TOCOTRIENOLS: THE “NEW FRONTIER” FOR A HEALTHY LIVER

By Enrico Magosso, PhD
Advanced Medical & Dental Institute - Universiti Sains Malaysia
Outline:

• Introduction to Non-Alcoholic Fatty Liver
• Natural History & Epidemiology
• Management & Proposed Treatment
• Tocotrienols for Fatty Liver
• Conclusions
Nonalcoholic Fatty Liver (NAFLD)

- Group of liver disorders: Fat deposition in the liver in absence of significant alcohol intake
- Hepatic component of the Metabolic Syndrome
- Pathogenesis not fully understood
- Absence of a proven pharmacological treatment
- Absence of biomarkers (ALT & AST not sufficient!)
Nonalcoholic Fatty Liver (NAFLD)

- Initial stage is asymptomatic
- Suspected when blood Liver Function Test inexplicably abnormal (cryptogenic)
- Confirmed by USG, MRI or biopsy
Natural History of NAFLD

Underlying Patient Condition:

★ Overall Dietary Imbalance
  • High Fat & Sugar & Carbohydrates Intake
  • Lower Vitamin E Intake [Allard et al, 2008]
★ Sedentary Lifestyle [WHO, 2002]
Natural History of NAFLD

**First Hit:**

★ Metabolic Imbalance

- (Abdominal) Obesity [Garcia-Monzon et al, 2000]

⇒ Leads to Primary Hepatic Steatosis
Natural History of NAFLD

**Second Hit:**

- ★ Insulin Resistance
- ★ Increased Oxidative Stress & Lipid Peroxidation
- ★ ↑ TNF-α; ↓ Adiponectine; ↑ Interleukines

⇒ Leads to Chronic Inflammation; Ballooning; Necrosis = NASH
Natural History of NAFLD

From Fatty Liver to Death...

Simple Steatosis $\rightarrow$ 12 - 40% $\rightarrow$ NASH [de Alwis & Day, 2008]

NASH $\rightarrow$ 5 - 40% $\rightarrow$ Fibrosis

Fibrosis $\rightarrow$ 5 - 10% $\rightarrow$ Cirrhosis

1) Cirrhosis $\rightarrow$ 10 - 40% $\rightarrow$ Liver Failure (up to 80% need transplant)

2) Cirrhosis $\rightarrow$ 1 - 30% $\rightarrow$ Liver Cancer

However: Steatosis $\rightarrow$ 10 - 40% $\rightarrow$ CV Mortality
NHANES III (6709 ppl): NAFLD individuals with an associated metabolic disease have higher mortality than control [Younussi et al, 2013]
Natural History of NAFLD without MetS

No MetS Participants Survival Curve: NAFLD+ vs NAFLD - [Younussi et al, 2013]
Natural History of NAFLD with MetS

MetS Participants Survival Curve: NAFLD+ vs NAFLD - [Younussi et al, 2013]
Epidemiology & Susceptibility

Major Risk Factors: Dyslipidaemia; Diabetes; Obesity

Metabolic Syndrome morbidity on the rise!
Epidemiology & Susceptibility

• Most common Non-communicable liver disease [Collier et al, 2006]

• 10 - 30% Adults worldwide estimated to be affected [Neuschwander-Tetri, 2001; Cave et al, 2007]
### Epidemiology & Susceptibility

<table>
<thead>
<tr>
<th>Author</th>
<th>Country</th>
<th>Year</th>
<th>No.</th>
<th>Normal weight</th>
<th>Obese</th>
<th>IR/ Diabetes</th>
<th>Hypertension</th>
<th>Others</th>
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<tbody>
<tr>
<td>Bellentani</td>
<td>Italy</td>
<td>2000</td>
<td>257</td>
<td>16%</td>
<td>76%</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Matteoni</td>
<td>USA</td>
<td>1999</td>
<td>132</td>
<td>-</td>
<td>70%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Angulo</td>
<td>USA</td>
<td>1999</td>
<td>144</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
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<tr>
<td>Tolman</td>
<td>USA</td>
<td>2007</td>
<td>5000</td>
<td></td>
<td>70%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gaiani</td>
<td>Italy</td>
<td>2009</td>
<td>100</td>
<td>all</td>
<td>-</td>
<td>Type 1=24%</td>
<td>Type 2= 80%</td>
<td>-</td>
</tr>
<tr>
<td>Dixon</td>
<td>USA</td>
<td>2001</td>
<td>105</td>
<td>10%</td>
<td>-</td>
<td>60%</td>
<td>30%</td>
<td>Diabetes + HT = 80%</td>
</tr>
</tbody>
</table>
### Table 4. Risk Factors Associated with NAFLD

<table>
<thead>
<tr>
<th>Conditions with established association</th>
<th>Conditions with emerging association*</th>
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<tbody>
<tr>
<td>Obesity</td>
<td>Polycystic ovary syndrome</td>
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<td>Type 2 diabetes mellitus</td>
<td>Hypothyroidism</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>Obstructive Sleep apnea</td>
</tr>
<tr>
<td>Metabolic syndrome**</td>
<td>Hypopituitarism</td>
</tr>
<tr>
<td></td>
<td>Hypogonadism</td>
</tr>
<tr>
<td></td>
<td>Pancreato-duodenal resection</td>
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</table>
Epidemiology & Susceptibility

NAFLD in Asia—as common and important as in the West
Geoffrey C. Farrell, Vincent Wai-Sun Wong and Shiv Chitturi

NAFLD: an Underestimated Disease in Malaysia
Previous Studies in Malaysia

• 75 Liver Patients:
  • Steatosis (4.3%); NASH (84.3%); Cirrhosis (11.4%)  [Malik et al, 2007]

• 180 Mildly Hypercholesterolemic adults:
  • Normal LFT
  • 102 (56.7%) NAFLD  [Magosso et al, 2010]
3rd EAST COAST GASTROHEPATOLOGY CONFERENCE 2013

VENUE: KELANTAN TRADE CENTER (KTC)
DATE: 29 APRIL 2013 (MONDAY)
TIME: 0815 – 1730 HRS

CONCURRENT SESSION 1
GI MALIGNANCIES

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<td>0745hrs-0800hrs</td>
<td>REGISTRATION</td>
</tr>
<tr>
<td>0815hrs – 0900hrs</td>
<td>RISK FACTOR FOR GI MALIGNANCIES</td>
</tr>
<tr>
<td>0900hrs – 0945hrs</td>
<td>EARLY DETECTION STRATEGIES FOR GI MALIGNANCY</td>
</tr>
<tr>
<td>0945hrs – 1030hrs</td>
<td>PREVENTION OF GI MALIGNANCIES</td>
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CONCURRENT SESSION 2
HEPATOLOGY

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<td>REGISTRATION</td>
</tr>
<tr>
<td>0815hrs – 0900hrs</td>
<td>HOW TO INTERPRET ABNORMAL LFT</td>
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<tr>
<td>0900hrs – 0945hrs</td>
<td>FATTY LIVER – WHAT IS IT?</td>
</tr>
<tr>
<td>0945hrs – 1030hrs</td>
<td>AUTOIMMUNE HEPATITIS</td>
</tr>
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Consequences of Underestimating NAFDL in Malaysia
Malaysia

• The management of other liver diseases and the complications of cirrhosis have also changed significantly, requiring highly skilled and high intensity inpatient or intensive care.

• As for liver transplants, data for Selayang Hospital shows that the total number of liver transplants performed as of May 2007 were 27, with 21 of the recipients still alive (78%). The 1-year survival rate is 85%, which is at par with the international benchmark of 80-90%.

• Hospital Selayang, is the national, tertiary referral centre for liver diseases

YB DATUK SERI DR CHUA SOI LEK
(former) MINISTER OF HEALTH MALAYSIA
AT THE OPENING CEREMONY & LAUNCH OF CLINICAL PRACTICE GUIDELINES, LIVER UPDATE 2007, SUNWAY RESORT HOTEL AND SPA, 12 JULY 2007
Management of Simple Steatosis

• Tackle associated conditions
  ✓ Sedentary Lifestyle
  Treat MetS related co-morbidities
    ✓ IR / Diabetes
    ✓ Obesity
• Therapeutic Agents (?)
Management of NASH Patients

- Simple Steatosis
  - Treat MetS related co-morbidities
  - Follow-up for HCC
Management of NAFLD

- **Insulin sensitising agents** (Metformin, Pioglitazone, Rosiglitazone)
- **Lipid lowering drugs** (Atorvastatin, Pravastatin, Gemfibrozil, Clofibrate)
- **Hepatoprotective agents** (UDCA, Betaine, Probucol, Pentoxifylline, Losartan, Omega-3, Phosphatidil Choline, Vitamin E)
- **Combinations** (e.g. Pioglitazone+Vit E)
<table>
<thead>
<tr>
<th>Author</th>
<th>Intervention</th>
<th>No.</th>
<th>Study type</th>
<th>Comparison</th>
<th>Duration (months)</th>
<th>ALT</th>
<th>Histology</th>
<th>Inflammation</th>
<th>Fibrosis</th>
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<tbody>
<tr>
<td>Hasegawa</td>
<td>vit E</td>
<td>22</td>
<td>pilot</td>
<td>baseline</td>
<td>12</td>
<td>improved</td>
<td>no change</td>
<td>no change</td>
<td>no change</td>
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<tr>
<td>Kugelmas</td>
<td>Vit E + diet &amp; exercise</td>
<td>16</td>
<td>open label, randomised</td>
<td>diet &amp; exercise</td>
<td>3</td>
<td>no diff</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Vajro</td>
<td>Vit E</td>
<td>28</td>
<td>blinded RCT</td>
<td>placebo</td>
<td>5</td>
<td>no diff</td>
<td>no difference</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Harrison</td>
<td>Vit E + Vit C</td>
<td>45</td>
<td>blinded RCT</td>
<td>placebo</td>
<td>6</td>
<td>no diff</td>
<td>NA</td>
<td>no diff</td>
<td>no diff</td>
</tr>
<tr>
<td>Lavine</td>
<td>Vit E</td>
<td>11</td>
<td>pilot</td>
<td>baseline</td>
<td>5</td>
<td>improved</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>PIVENS</td>
<td>Vit E or Pioglitazone</td>
<td>247</td>
<td>blinded RCT</td>
<td>placebo</td>
<td>24</td>
<td>improved</td>
<td>improved</td>
<td>improved</td>
<td>no diff</td>
</tr>
<tr>
<td>TONIC</td>
<td>Vit E or Metformin</td>
<td>173</td>
<td>blinded RCT</td>
<td>placebo</td>
<td>24</td>
<td>no diff</td>
<td>no diff</td>
<td>no diff</td>
<td>no diff</td>
</tr>
</tbody>
</table>
PIVENS Summary

247 non-diabetic adults for 96 weeks
30 mg Pio; 800 IU vit E; Placebo
Primary Endpoint: ↓ NAS by 2 degrees & No ↑ Fibrosis

Results

• Only vit E ↓ ballooning
• None ↓ fibrosis
Nevertheless....
Vitamin E!

✓ Vitamin E (alpha-tocopherol), BUT...
  ➡ Limited to NASH
  ➡ Non-Diabetic
  ➡ High dose (800 IU/day)
  ➡ How Long??
PIVENS Summary

One concern with vitamin E is the controversial issue of whether it increases all-cause mortality. Some meta-analyses have reported an increase in all-cause mortality with high dose vitamin E,\textsuperscript{131,132} but others failed to confirm such an association.\textsuperscript{133-135} A recently published RCT showed that vitamin E administered at a dose of 400 IU/day increased the risk of prostate cancer in relatively healthy men (absolute increase of 1.6 per 1000 person years of vitamin E use).\textsuperscript{136}

**Recommendation**

21. Vitamin E (\(\alpha\)-tocopherol) administered at daily dose of 800 IU/day improves liver histology in non-diabetic adults with biopsy-proven NASH and therefore it should be considered as a first-line pharmacotherapy for this patient population. (Strength - I, Quality - B)
Why Tocotrienols? (I)

- up to 60 times more potent activity against liver microsomal lipid peroxidation [Serbinova et al, 1991]
- Higher affinity for α-TTP [Hosomi et al, 1997]
- Liver is a preferential site for biodistribution [Yuen et al, 2009]
- Reduced liver neoplastic lesions in animals treated with acetaminofluorene [Ngah et al, 1991]
Why Tocotrienols? (2)

- Less hepatic TG accumulation in animals treated with CCL4 [Yachi et al, 2010]
- Reduced ALT activity, especially with γ-T3 [Yachi et al, 2010]
- Less hepatic TG accumulation in animals and HepG2 cells treated with T3 [Burdeos et al, 2012]
Why Tocotrienols? (3)

• γ-T3 may regulate fatty acid syntase and carnitine palmitoyltransferase [Muto, 2012]

• γ-T3 may reduce hepatic inflammation & endoplasmic reticulum stress [Muto, 2012]
Tocotrienols: our study

- 6-month study in Swiss Albino Mice, fed *ad libitum*
- 3 Groups (n=9)
  - Mice fed standard pellets
  - Mice fed High Fat-High Fructose diet
  - Mice fed High Fat-High Fructose diet + Tocotrienols
- HPLC determination of Tocotrienols in liver grafts
## Tocotrienols: our results

<table>
<thead>
<tr>
<th></th>
<th>Mice (g)</th>
<th>Liver (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td>40.3±4.2</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td><strong>Steatogenic Diet</strong></td>
<td>46.3±2.1</td>
<td>2.3±0.4</td>
</tr>
<tr>
<td><strong>Steatogenic Diet + Tocotrienols</strong></td>
<td>45.3±7.2</td>
<td>2.2±0.1</td>
</tr>
</tbody>
</table>
Histology Results

Mice fed HED

Hematoxylin/Eosin Stain (x40);
Scored by NAK, blinded

control

Steatogenic Diet

Tocotrienols

Mice fed HED

Hematoxylin/Eosin Stain (x40);
Scored by NAK, blinded
Tocotrienols: our results

- **All** Animals Fed Steatogenic Diet Developed NAFLD
- **No** Animal Fed Tocotrienols developed NAFLD
Potential in animals and cells...

But in Humans??
Aim of the Clinical Trial

Amelioration of NAFLD Grading on USG

Helmert-Pearson’s Chi-square
Scheffé’s ANOVA for split-plot
HPLC quantification of plasma Tocotrienols
Study Design

✦ Male & Female Patients (above 35-year old)
  ✦ With untreated Hypercholesterolaemia
  ✦ Liver enzymes up to 3-time the normal values
  ✦ Insignificant alcohol intake

✦ ULTRASOUND EXAMINATION
  ✦ Experienced radiologists; validated scoring system

✦ PLASMA TOCOTRIENOLS LEVELS (HPLC)
Methods

Double-Blind randomisation

- Placebo (2 identical looking cps)
- Mixed Tocotrienols (Tocovid Suprabio) 400 mg/day (1 cps 200mg twice a day)
Study Highlights

- 87 ppl enrolled: 43 T3; 44 Placebo
- Baseline Biochemical parameters: Homegenous (including α-tocopherol) \( (P>0.05) \)
- Baseline Anthropometric parameters: Homegenous \( (P>0.05) \)
Baseline Clinical Parameters

- **mild**
  - T3: 23
  - Placebo: 30

- **moderate**
  - T3: 8
  - Placebo: 15

- **severe**
  - T3: 0
  - Placebo: 0

- **overweight**
  - T3: 15
  - Placebo: 23

- **obese**
  - T3: 8
  - Placebo: 15

- **IFG**
  - T3: 0
  - Placebo: 0
180 Subjects were assessed for eligibility

102 met inclusion criteria for NAFLD

14 were excluded
- 1 for metastatic HCC
- 1 for brain infarct
- 2 for other medical reasons
- 10 declined to participate

88 were randomised

44 were assigned & received tocotrienols
- 14 stopped treatment with tocotrienols before 52 wk
  - 8 were lost at follow
  - 2 withdrew from study
  - 4 were excluded for protocol violation

30 completed the study and underwent baseline & final USG

44 were assigned & received placebo
- 10 stopped treatment with placebo before 52 wk
  - 6 were lost at follow
  - 1 withdrew from study
  - 3 were excluded for protocol violation

34 completed the study and underwent baseline & final USG
Results after 1-year

Placebo group (N=34)

- 14 improved
- 8 normalised (23%)
- 2 worsened

TOCOTRIENOLS group (N=30)

- 20 improved
- 15 normalised (50%)
# Vit E Plasma Concentrations

<table>
<thead>
<tr>
<th></th>
<th>Tocotrienols (n=30)</th>
<th>Placebo (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Conclusion of the Study</td>
</tr>
<tr>
<td>alpha-tocopherol (μg/ml)</td>
<td>14.6±6.0</td>
<td>18.5±6.8</td>
</tr>
<tr>
<td>alpha-tocotrienol (ng/ml)</td>
<td>25.0±21.0</td>
<td>212.0±379.3</td>
</tr>
<tr>
<td>gamma-tocotrienol (ng/ml)</td>
<td>below LOQ</td>
<td>181.4±440.8</td>
</tr>
<tr>
<td>delta-tocotrienol (ng/ml)</td>
<td>below LOQ</td>
<td>41.4±98.4</td>
</tr>
</tbody>
</table>

**ULTRASOUND RESULTS CORRELATED WITH USE OF MIXED TOCOTRINOLS**
Intention to treat analysis (n=87)

- Drop-out participants calculated as non-responders
- T3 vs Placebo: $P = 0.039$; NNT = 6
Per protocol analysis (n=64)

Participants that concluded the study

- Tocotrienols shown to have significant hepatoprotective activity compared to Placebo
  - Improved 67% of subjects (p=0.021; NNT=3.9)
  - Cured 50% of subjects (p=0.014; NNT=3.8)
↑ Post-treatment with Mixed Tocotrienols (MILD)

↑ Pre-treatment (SEVERE)
↑ Pre-treatment (SEVERE)

↑ Post-treatment with Mixed Tocotrienols 400mg/day (MILD)
↑ Pre-treatment (MILD)

↑ Post-treatment with Mixed Tocotrienol (CURED)
Summary

• 50% Cured with T3
• Nobody on T3 worsened
• Well tolerated, NO adverse reaction
• No significant changes in major metabolic parameters

[Magosso et al, 2010]
Advantages of Mixed T3

- α-tocopherol: recommended dose in NASH is 800 IU/day ($\approx 560$ mg) [AASLD Guidelines, 2012]

- Mixed T3: highest is $\gamma$-T3 $\approx 250$ mg/day
Present Day....

- Ongoing: USM funded MRI pilot clinical trial [Magosso, Yuen, Ibrahim: ST grant]
- Proposed: Large MRI based RCT
  - We need a direct comparison with α-Tocopherol to win the NAFLD war...
Take home message

FATTY LIVER:

✓ is a very common disease of GROWING INCIDENCE
✓ is a debilitating & deadly disease
✓ vit E has been indicated as possible treatment
✓ Tocotrienols proven effective
✓ THE WORLD MARKET IS THERE....
Acknowledgements

- MPOB for financial support
- Volunteers, nurses, radiographers, USM staff
- Dr Nor Azlina Khalil (Vet-AMDI)
- Malaysian Palm Oil Council (MPOC)

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