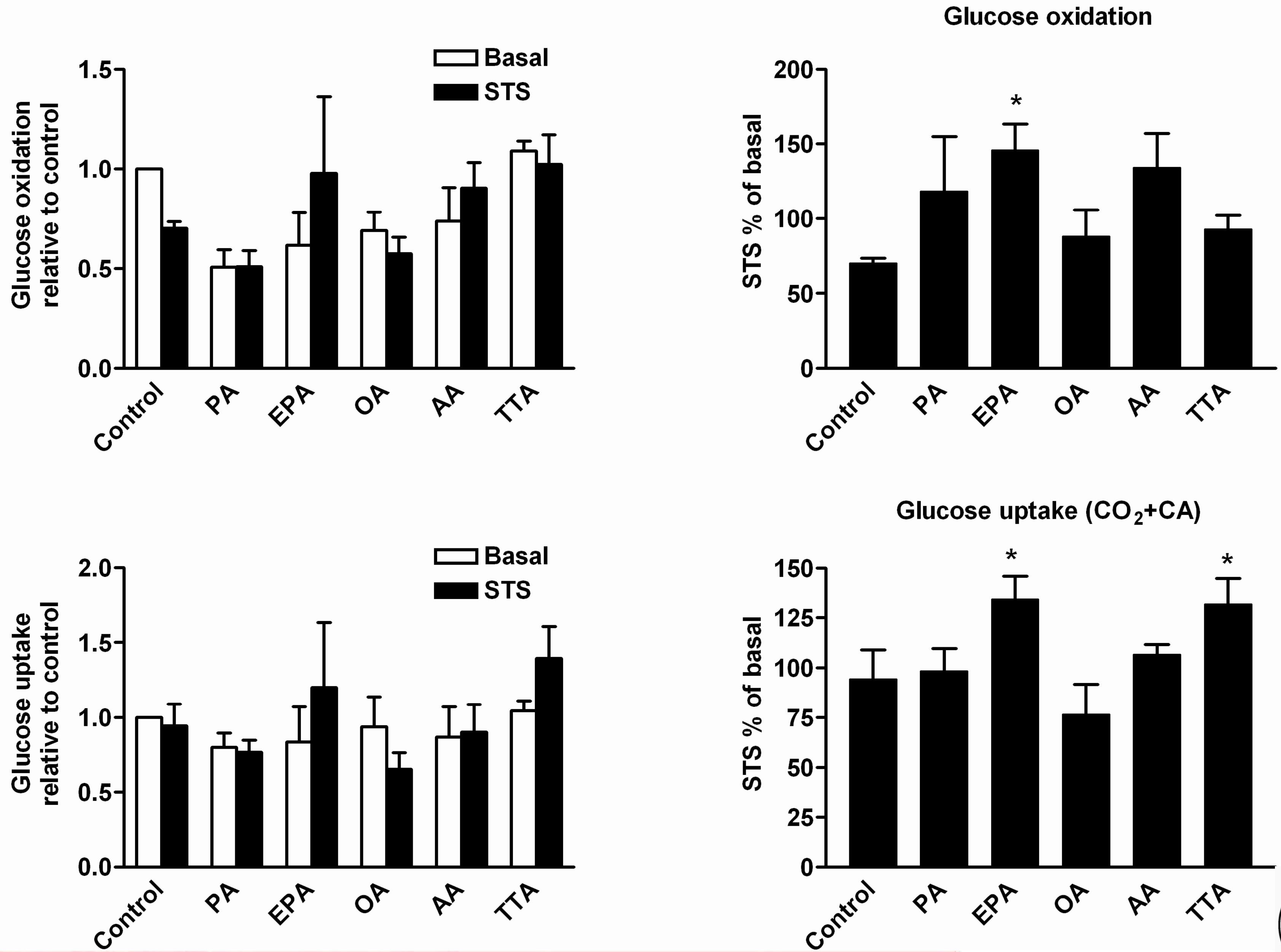


Fig. 3. The effect of TTA on H^3-Thymidine incorporation into human smooth muscle cells. (■) No addition, (□) palmitic acid 100 μM and () TTA 100 μM incubated for the indicated time periods. ¶ Compared with palmitic acid; * compared with no addition, $P < 0.05$. Data are mean ± S.D. of seven to eight experiments.

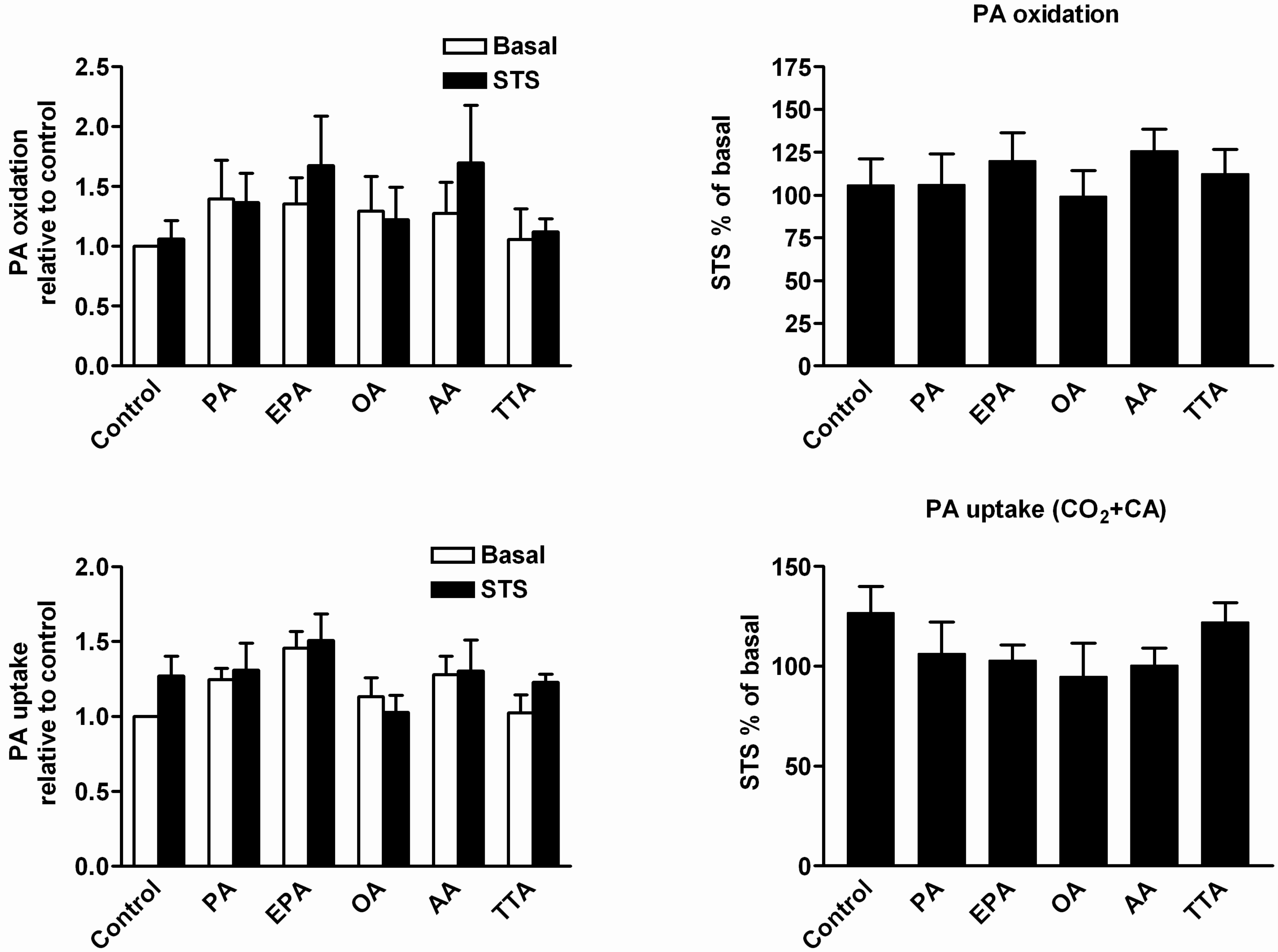
TTA reduces TNFα-induced endothelial activation



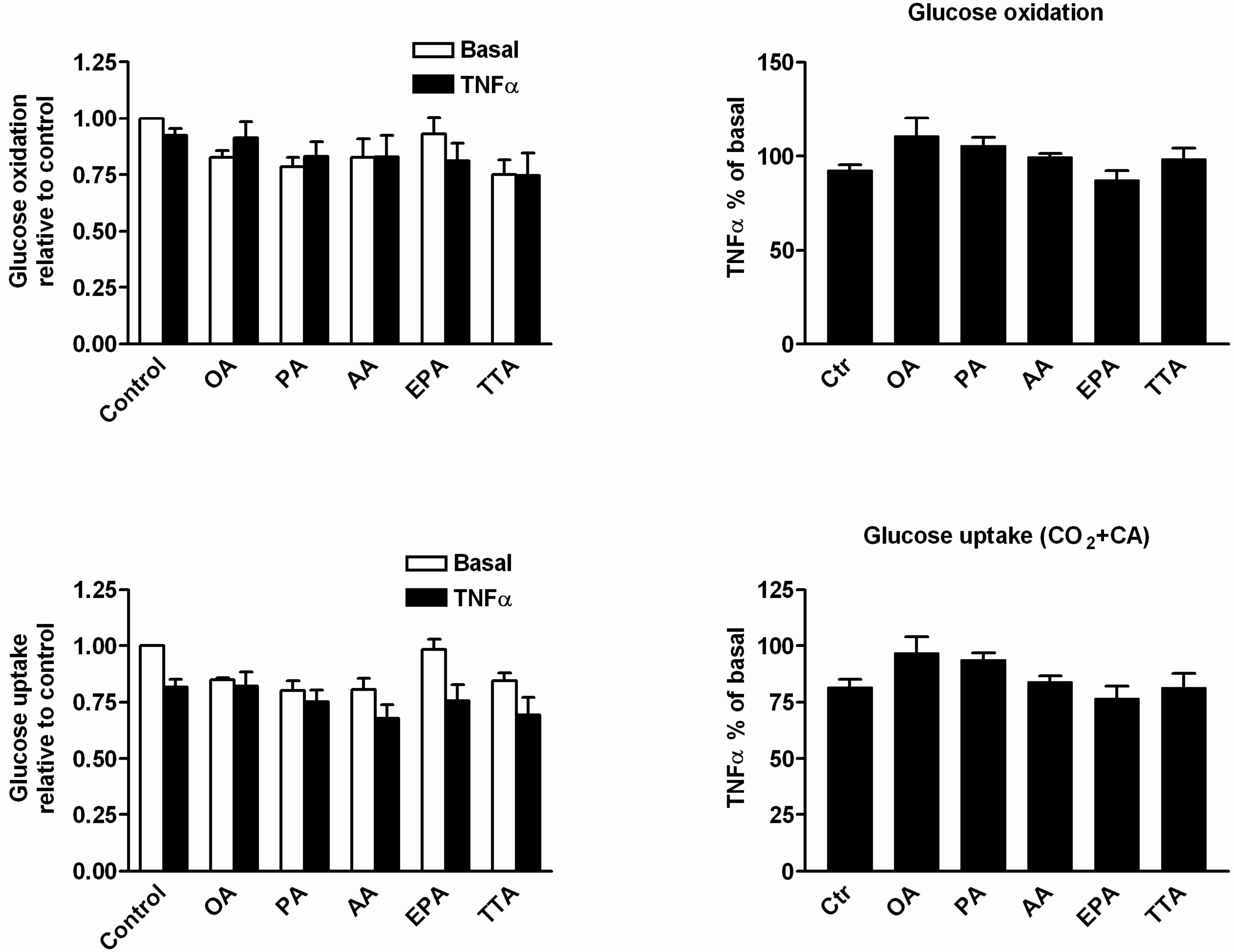
Staurosporine, fatty acids, [^{14}C]-glucose (n=4) *p<0.05 vs. control (T-test)



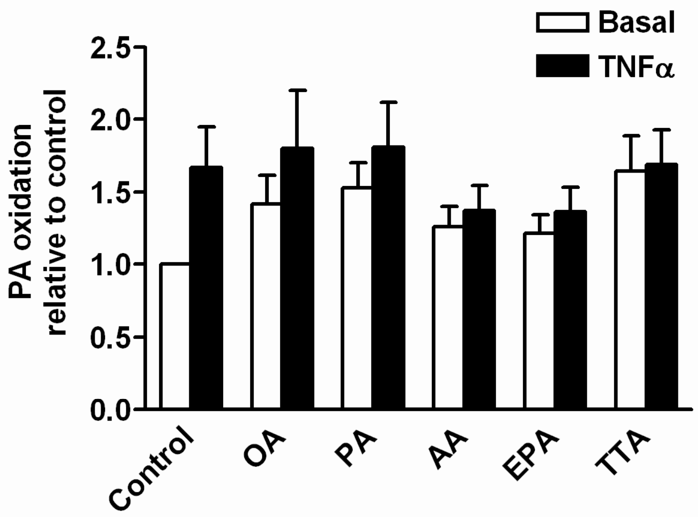
Staurosporine, fatty acids, [^{14}C]-PA (n=4)

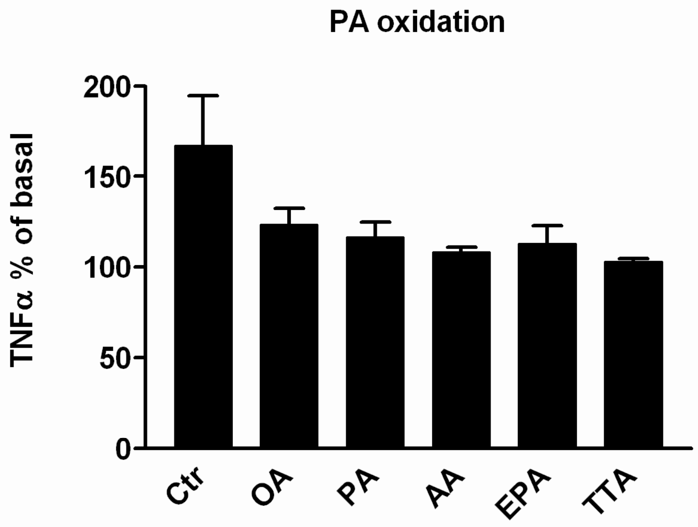


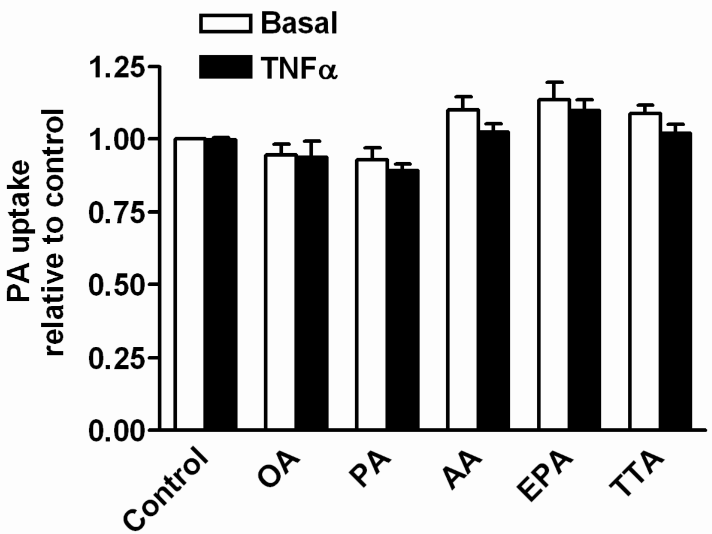
TNFa, fatty acids, [^{14}C]-glucose (n=4)

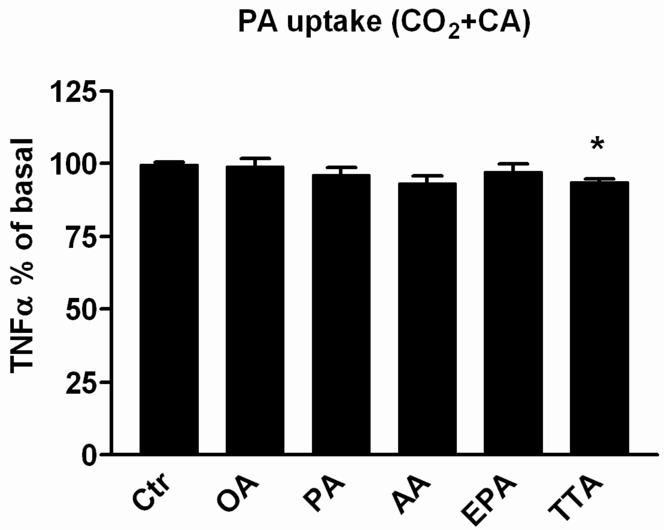


TNFa, fatty acids, [^{14}C]-PA (n=4)

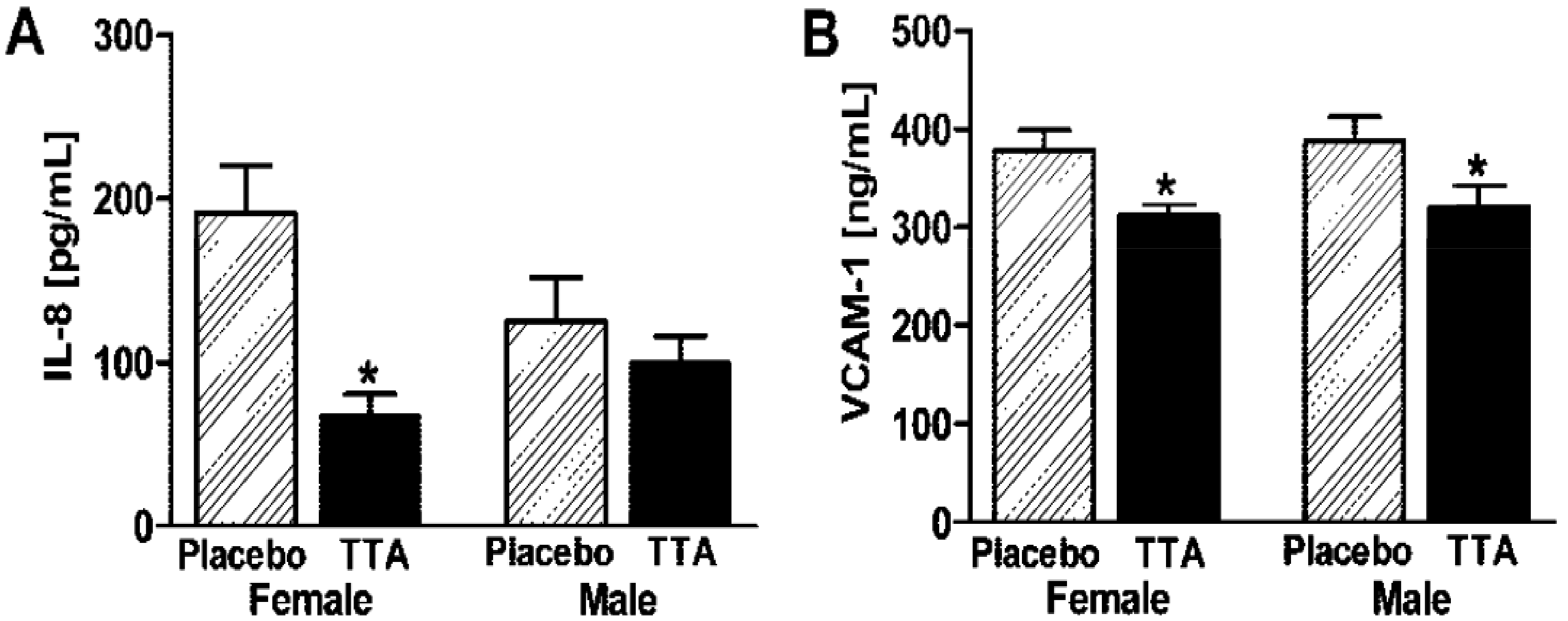








TTA decreases serum levels of sVCAM-1 And IL-8 in human patients with Psoriasis



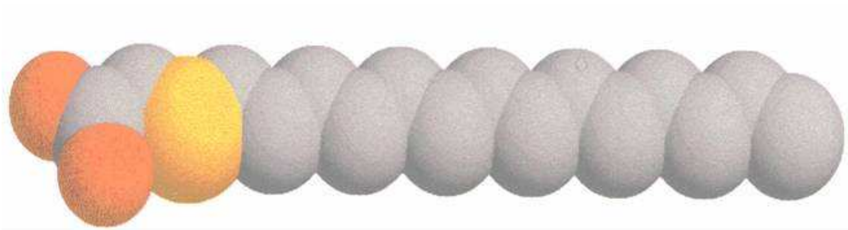
PLA_2 signaling and cross talk to mitochondria

- associated to mitochondria
- decline in mitochondrial function
- ATP production
- mitochondrial membrane potential
- ROS production

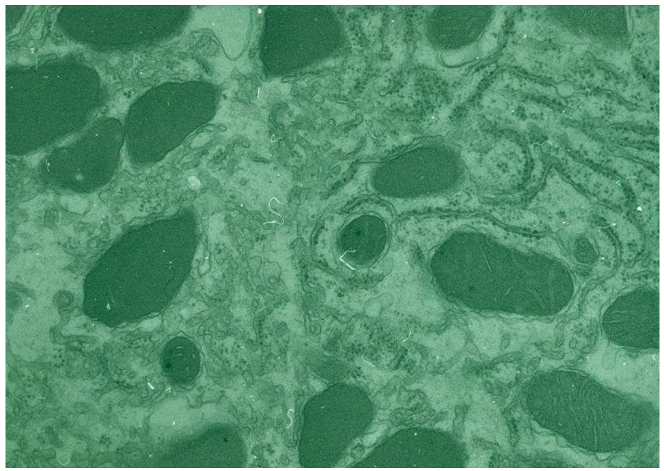
Mitochondrial dysfunction – impairment in the pathophysiology of AD patients

Mitochondria as target organelles

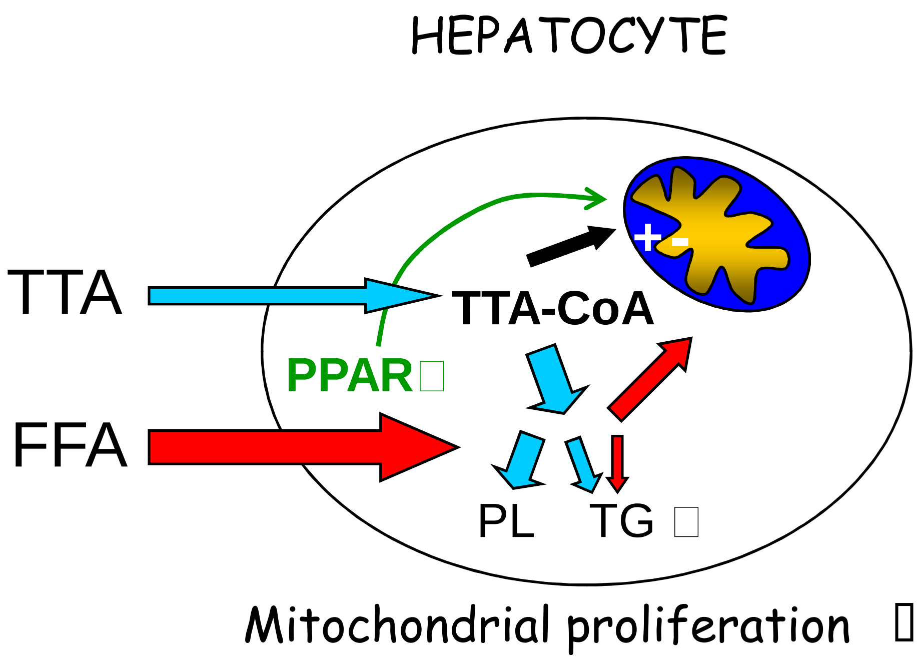
Increased mitochondrial β-oxidation followed by a decrease in triglyceride synthesis and secretion seems to be the primary mechanism underlying the hypolipidemic effect of EPA and fibrates in rats, rabbits and possibly also humans.



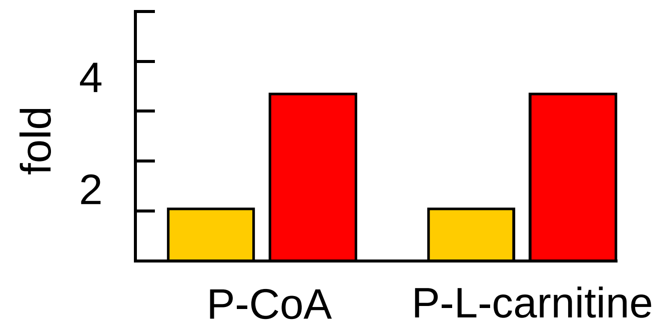
TTA (tetradecylthioacetic acid)



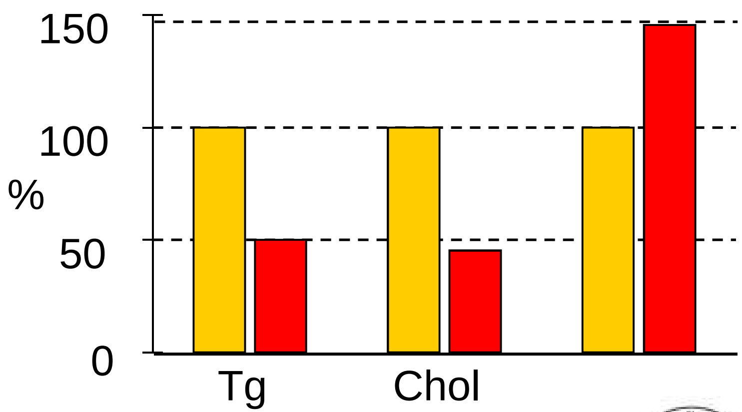
Increased hepatic β-oxidation and decreased levels of plasma lipids



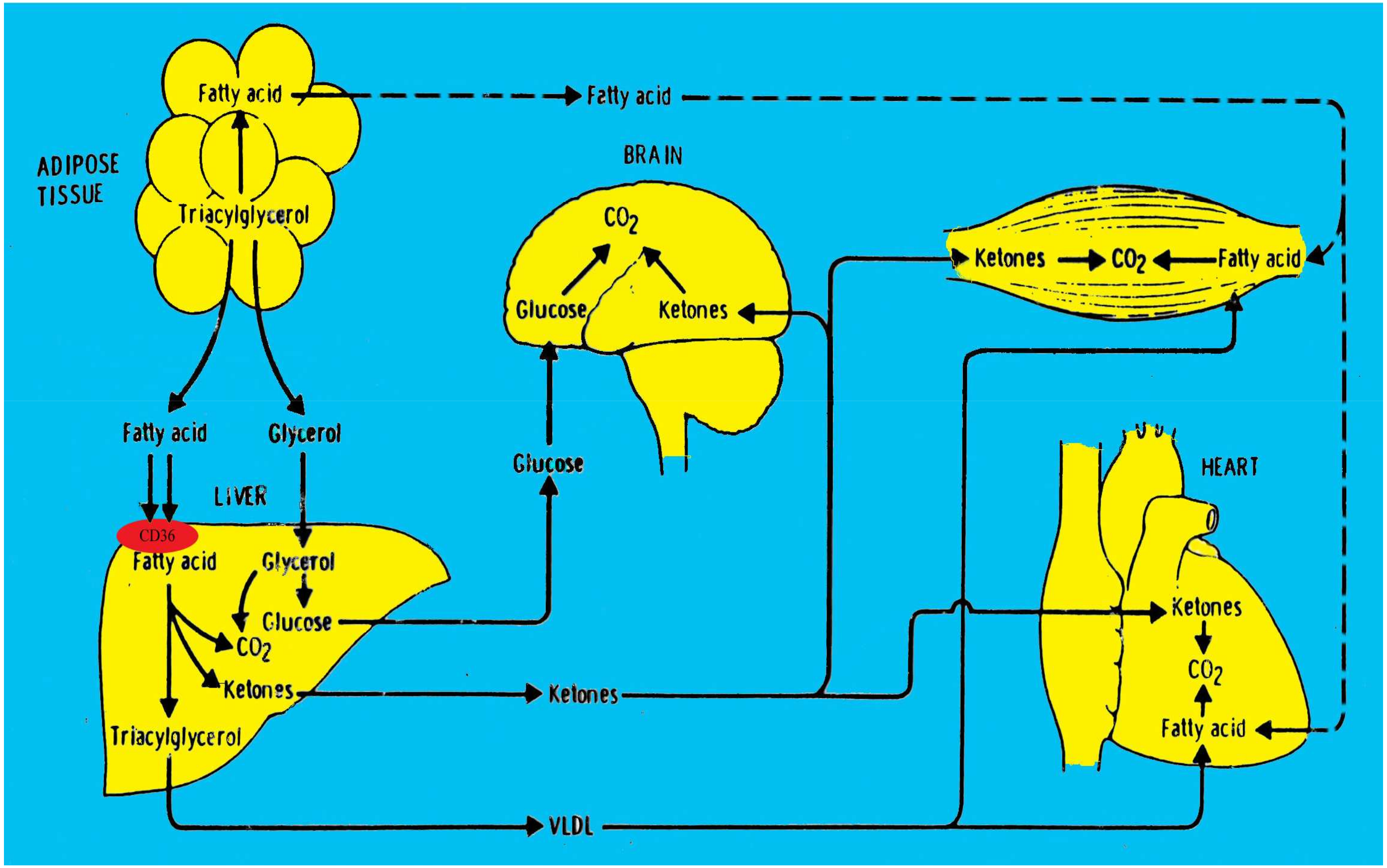
Fatty acid oxidation



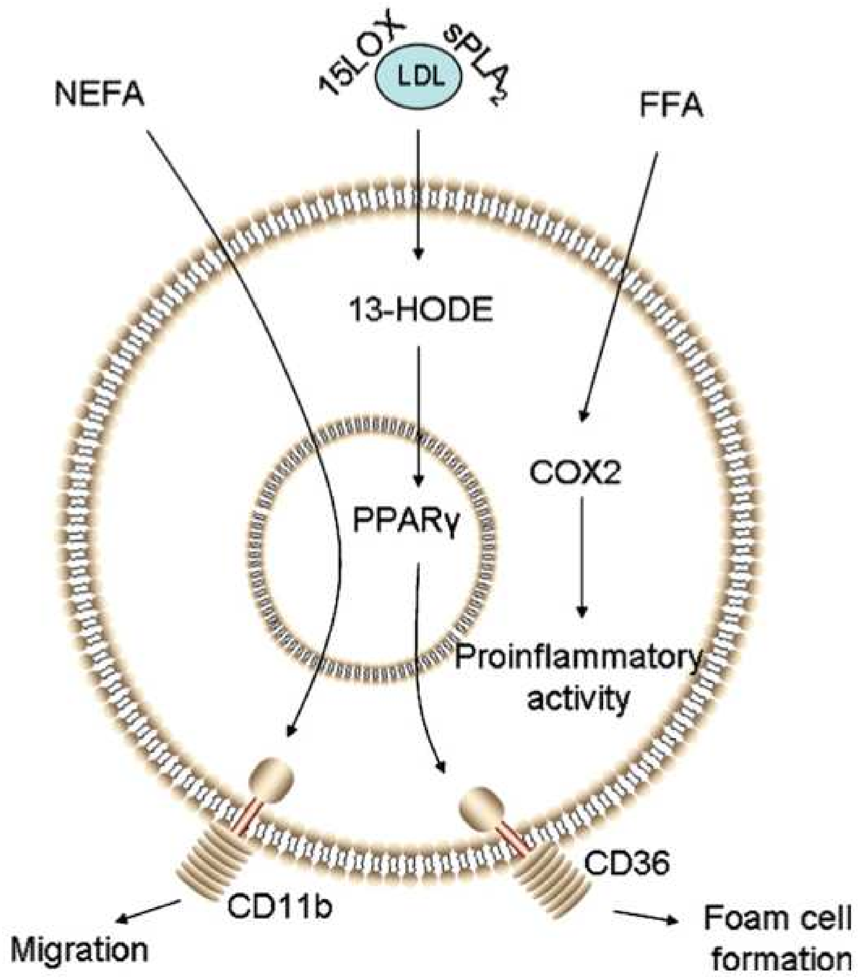
Plasma lipid levels



Transport of energy in the form of fatty acids



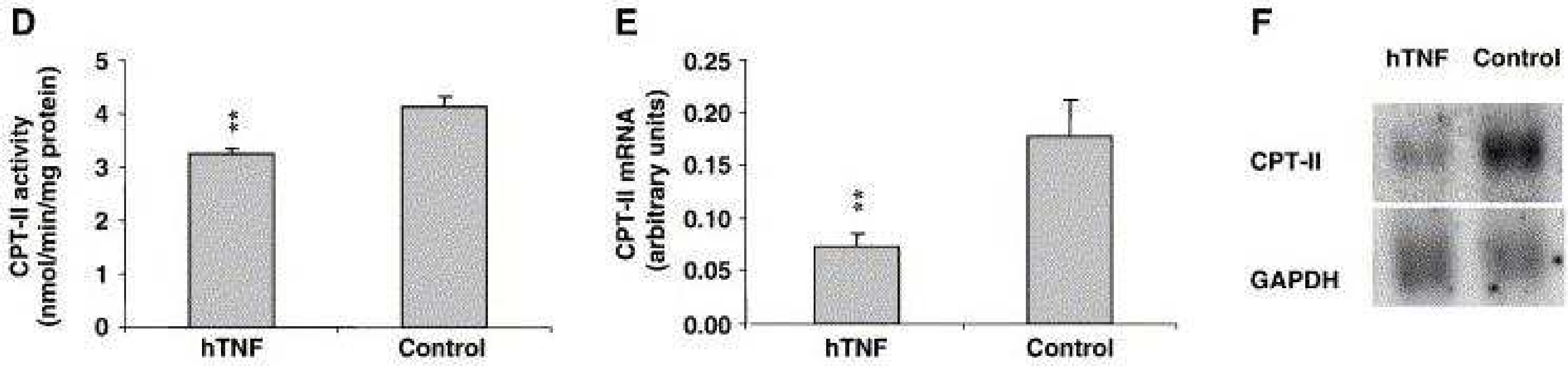
Cell signaling induced by fatty acids

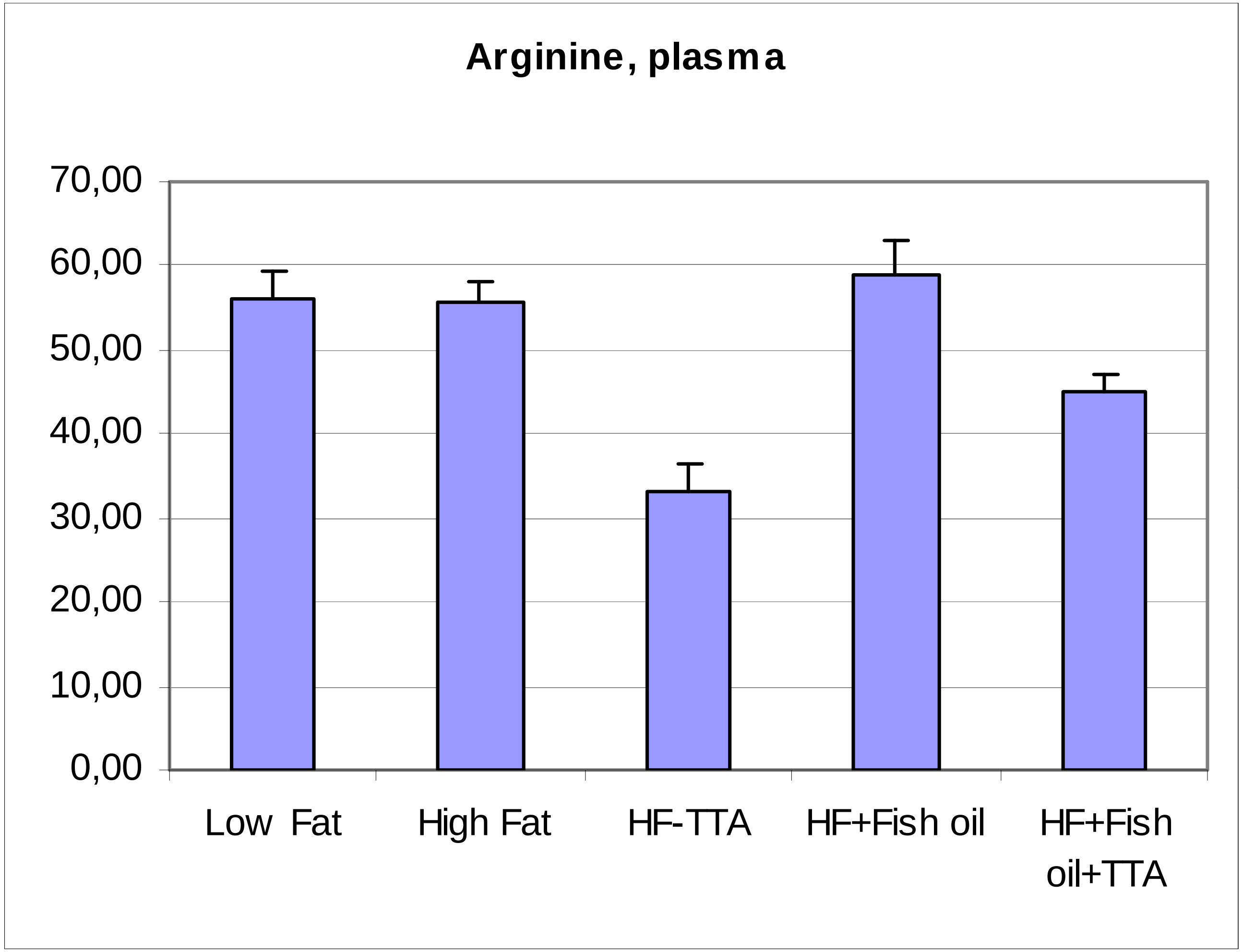


Oestevang and Johansen, 2006

- Systemic TNFalpha-exposure influence on the lipid metabolism in liver

- PPARs are key regulators of energy balance and genes involved in inflammatory disorders





Arginine, plasma
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
Low Fat
High Fat
HF-TTA
HF+Fish oil
HF+Fish oil+TTA

Cell-based multiwell assays for the detection of substrate accumulation and oxidation[1]

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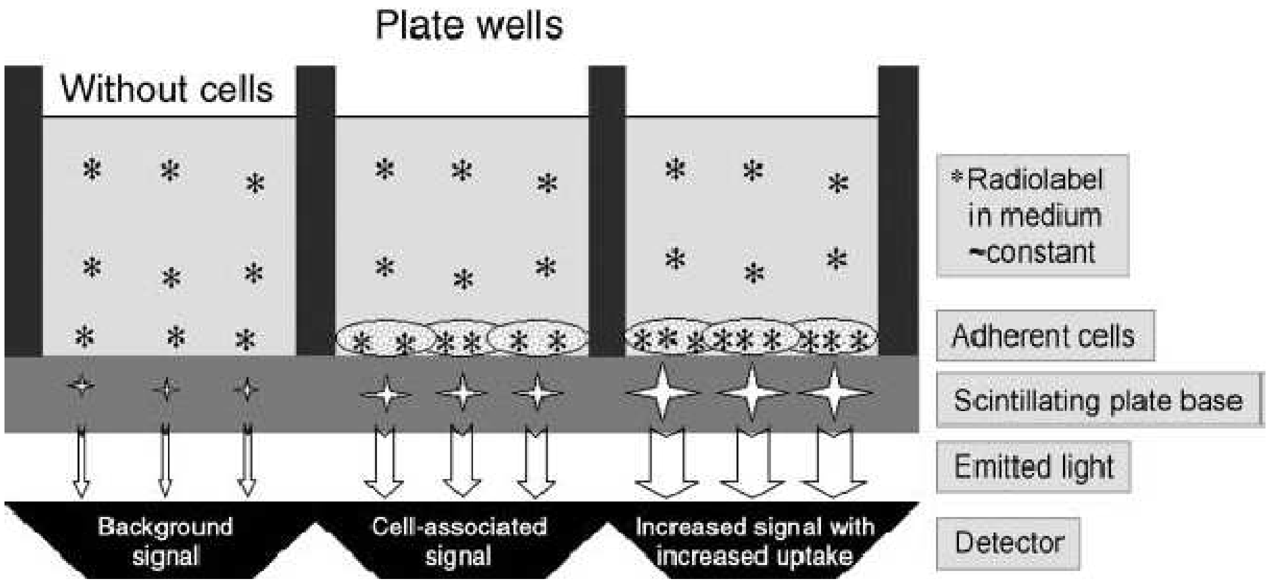


Fig. 1. Principles of the scintillation proximity assay (SPA).

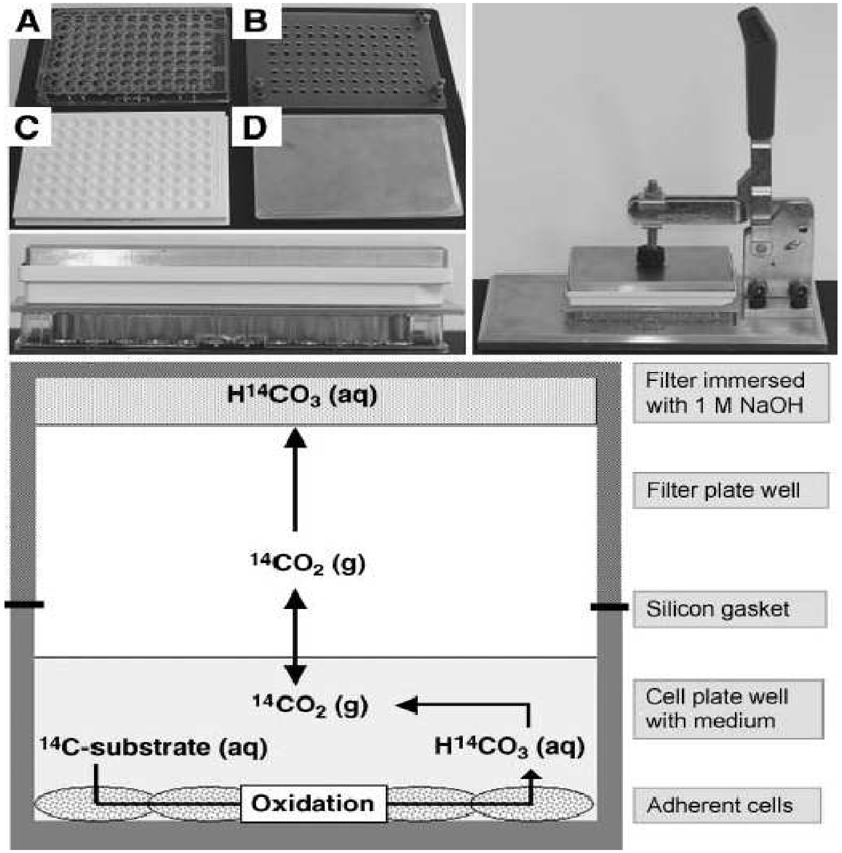
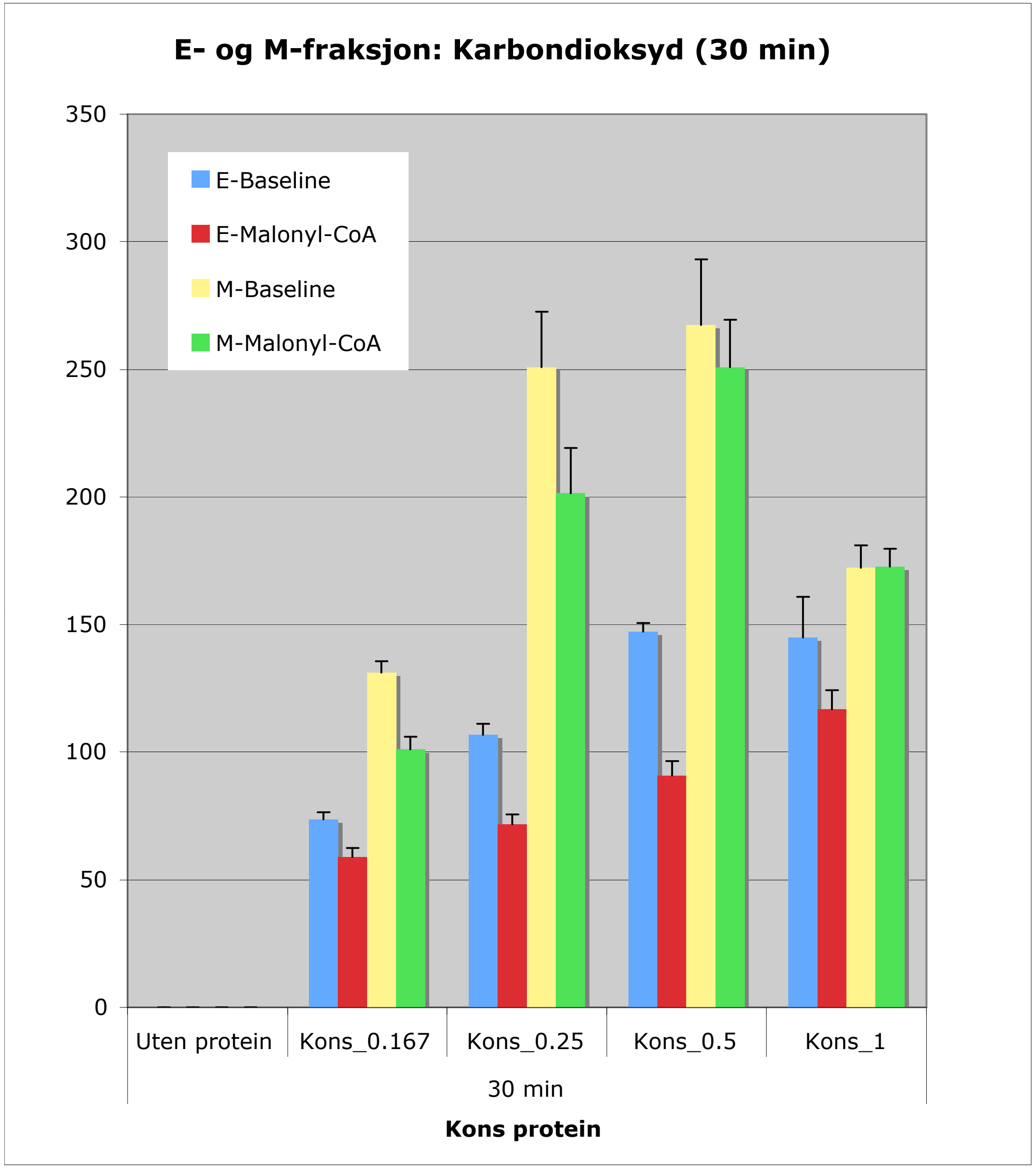
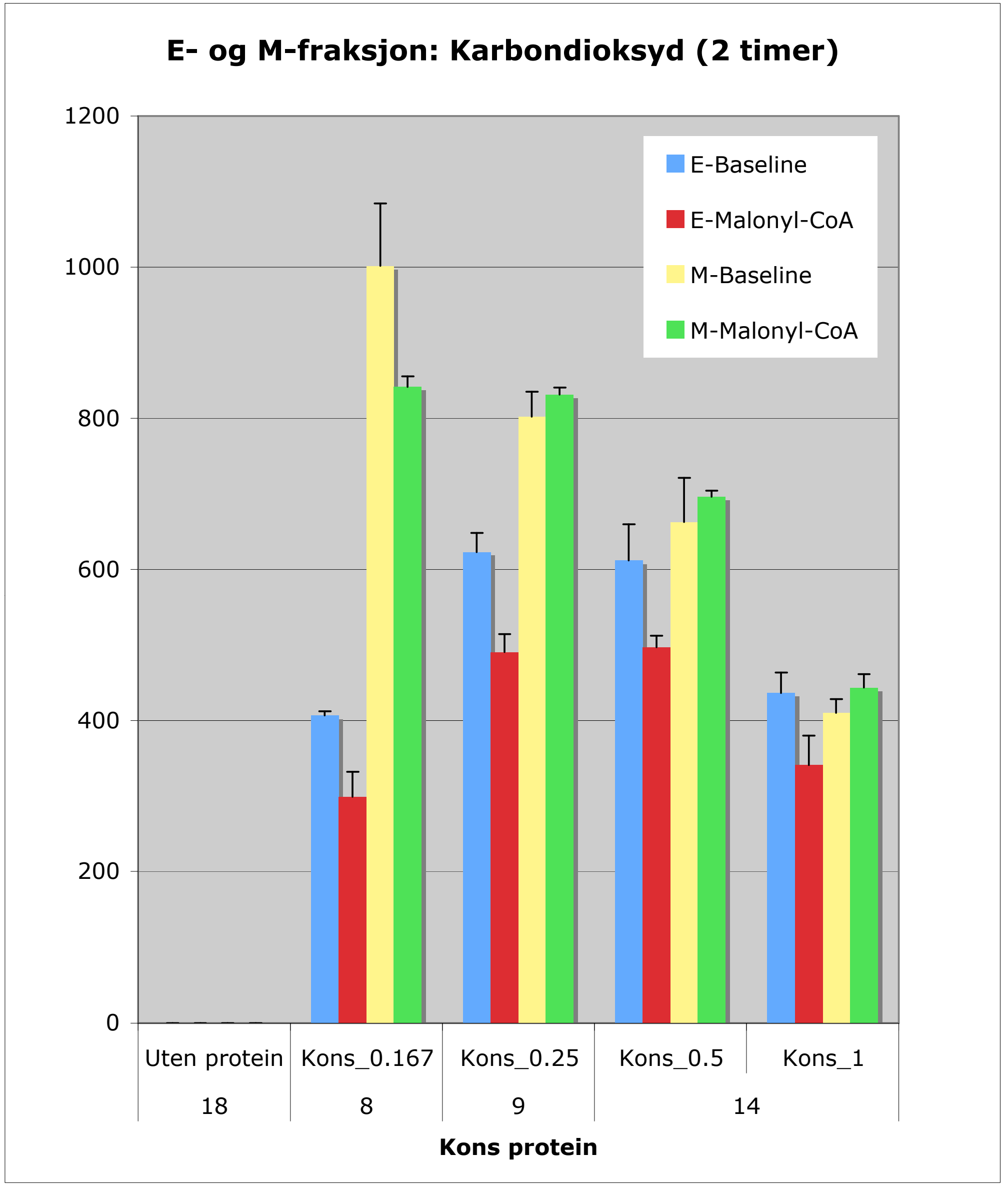
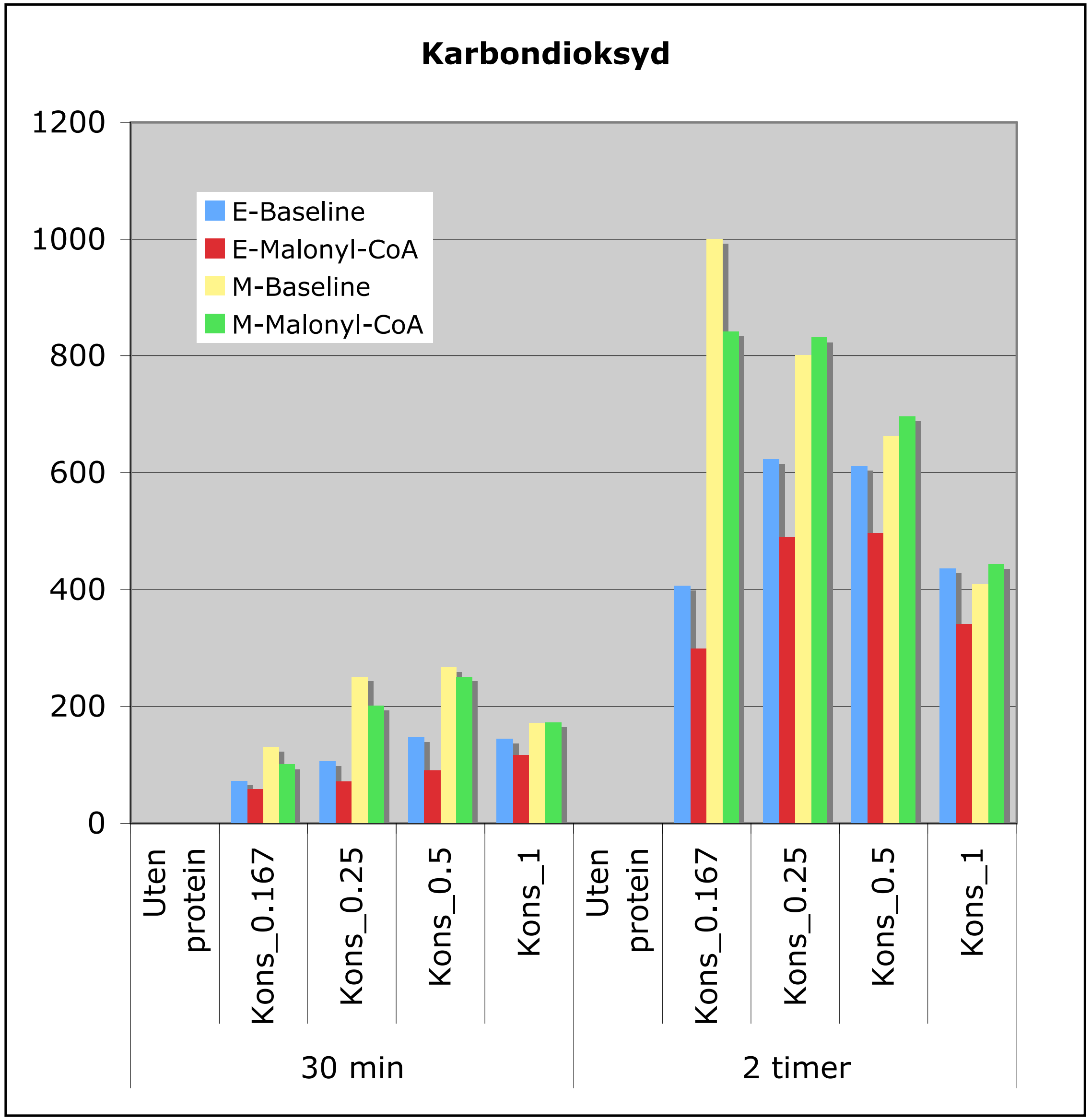


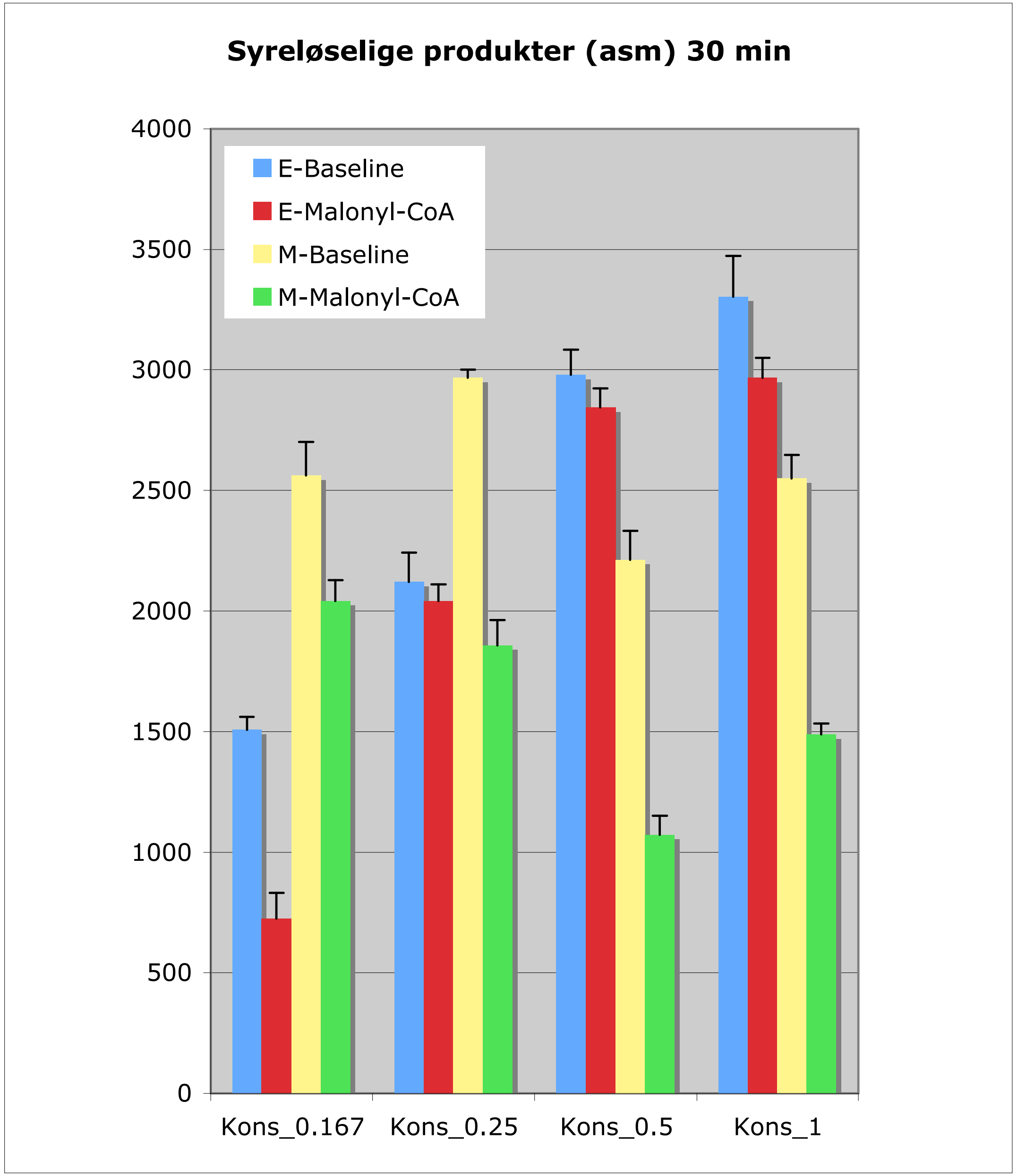
Fig. 2. The substrate oxidation appliance. The top left panel shows all of the different parts before assembly of the apparatus. A: 96-well tissue culture plate; B: silicon gasket with stabilizing knobs for each corner well; C: 96-well filter plate; D: metal plate for applying even pressure. The parts are assembled into a sandwich (left panel below) and put under pressure by the apparatus (top right panel). The lower panel illustrates the principles of the CO_2 trapping system. When a ^{14}C-labeled substrate is oxidized, the released CO_2 will be trapped in the alkaline suspension in the top filter.

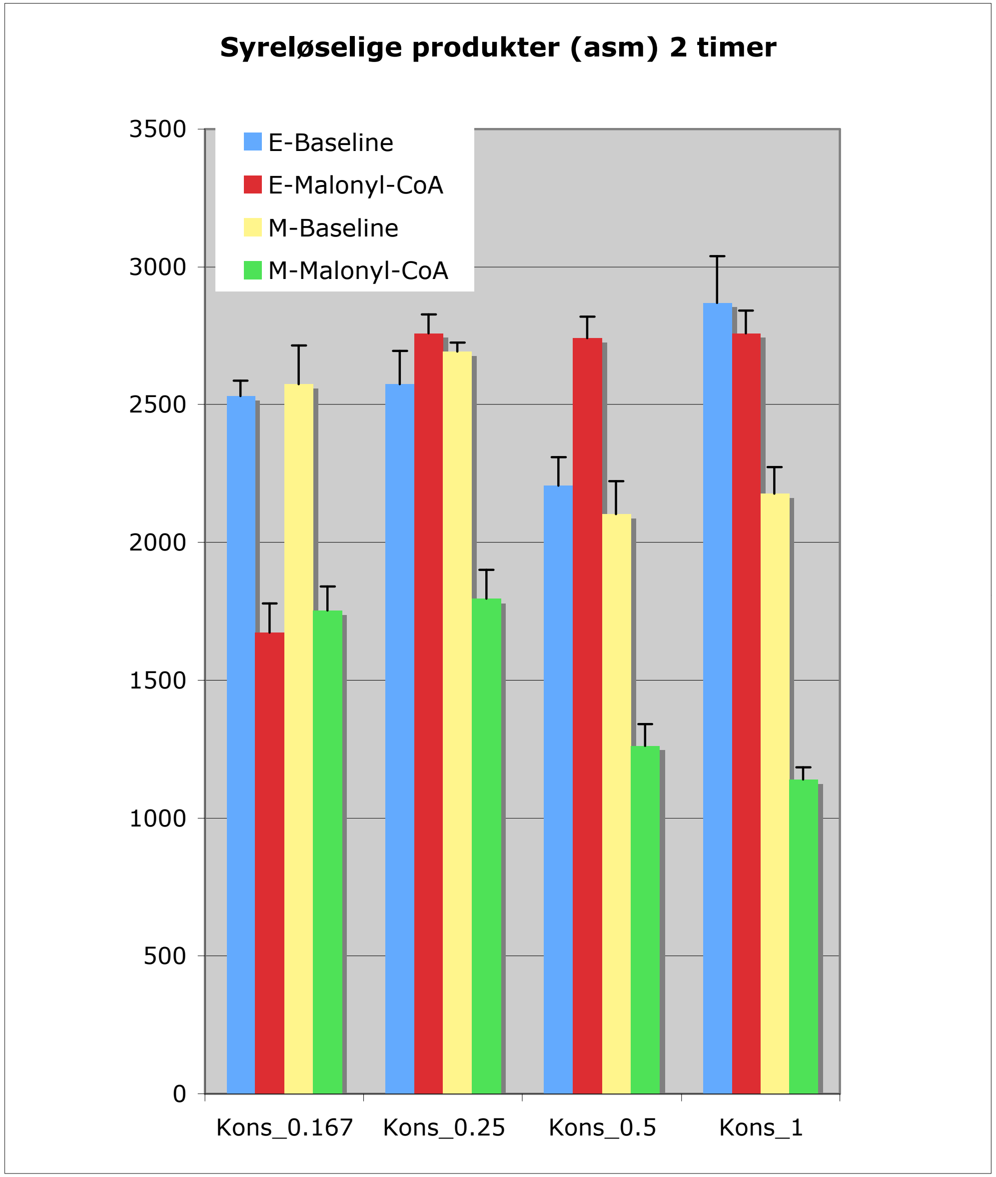

E- og M-fraksjon: Karbondioksyd (30 min)
350
300
250
200
150
100
50
0
E-Baseline
E-Malonyl-CoA
M-Baseline
M-Malonyl-CoA
Uten protein
Kons_0.167
Kons_0.25
Kons_0.5
Kons_1
30 min
Kons protein

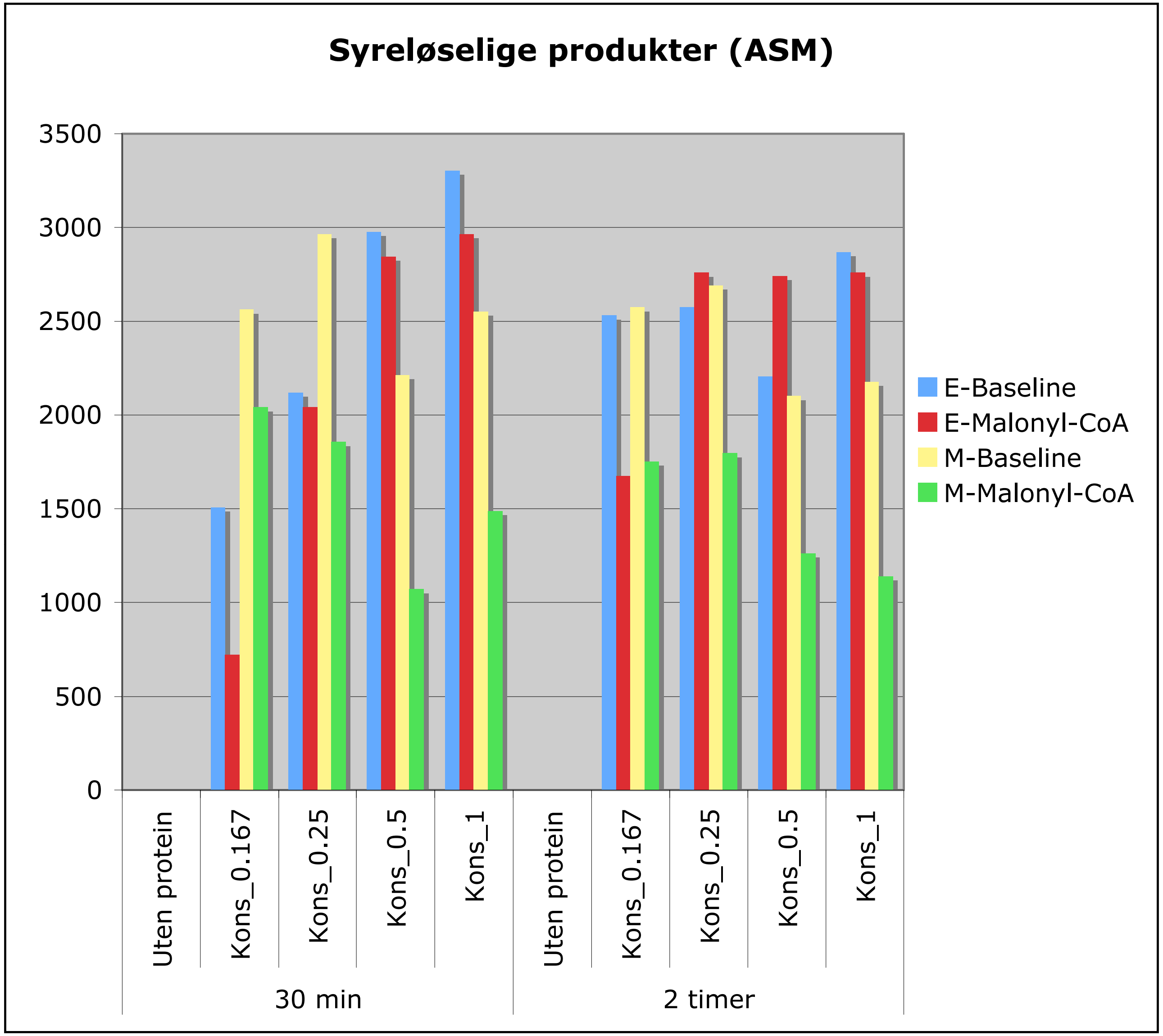

E- og M-fraksjon: Karbondioksyd (2 timer)
1200
1000
800
600
400
200
0
E-Baseline
E-Malonyl-CoA
M-Baseline
M-Malonyl-CoA
Uten protein
Kons_0.167
Kons_0.25
Kons_0.5
Kons_1
18
8
9
14
Kons protein

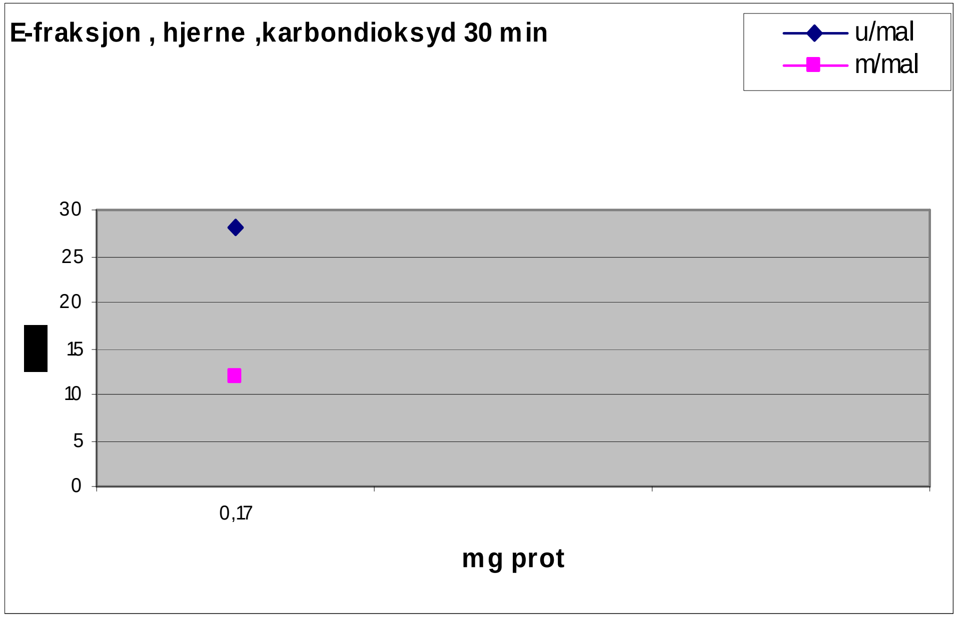


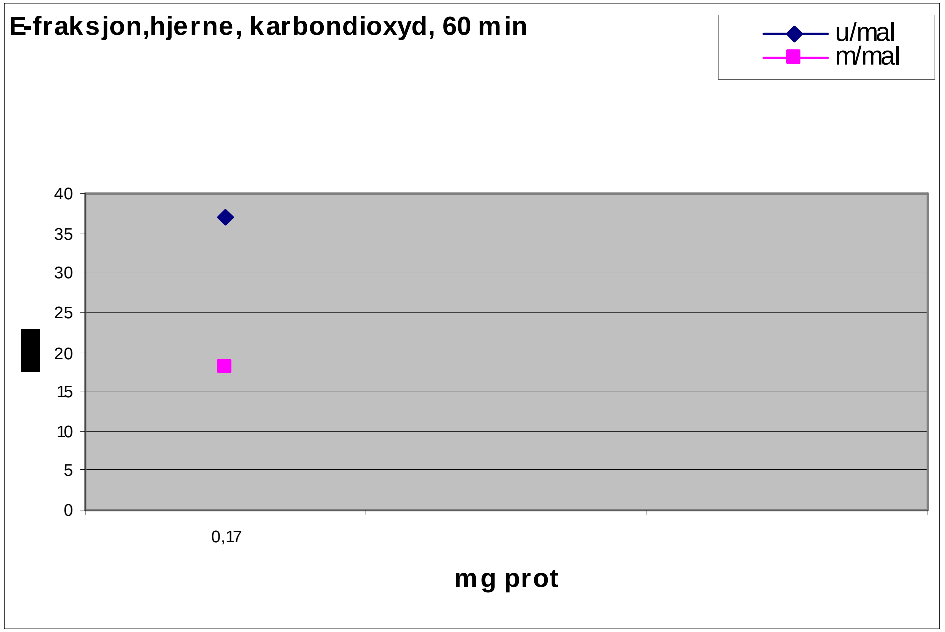
Karbondioksyd
1200
1000
800
600
400
200
0
E-Baseline
E-Malonyl-CoA
M-Baseline
M-Malonyl-CoA
Uten protein
Kons_0.167
Kons_0.25
Kons_0.5
Kons_1
Uten protein
Kons_0.167
Kons_0.25
Kons_0.5
Kons_1
30 min
2 timer


Syreløselige produkter (asm) 30 min
4000
3500
3000
2500
2000
1500
1000
500
0
E-Baseline
E-Malonyl-CoA
M-Baseline
M-Malonyl-CoA
Kons_0.167
Kons_0.25
Kons_0.5
Kons_1


Syreløselige produkter (asm) 2 timer
3500
3000
2500
2000
1500
1000
500
0
E-Baseline
E-Malonyl-CoA
M-Baseline
M-Malonyl-CoA
Kons_0.167
Kons_0.25
Kons_0.5
Kons_1


Syreløselige produkter (ASM)
3500
3000
2500
2000
1500
1000
500
0
Uten protein
Kons_0.167
Kons_0.25
Kons_0.5
Kons_1
Uten protein
Kons_0.167
Kons_0.25
Kons_0.5
Kons_1
30 min
2 timer
E-Baseline
E-Malonyl-CoA
M-Baseline
M-Malonyl-CoA


E-fraksjon , hjerne ,karbondioksyd 30 min
u/mal
m/mal
30
25
20
15
10
5
0
0,17
mg prot


E-fraksjon,hjerne, karbondioxyd, 60 min
u/mal
m/mal
40
35
30
25
20
15
10
5
0
0,17
mg prot

Can bioactive peptides and TTA abolish the PLA_2 mediated inflammation in atherosclerosis?

A collaborative AtheroRemo project.
Partners: Berge,Chiesa,Laksonen

Objectives

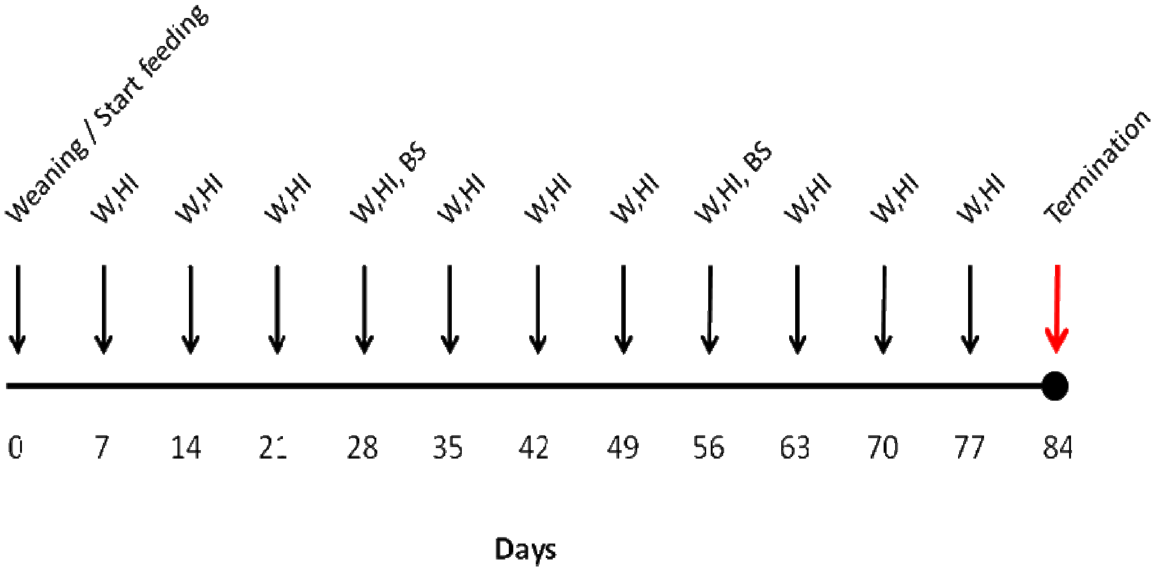
In this study we will test the effect of bioactive peptides and TTA on PLA_2 mediated atherosclerosis in mouse.

Hypothesis

Administration of TTA or cytosolic or secretory Phospholipase A_2 inhibitor ameliorates atherogenesis. We will also investigate whether a bioactive peptide ameliorates atherogenesis through anti-apoptotic mechanisms.

Group	ID		Dose of test substance	Administration route	Number of animals
1	LF	Low fat	7%	diet	8
2	HF	High Fat	21%	diet	8
3	HF-FO	High fat + Fish oil	5%	diet	8
4	HF-TTA	High fat + TTA	0.75%	diet	8
5	HF-K	High Fat + Krill oil	5%?	diet	8
6	HF-FP	High fat + Fish peptide	10%?	diet	8
7	HF-HNA	High fat + Humanin A	5 or 10 ng/g bw	injection ip	8
8	HF-HNB	High fat + Humanin B	5 or 10 ng/g bw	injection ip	8
9	HF-LA	High fat + L-arginine	1%	drinking water	8
10	HF-TNaI	High fat + TNFα inhibitor	Thalidomide?	?	8
11	HF-PLAI	High fat + sPLA2 inhibitor	? (A-002)	diet	8

During experiment	Method	Parameter (Frequency)
Feed intake		(When fed)
Animal weight		(Weekly)
Blood samples	Punctuation of tail vein?	LP, Cyt (Day 0, 28, 56)
At time of sacrifice		(Day 84)
Blood	Cardiac punctuation	LP, Cyt
Brain	D, W, SF	GE, LP, MF
Heart (cardiac tissue)	D, W, SF	GE, LP
Aorta	D, SF	GE, Atherosclerosis
Liver	D, W, SF	GE, LP, MF
Kidney	D, W, SF	GE
Spleen	D, W, SF	GE
Fat tissue	D, W, SF	GE, LP, MF
Intestine	D, W, SF	GE
Soleus?		



W-Weight; HI-Humanin; BS-Blood sample

Effect of TTA in lipid rich diet on inflammation in diabetes sensitive male mice

Oslo - Bergen

Objective

In this study we will test the effect of TTA in the diet on diabetes (glucose tolerance), weight development and inflammation in mice disposed to diabetes and obesity.

Hypothesis

Mice sensitive to diabetes (transgen human ApoE3, male mice) will show less weight increase and LPS induced inflammation as well as increased tolerance to glucose changes, if fed a lipid rich diet containing TTA than without TTA.

Groups

	Group	Description	ApoE+/Luc+	ApoE+/Luc-
1	Control	High fat diet	6	5
2	TTA	High fat diet + TTA	6	5

Readout	Handling of tissue/ assay	Performed by
β-oxiadation	Snap freeze Homogenization and PNS	Arild, Rolf?
Cytokines	?	?
Brain markers	?	Rolf
Luciferase activity	Snap freeze Homogenization Protein and luciferase assay	Torunn
Weight	During experiment	Torunn
Inflammation	In vivo imaging after LPS treatment	Torunn
Glucose tolerance	In vivo during experiment	Torunn

	ApoE+/luc+ **6 mice in each group**	**ApoE+/luc-** **5 mice in each group**
Brain **Heart** **Liver**	Betaox Cytokines Brain markers Luc	Betaox Cytokines Brain markers
adipose	?	?
muscle	?	?
lung	?	?
spleen	?	?
kidney	?	?
blood	?	?

Can bioactive peptides, marine oils or TTA put out the TNF alpha mediated inflammation in mouse brain?

A project in the KRAKOW research

Objectives

In this study we will test the effect of bioactive peptides, fish oil and TTA on inflammation in brain of mouse over expressing tumor necrosis factor alpha (TNFα).

Hypothesis

Administration of bioactive peptides, fish oil, TTA or Humanin ameliorates inflammation and impaired mitochondrial function mediated by TNF α.

Protocol design

Mouse model

At 4 weeks of age apoE$^{-/-}$ mice spontaneously <u>start</u> developing atherosclerotic lesions

Diet and administration

Group	ID		Dose of test substance	Administration route	Number of animals
1-2	WHF-C	Western High Fat (WHF)		Diet	6-6
3-4	WHF-TTA	WHF + TTA	0.75%	Diet	6-6
5-6	WHF-HN	WHF + sPLA2-inhibitor	? U/g bw	diet	6-6
7-8	WHF-HN	WHF + cPLA2-inhibitor	? U/g bw	diet	6-6
9-10	WHF-FP	WHF + Fish peptide	5%	Diet	6-6