

A Carotenoid/Tocotrienol rich palm oil protects against induced inflammation in a rodent model

Jacques van Rooyen^{1,3}

E Katengua-Thamahane^{1,4}, JL Marnewick¹, G Szucs², P Ferdinandy³, T Csont², C Csonka²

¹Cape Peninsula University of Technology, South Africa, ²University of Szeged, ³Semmelweis University, Hungary, ⁴Botho University, Botswana



Talk structure

A. Inflammation study 2015



B. Combination Therapy 2014



C. Heart studies 2005-2014



D. Product Development 2012-2015



E. USA Market Experience 2012-2015

Inflammation and Heart: Markets

2000 Top Supplements in USA

Arthritis/Inflammation
Heart
Digestion

2000 Top Herbs in USA

Anti-oxidant
Cholesterol
Prostate
Inflammation

2010 Top Drugs Revenue World

Lipitor - Cholesterol
Embrel - Inflammation
Celebrex - Arthritis
\$16.2 out of \$58
(28%)

2012 Top Prescription World

Zocor - statin - 94 mil
Lisinopril - BP - 87 mil

Synergistic effects of anti-oxidants

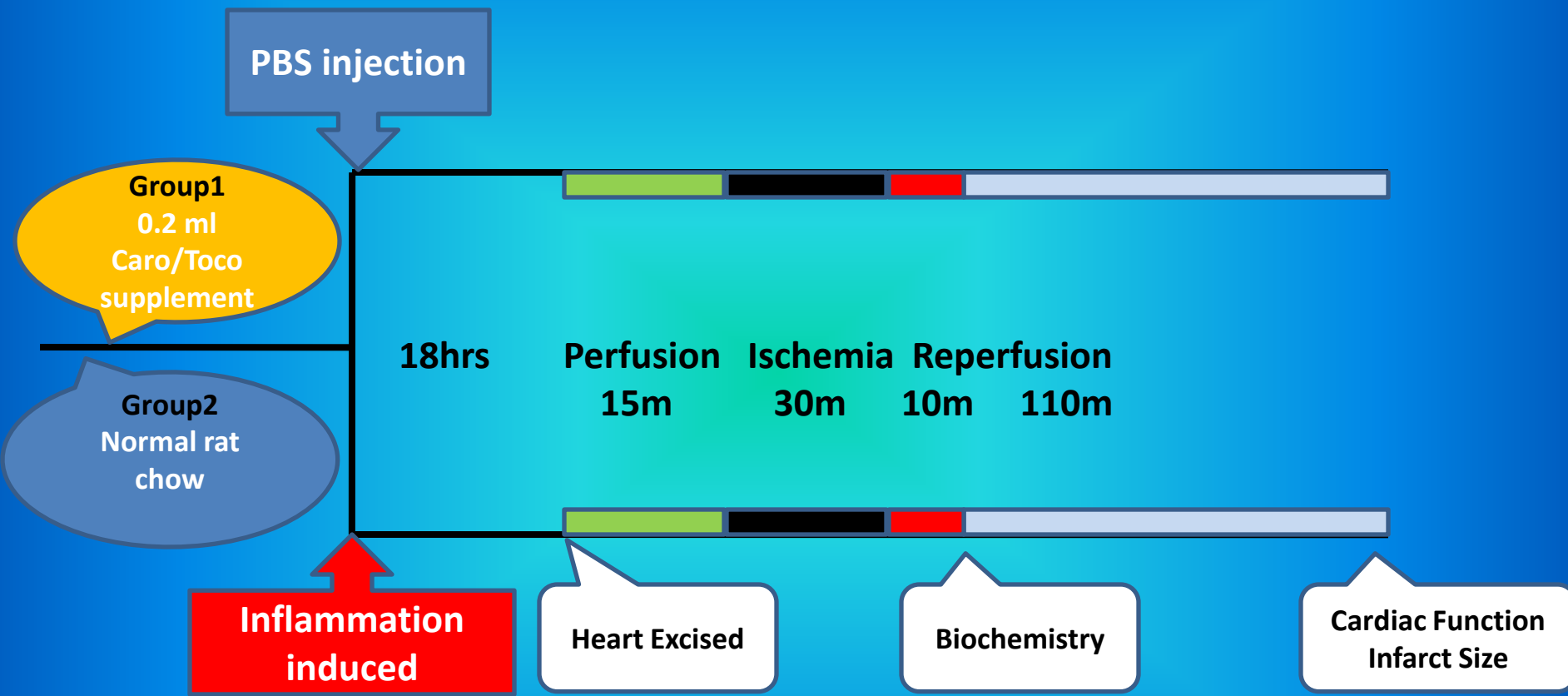
“A cocktail of anti-oxidants may have a far more profound anti-oxidative effect due to the synergistic action of individual compounds”. Stahl and Sies 2005

Therefore

A cocktail of carotenoid and vitamin E tocotrienol embedded in mono- and poly-unsaturated fatty acids might provide the ultimate combinations for a dietary supplement.

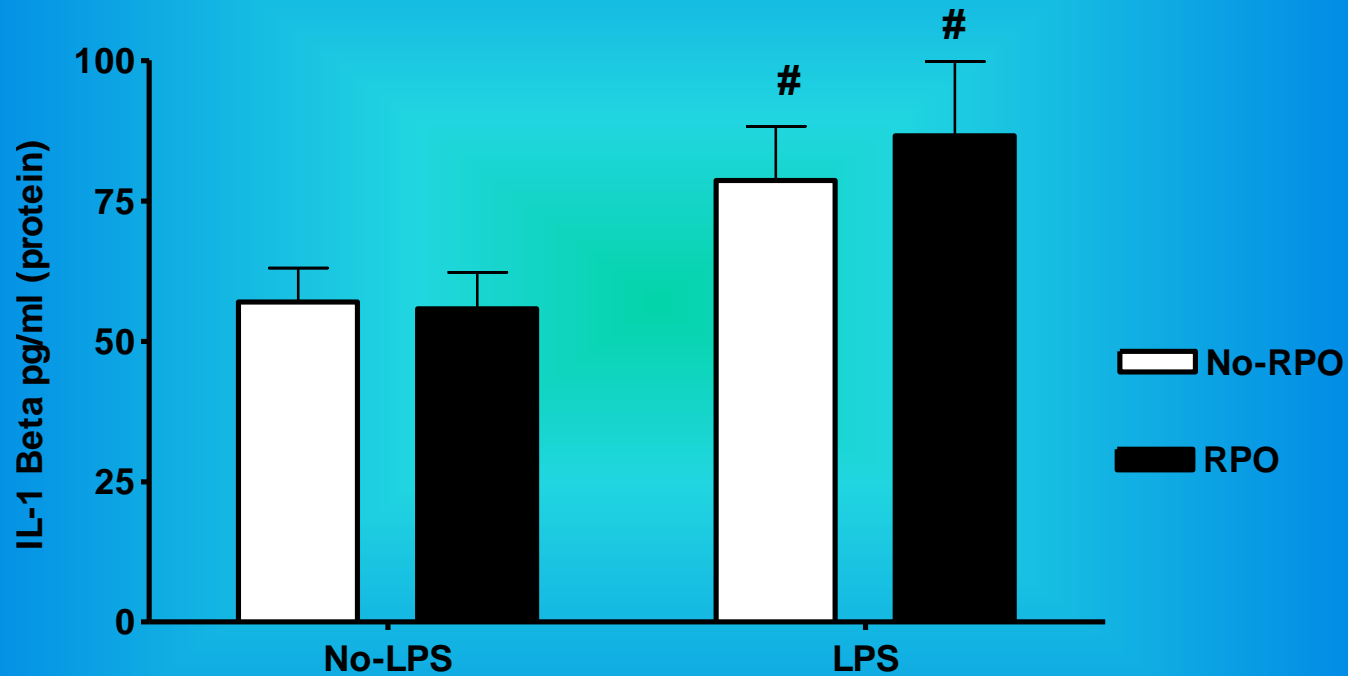
A

Study Design



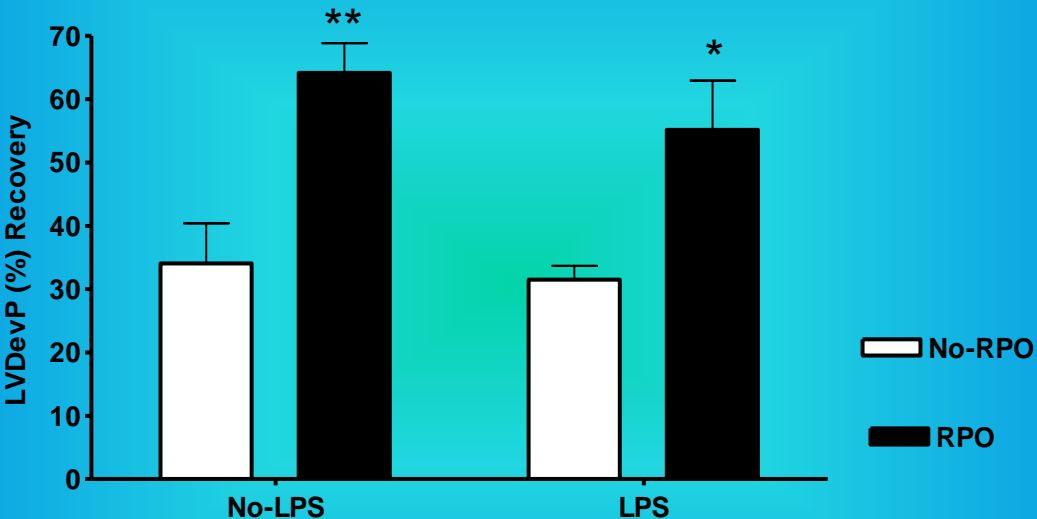
A

Results



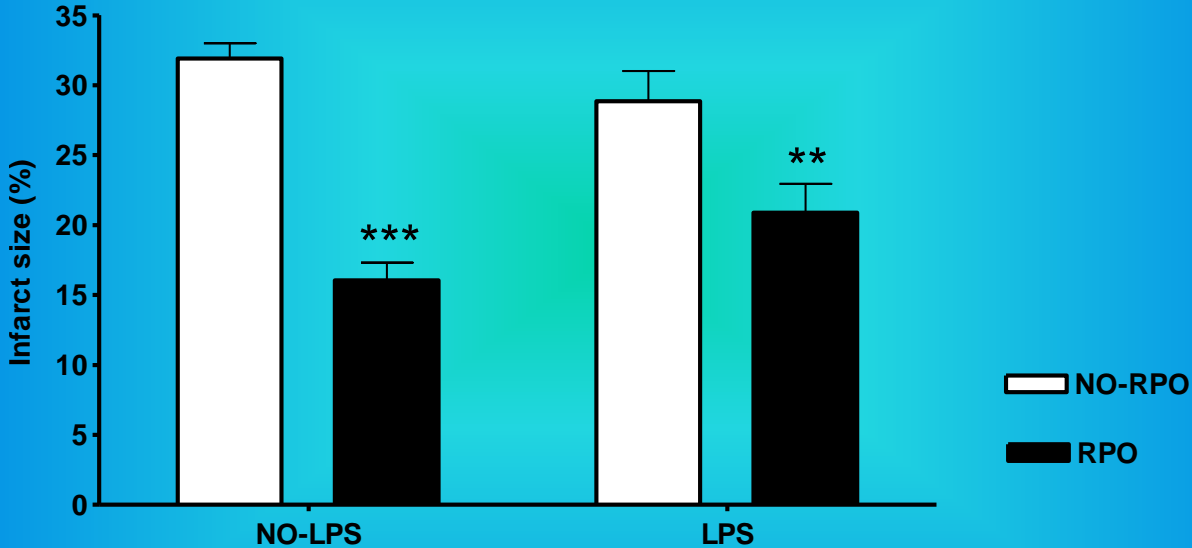
IL-1 β is an indicator of the presence of inflammation. In this case it shows that our model of inducing inflammation with LPS was successful

Results



This graph shows that a carotenoid/tocotrienol supplement improved recovery significantly in the inflammation induced group

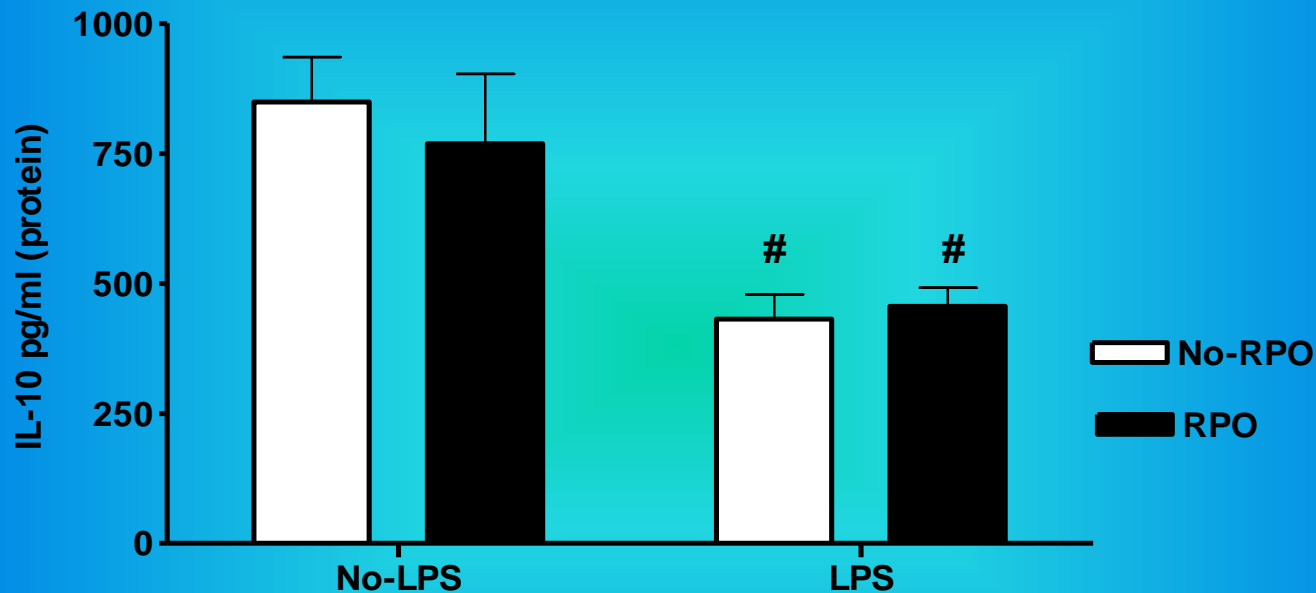
Results



Infarct size was significantly reduced by carotenoid/tocotrienol rich palm

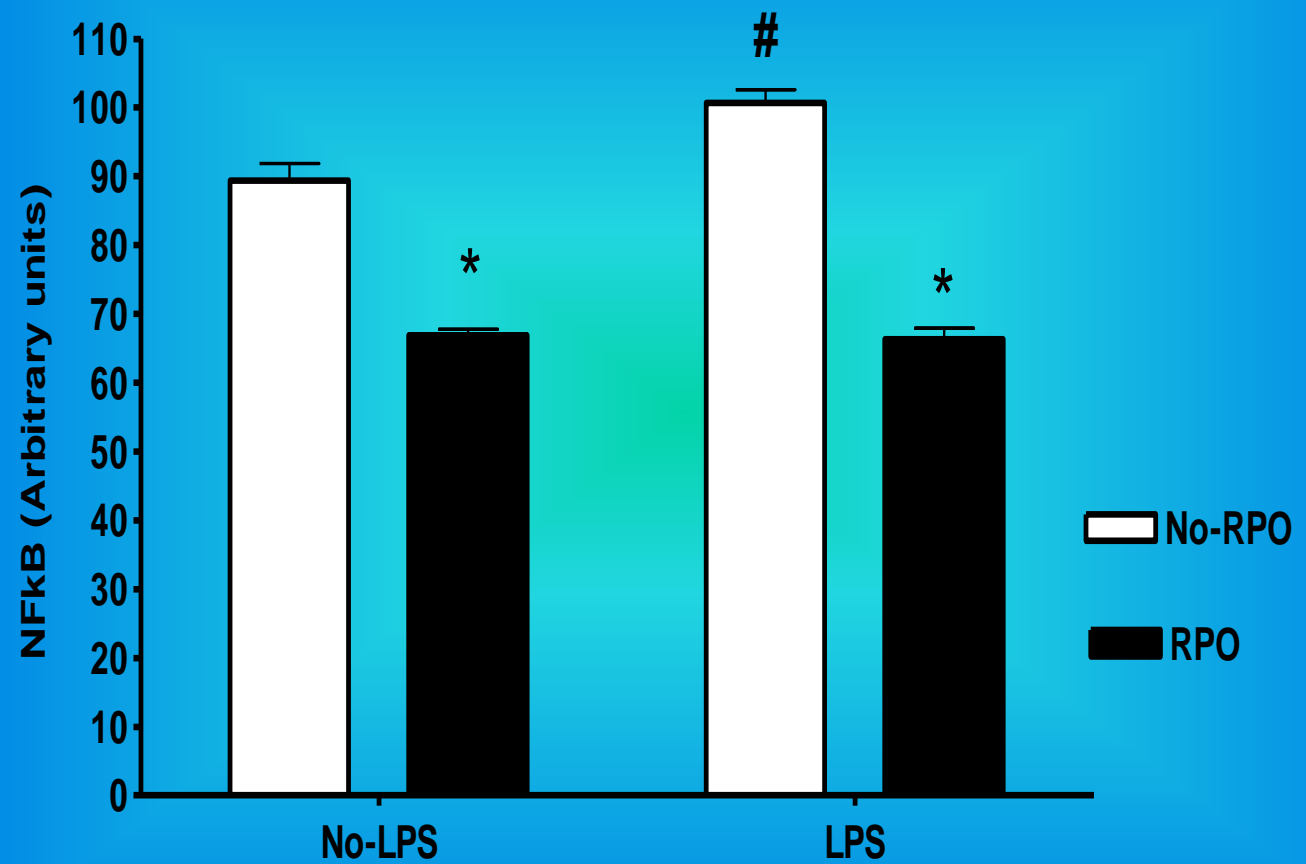
A

Results



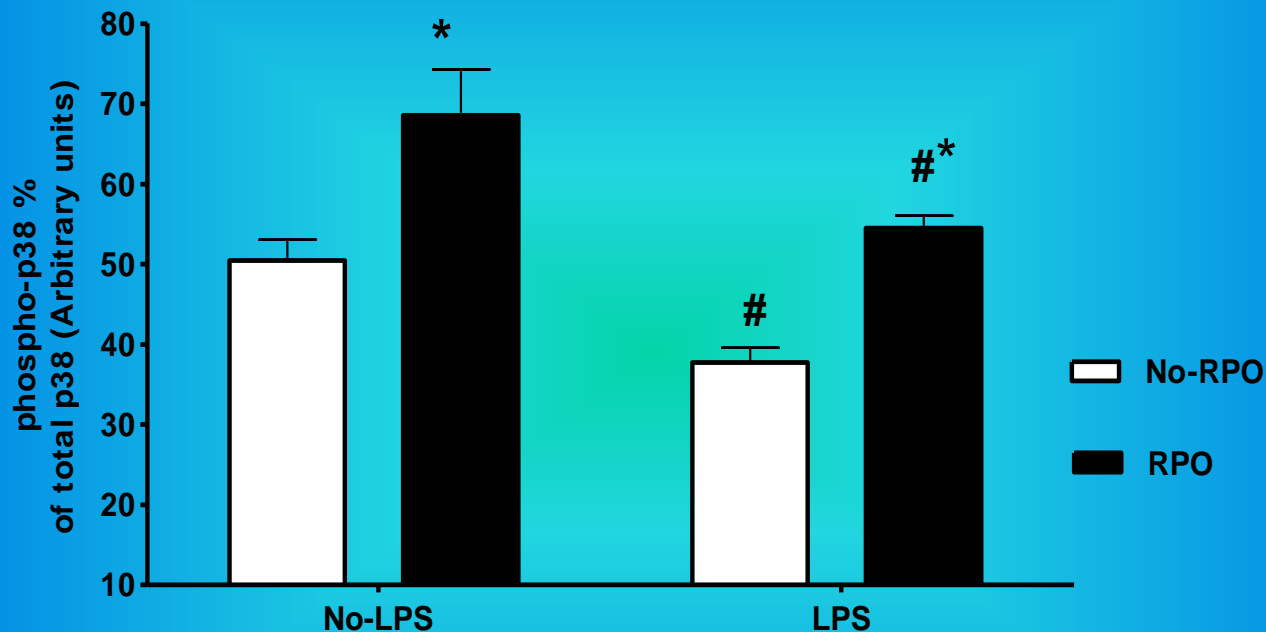
IL-10 is an anti-inflammatory cytokine. It blocks pro-inflammatory NF κ B and regulates the JAK-STAT Pathway. This result shows that carotenoid/tocotrienol protection did not occur through this mechanism.

Results



NFk β is a pro-inflammatory cytokine and linked to septic shock, infections and cancers

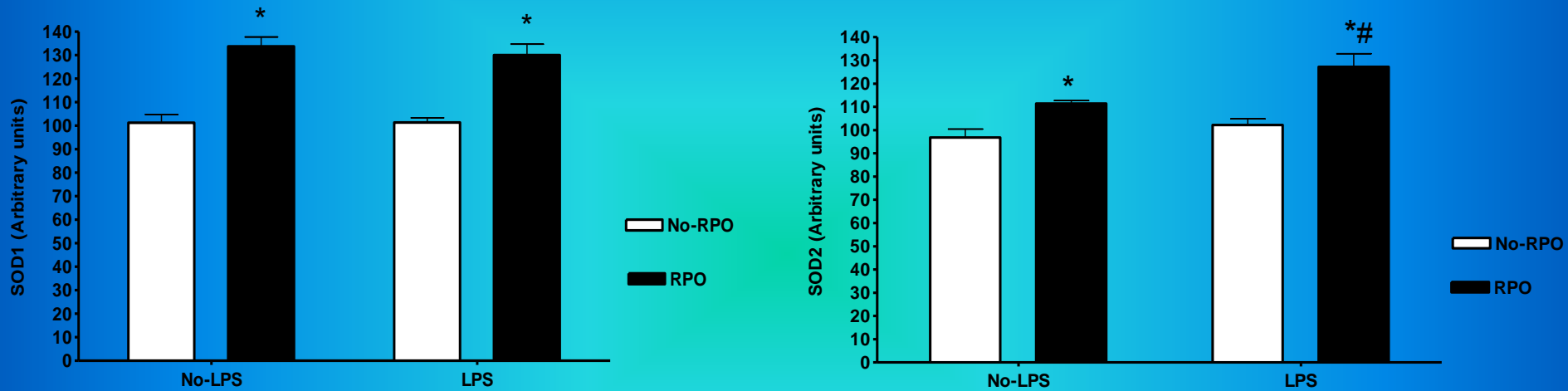
Results



p38MAP Kinase consist of 4 isomers with α and β the most common. α has been associated with harm and β with protection. This portrays total p38MAP Kinase and by implication p38- β MAP Kinase phosphorilation.

A

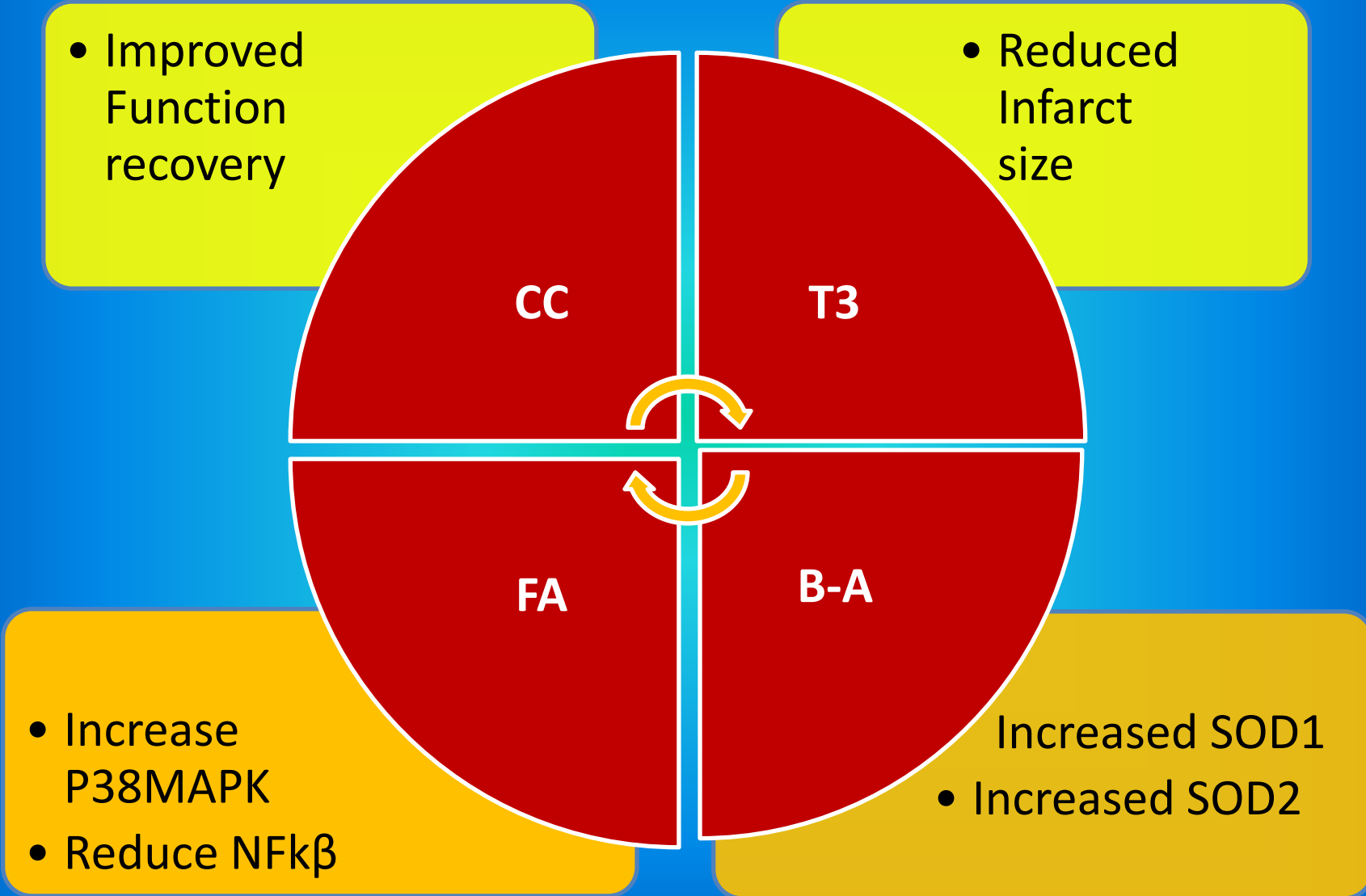
Results



Superoxide dismutase scavenge free radicals. SOD1 is found in the cytoplasm and SOD2 in the mitochondria. They have a highly protective function

A

Summary and Conclusion



Combination Therapy Fat soluble carotenoid/tocotrienol and water soluble flavonoids

Katengua-Thamahane et al. *Journal of Inflammation* (2014) 11:41

DOI 10.1186/s12950-014-0041-4



JOURNAL OF
INFLAMMATION

RESEARCH

Open Access

The combination of red palm oil and rooibos show anti-inflammatory effects in rats

Emma Katengua-Thamahane^{1*}, Jeanine L Marnewick², Olawale R Ajuwon³, Novel N Chegou³, Gergő Szűcs⁴, Péter Ferdinandy^{4,5}, Tamás Csont⁴, Csaba Csonka⁴ and Jacques Van Rooyen¹

Combination Therapy

Fat soluble carotenoid/tocotrienol and water soluble flavonoids

Table 2 Study design illustrating the experimental groups and study protocol

Groups	NO-LPS				LPS			
	Control	Rooibos	RPO	RB + RPO	Control	Rooibos	RPO	RB + RPO
Feeding time	28 days	28 days	28 days	28 days	28 days	28 days	28 days	28 days
Treatment	PBS	PBS	PBS	PBS	LPS	LPS	LPS	LPS
	*Heart excision and perfusion protocol				*Heart excision and perfusion protocol			

RB: rooibos.

RPO: red palm oil.

LPS: liposaccharide.

Treatment: 0.5 mg/kg LPS was injected intraperitoneally to induce inflammation while 0.1 ml PBS was injected as a vehicle in control groups 18 hours prior to sacrificing.

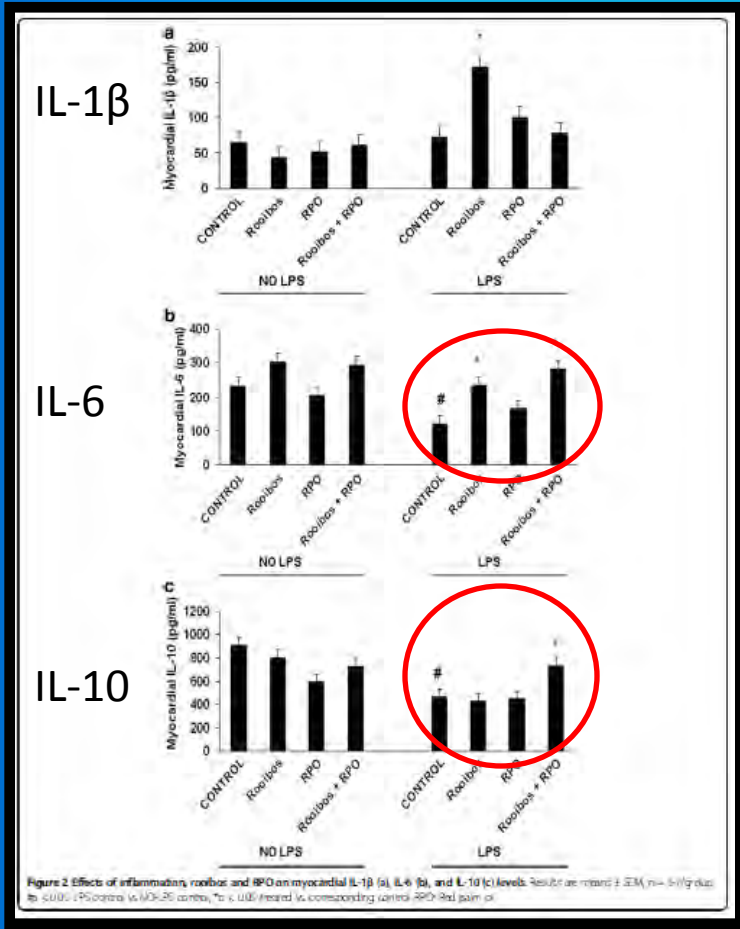
*Hearts were excised and perfused for 20 minutes in a Langendorff mode, baseline heart function was recorded at 20 minutes after which hearts were freeze clamped for cytokine analysis.

Katengua-Thamahane *et al.* *Journal of Inflammation* (2014) 11:41

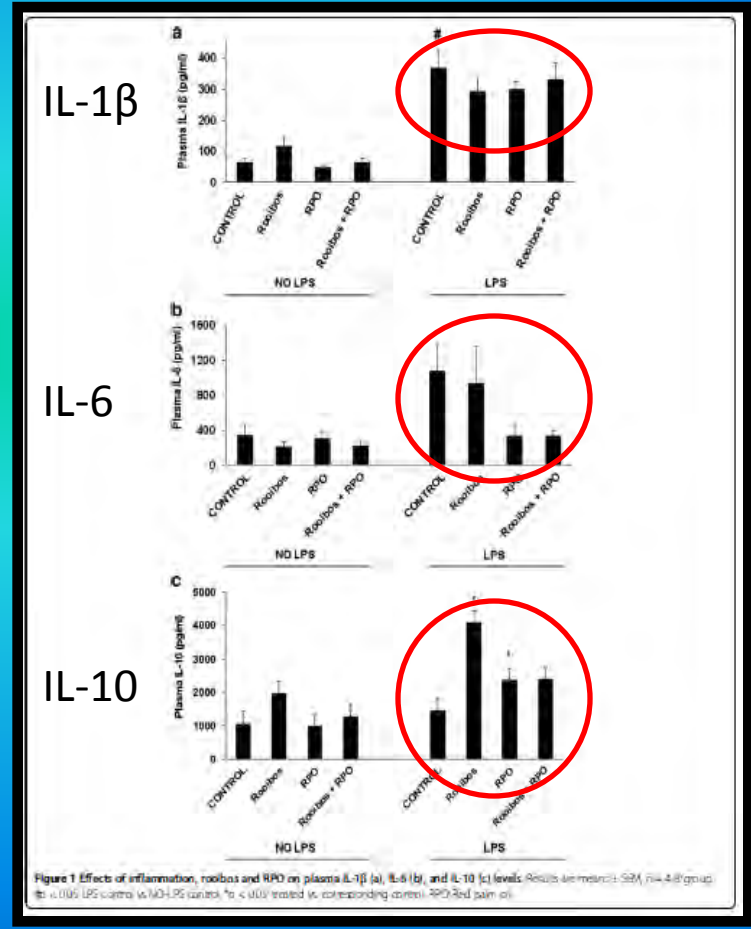
Rooibos contains Aspalathin – polyphenolic flavonoid (tannins)

Combination Therapy Fat soluble carotenoid/tocotrienol and water soluble flavonoids

Myocardial levels



Plasma levels



Combination Therapy

Fat soluble carotenoid/tocotrienol and water soluble flavonoids

Table 3 Effects of inflammation, rooibos and RPO on baseline cardiac functional parameters in the NO-LPS group and the LPS group

	LPS				NO-LPS			
	Control	Rooibos	RPO	RB + RPO	Control	Rooibos	RPO	RB + RPO
CF (ml/min)	13.92 ± 1.00	13.84 ± 1.00	14.6 ± 1.00	15.2 ± 1.00	14.30 ± 0.60	13.70 ± 0.70	14.60 ± 0.70	15.10 ± 0.20
HR bpm (1/min)	294.33 ± 6.00	277.87 ± 13.00	279.69 ± 15.00	296.313 ± 8.00	293.14 ± 14.00	302.39 ± 12.00	296.86 ± 10.00	300.00 ± 13.00
LVDvP (mmHg)	92.804 ± 6.00	86.40 ± 6.00	86.47 ± 5.00	97.00 ± 3.00	106.25 ± 4.30	89.60 ± 2.40	95.500 ± 4.50	100.90 ± 4.410
RPP (Bpm*mmHg)	27593.83 ± 1814.00	23706.98 ± 660.00	24383.99 ± 2503.00	28319.93 ± 764.00	30133.51 ± 1394.00	24805.68 ± 1366.00	24701.52 ± 1551.00	26176.72 ± 1212.00
dp/dt (+) (mmHg/sec)	2822.951 ± 149.00	2829.72 ± 84.00	2661.67 ± 198.00	2664.75 ± 112.00	3065.48 ± 103.00	2888.66 ± 129.00	2885.71 ± 80.00	2814.45 ± 113.00
dp/dt (-) (mmHg/sec)	1933.91 ± 70.00	1906.73 ± 82.00	1918.94 ± 103.00	1996.27 ± 94.00	2140.53 ± 90.00	1969.86 ± 69.00	2024.86 ± 120.00	1952.45 ± 51.00
EDLVP (mmHg)	10.256 ± 0.92	13.424 ± 2.20	11.558 ± 0.72	12.29 ± 1.34	13.74 ± 2.36	12.93 ± 1.33	12.22 ± 2.24	11.78 ± 1.16
HW (g)	1.17 ± 0.09	1.16 ± 0.05	1.11 ± 0.041	1.09 ± 0.03	1.32 ± 1.0	1.11 ± 0.0	2.82 ± 1.4	1.28 ± 0.1
BW (g)	333.70 ± 4.53	343.90 ± 10.02	346.60 ± 6.80	348.20 ± 3.40	350.20 ± 7.5	352.40 ± 7.4	334.20 ± 8.8	340.40 ± 9.0

No significant differences were observed in baseline cardiac function between the groups. Results are expressed as SEM, n = 5-7. CF- Coronary flow, HR- Heart rate, LVDvP- Left ventricular developed pressure, RPP- Rate pressure product, dp/dt (+) - maximum of LVDvP derivative, dp/dt (-) - minimum of LVDvP derivative, EDLVP- End diastolic left ventricular pressure, HW- Heart weight, BW- Body weight.

Carotenoid/Tocotrienol rich palm oil Publications:2005-2014

1. Esterhuysen AJ, Du Toit EF, Rooyen J. Asia Pac J Clin Nutr. 2005;14(4):340-7
2. Esterhuysen AJ, Du Toit EF, Benadè AJS, Van Rooyen J (2005). Prostaglandins, Leukotrienes and Essential Fatty Acids Prostaglandins, Leukotrienes and Essential Fatty Acids 72(3):153-161
3. Engelbrecht AM, Esterhuysen J, Du Toit EF, Lochner A, Van Rooyen J (2006): Journal of Nutritional Biochemistry 17: 265-271
- 4) Esterhuysen AJ, Van Rooyen J, Strijdom H, Bester D, Du Toit EF (2006). Prostaglandins, Leukotrienes and Essential Fatty Acids 75: 375-384
- 5) Bester D, Van Rooyen J, Du Toit E, Esterhuysen J (2006): Medical Technology South Africa 20(1):3-10
- 6) Kruger M, Engelbrecht AM, Esterhuysen J, Du Toit E, Van Rooyen J (2007). British Journal of Nutrition 97(4): 653-60
- 7) J van Rooyen, AJ Esterhuysen AM Engelbrecht, EF Du Toit (2008). Asian Pacific Journal of Clinical Nutrition 17(S1):316-319
- 8) Engelbrecht AM, Odendaal L, Du Toit EF, Kupai K, Csont T, Ferdinandy P, Van Rooyen J (2009). Lipids in Health and Disease 8:18
- 9) Dirk J Bester, Krisztina Kupai, Tamas Csont, Gergo Szucs, Csaba Csonka, Adriaan J Esterhuysen, Peter Ferdinandy and Jacques Van Rooyen (2010). Lipids in Health and Disease 9:64
- 10) Bester D, Esterhuysen AJ, EJ Truter, Van Rooyen J (2010). Nutrition Research Reviews 23(2):334-48
- 11) Wergeland A, Bester DJ, Sishi BJN, Engelbrecht AM, Jonassen AK, Van Rooyen J (2011). Cell Biochemistry and Function 29(5):356-364
- 12) Gergo Szucs, Dirk J Bester, Krisztina Kupai, Tamas Csont, Csaba Csonka, Adriaan J Esterhuysen, Peter Ferdinandy and Jacques Van Rooyen (2011). Journal: Lipids in Health and Disease 10:103
- 13) Bester DJ, Jonassen AK, Du Toit EF, Esterhuysen AJ, Van Rooyen J (2012). Journal of Food and Agricultural Exp (2012) Part I Vol 10 No 1.p29 ISSN 1459-0263.
- 14) Jacques van Rooyen (2013). Bioactive Food as Dietary Interventions for Cardiovascular Disease. Ed Ron Watson, Elsevier Publishers. Publication Date: November 2012 (2013 copyright year) Editor: Ronald R. Watson
- 15) Katengua-Thamahane E, AM Engelbrecht, AJ Esterhuysen, J van Rooyen (2012). Cardiology in Research and Practice 2012;2012:392457.
- 16) Barbara Bacova, Jana Radosinska, Csilla Viczenczova, Vladimir Knezl, Victor Dosenko, Tamara Benova, Jana Navarova, Jacques van Rooyen, Narcis Tribulova (2012). Canadian Journal of Physiology and Pharmacology 90(9):1235-45
- 17) Olawale Razaq Ajuwon, Katengua-Thamahane Emma, Jacques Van Rooyen, Oluwafemi O. Oguntibeju and Jeanine L. Marnewick (2013). Evidence Based Complementary and Alternative Medicine 2013:984273
18. Emma Katengua-Thamahane, Jeanine L Marnewick, Owale R Ajuwon, Novel N Chegou, Gergu Szucs, Peter Ferdinandy, Tamas Csont, Csaba Csonka and Jacques Van Rooyen (2014). Journal of Inflammation 11(1):41
- 19-20. 2 manuscripts submitted from the D.Tech thesis of Dr Emma Thamahane-Katengua on red palm in hypertension and inflammation model. D.Tech degree awarded in April 2014

C Palm Carotenoids/Tocotrienol

Summary of 15 years of results in heart and sperm

Cardiovascular

- Improved functional recovery after ischaemia/reperfusion
- Decreased infarct size in hypercholesterolemic model
- Decreased reperfusion arrhythmias in hypertension model
- Decreased blood glucose and blood pressure in animal hypertension model.
- Reversed the negative adverse effects of chemotherapy on heart function

Mechanisms of protection

- Increased phosphorylation of the protective Akt signalling pathway
- Reduced apoptosis
- Increased SOD1&2 in chemotherapy model
- Protected endothelium function
- Reduce MDA (oxidative stress)

Sperm motility

- Dr Oboua et al published several papers to show that red palm oil could improve sperm Motility in a laboratory model



Product Development



- Palm oil bio-actives is suitable for supplement use:
- Research
- Concentrated bio-actives
- Fat soluble and water soluble
- Combination supplements

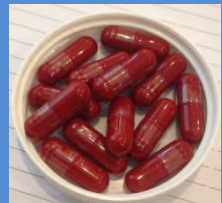


Product Development

wet dry
Capsule Technology



Branding & Positioning



Proprietary Formula

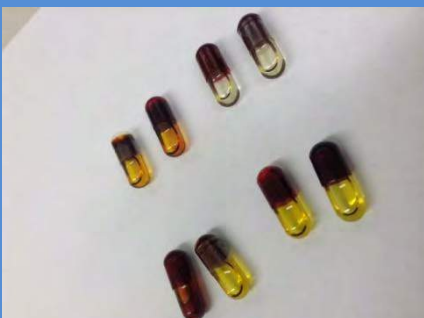


wet & dry



Separate but together

wet & wet





ActiRed Palm CASE STUDY SOUTH AFRICA



Subject: 65 year old male
Practitioner no: 5200539
Pathology: Pathcare

Product



	Pre 2014	2014/7/11	2014/10/30	2015/02/23	Normal
Prostate Specific Antigen (PSA) (µg/L)		0.53	1.21	0.45	0.01-4
Blood glucose (mmol/L)	5.7	5.2	5.2	4.8	3.5-5.5
Total Cholesterol (mmol/L)	3.8 With statin	7.3	6.2	5.6	5.0
LDL (measured) (mmol/L)		5.5	4.5	3.6	3.0
Hematocrit (L/L)		0.49	0.45	0.46	0.4-0.5

D



Planned Clinical Studies

wet/dry
Capsule Technology



ActiRed Palm

Cardio study in Hungary

ARV study in South Africa on rodents

HIV/ARV study in South Africa



Marketing strategy



USA Supplement Industry experience: Palm Bioactives

Dr Oz effect

Clarity in positioning

Tocotrienol vs red palm oil

- Complexity of palm oil ingredients

- Clients and consumers do not know difference

Research

Tocotrienol vs red palm oil

- Tocotrienol research vs Oz statement

Sustainability

Currently the biggest challenge

- Source

- Traceability

- Programs, certification and endorsement

Acknowledgements

The people: Global contribution

2007-2015



Prof Csont: Szeged

2000-2015



Profs DuToit Engelbrecht
Esterhuyse : Stellenbosch

Prof Ferdinandy: Budapest

2007-2015



2008-2011



Prof Jonassen: Bergen

2007-2015



Drs Katengua & Bester: CT

2009-2015



Dr Tribulova: Bratislava

Thank you to the Sponsors

Global participants and contributors
Universities, business and government



**Cape Peninsula University of Technology URF grant
South Africa Government Collaborative NRF grant**



**Hungarian National Office for Research and Technology
Hungarian Government Collaborative grant**



**The Norwegian Centre for International Cooperation
Bergen Heart Foundation
Bergen Medical Foundation**



**Carotino SND BHD, Johor Bahru, Malaysia for provision
of the carotenoid/tocotrienol-rich supplements**

