AN OVERVIEW OF
FATTY ACID ETHYL ESTERS

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Massachusetts General Hospital
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OUTLINE OF PRESENTATION

Background - FAEE
Toxicity of FAEE
FAEE Synthesis
FAEE Secretion
FAEE Transport in Blood
FAEE Degradation
FAEE as Markers of Ethanol Intake
FAEE BACKGROUND
STRUCTURE OF FATTY ACID AND FATTY ACID ETHYL ESTER

\[
\text{FATTY ACID} \\
\text{(FA)} \\
\text{FATTY ACID ETHYL ESTER} \\
\text{(FAEE)}
\]
Organs most frequently damaged by ethanol abuse include:

Pancreas > Liver > Heart > Brain

After ethanol intoxication, these organs have the highest levels of fatty acid ethyl esters and fatty acid ethyl ester synthase.
Oxidative and Non-Oxidative Metabolism of Ethanol

**Oxidative**
- Ethanol
  - Alcohol Dehydrogenase
  - Microsomal Ethanol Oxidizing System
  - Acetaldehyde
  - Aldehyde Dehydrogenase
  - Acetate

**Non-Oxidative**
- Ethyl Ester Synthase I, II & III
- Ethanol O-Acyltransferase
- Carboxylesterase
- Lipoprotein Lipase
- Pancreatic Triglyceride Lipase
- Cholesterol Esterase
- Fatty Acid Ethyl Ester
- FA
- FA CoA
- FA / TG-FA
- TG-FA
- TG-FA
- FA
METHOD: FAEE SYNTHESIS AND LDL RECONSTITUTION

LDL ISOLATED

LDL CORE RECONSTITUTED WITH FAEE

FAEE ELUTED WITH HEXANE

CORE LIPIDS REMOVED WITH HEPTANE

FAEE PURIFIED BY SOLID PHASE EXTRACTION

RADIOLABELED TRIGLYCERIDE

0.5 M KOH IN ETHANOL

FAEE RADIOLABELED
Isolation and Quantitation of Fatty Acid Ethyl Esters

All samples were extracted with acetone / hexane and FAEEs were isolated by solid phase extraction.

FAEEs were then quantitated by gas chromatography-mass spectrometry (GC-MS).
FAEE TOXICITY
METHOD: ASSESSMENT OF CYTOXITY OF FAEE WITHIN LDL USING $^3$H-THYMIDINE INCORPORATION AS AN INDEX CYTOTOXICITY

HepG2 Cells Incubated with Delipidated Medium for 6 Hours to Increase Number of LDL Receptors

$^3$H-thymidine added for 5 hours

Cell monolayers harvested and $^3$H-thymidine incorporated into cells determined

HepG2 cells incubated with rLDL-FAEE or rLDL-TG or rLDL-CE or native LDL for 12 hours
ETHYL OLEATE AND ETHYL ARACHIDONATE INHIBIT PROLIFERATION OF HEPG2 CELLS

HEPG2 CELLS INCUBATED WITH rLDL OR NATIVE LDL FOR 12 HRS → 3H-THYMIDINE ADDED FOR 5 HRS → CELL MONOLAYERS RINSED & HARVESTED TO DETERMINE 3H-THYMIDINE INCORPORATION INTO CELLS

Graphs showing the incorporation of 3H-thymidine (DPM/mg of protein) in HEPG2 cells incubated with rLDL or native LDL in the presence of increasing concentrations of ethyl oleate or ethyl arachidonate.
METHOD

- **UNLABELED CHOLESTEROL ESTER**
  - LDL RECONSTITUTED WITH CHOLESTEROL ESTER
  - INTRA-ARTERIAL BOLUS AND INFUSION
  - ANIMALS SACRIFICED
  - BLOOD AND PANCREAS REMOVED FOR ANALYSIS
  - TOXICITY DETERMINED BY:
    - HISTOLOGICAL EXAMINATION AND ELECTRON MICROSCOPY FOR CELLULAR ABNORMALITIES
    - TRYSINOGEN ACTIVATION PEPTIDE (TAP) MEASUREMENT FOR PANCREATIC CELL INJURY
    - WET/DRY RATIO FOR EDEMA FORMATION

- **UNLABELED FAEE**
  - LDL RECONSTITUTED WITH FAEE
  - INTRA-ARTERIAL BOLUS AND INFUSION
Rats receiving FAEE showed markedly higher levels of trypsinogen activation peptide in the pancreas compared to rats receiving either cholesterol ester or saline.

Human LDL reconstituted with either FAEE or CE administered to rats intra-arterially → Rats sacrificed at 3, 6, and 12 hours → Toxicity determined by measuring trypsinogen activation peptide (TAP).

<table>
<thead>
<tr>
<th></th>
<th>3 HRS</th>
<th>6 HRS</th>
<th>12 HRS</th>
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</thead>
<tbody>
<tr>
<td>FAEE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nmol TAP/mg tissue weight</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>FAEE</td>
<td>250</td>
<td>100</td>
<td>350</td>
</tr>
<tr>
<td>CE</td>
<td>0</td>
<td>150</td>
<td>400</td>
</tr>
<tr>
<td>Saline</td>
<td>50</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

Toxicity determined by measuring trypsinogen activation peptide (TAP).

- P<0.001
- P<0.02
**RATIONALE FOR INHIBITOR STUDIES**

ETOH →

- ADH
- 4-methylpyrazole
- MEOS
  - Diallyl Sulfide
  - Catalase
- Aminotriazole

FAEE Synthase

FA → FAEE

Acetaldehyde → Organ Damage
THE INHIBITION OF THE OXIDATIVE PATHWAY OF ETHANOL METABOLISM LEADS TO AN INCREASED INFLUX THROUGH THE NON-OXIDATIVE PATHWAY IN VIVO

A Bolus of Either Saline or Inhibitors Administered to Rats

2.6 g/kg Ethanol Administered and Rats Sacrificed after 120 min

Liver and Pancreas Harvested, Lipid Extracted with GC-MS to Quantitate FAEE

FAEE (nmol/gm wet wt.)

Liver

Control 4 MP Triple

FAEE (nmol/gm wet wt.)

Pancreas

Control 4 MP Triple
Overview of Experiments: Hepatitis C Virus (HCV) and Ethanol

Ethanol + HCV

Incubate 7 Days

HEPG2 Cells

Trypsinize Cells

Ethanol Added in Increasing Concentration
0, 25, 50, 100, and 200 mM Daily to Cells

Real Time-PCR Assay

Extract Lipids and Quantitate FAEE by GC-MS

LDL Uptake Assay

In Situ Hybridization Assay
Ethanol increases the viral load in HCV - infected cells dose dependently.
Effect of Ethanol Treatment on HCV Viral Load in HepG2 Cells
Hepatitis C virus infection decreases FAEE production in ethanol treated cells.
Decreased FAEE Synthesis in Ethanol Treated-HCV Infected HepG2 Cells

![Graph showing decreased FAEE synthesis in ethanol treated-HCV infected HepG2 cells. The graph compares FAEE synthesis levels across different treatments: ETOH, ETOH+HCV, HCV, and No Treatment. The ETOH group has the highest FAEE synthesis, while the other groups show significantly lower levels.](image-url)
SYNTHESIS AND SECRETION OF FATTY ACID ETHYL ESTERS
METHOD

1.25 µM $^3$H-18.1 + HepG2 cells

12 Hrs
37°C

FAEE in cells and medium isolated by TLC

Cells rinsed and serum free medium containing 0, 50, or 100 mM ethanol added for 9 Hrs

FAEE quantitated by scintillation counting

Lipids extracted

Medium and cell monolayers harvested

FAEE in cells and medium isolated by TLC
INCREASE IN THE ACCEPTOR CONCENTRATION WILL INCREASE FAEE PRODUCTION AND SECRETION

FAEE
- Albumin
- Lipoprotein
- Fatty Acid Binding Protein

Addition of FAEE carriers

FAEE carriers are transported from the endoplasmic reticulum (ER) to the Golgi apparatus. The increase in acceptor concentration will increase FAEE production and secretion.
CELLS RETAIN A FIXED AMOUNT OF FAEE, INDEPENDENT OF ACCEPTOR CONCENTRATION & TIME OF INCUBATION

FAEE
Albumin
Lipoprotein
Fatty Acid Binding Protein

Addition of FAEE carriers
The addition of albumin to the culture medium markedly enhanced the secretion of FAEE.

3H-18:1 added to HepG2 cells for 12 hrs → Cells rinsed & serum free medium containing 100 mM ethanol and 1.5% BSA added for 9 hrs → FAEE isolated & quantitated by scintillation counting.

**Cellular Ethyl Oleate**

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<thead>
<tr>
<th>Serum Free Medium</th>
<th>BSA</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
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<td>100</td>
<td>100</td>
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<tr>
<td>150</td>
<td>150</td>
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<tr>
<td>200</td>
<td>200</td>
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</table>

**Cellular Ethyl Oleate**

<table>
<thead>
<tr>
<th>Serum Free Medium</th>
<th>BSA</th>
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<tr>
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<tr>
<td>1000</td>
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<td>5000</td>
<td>5000</td>
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<tr>
<td>6000</td>
<td>6000</td>
</tr>
</tbody>
</table>
LIPOPROTEIN PARTICLES IN THE CULTURE MEDIUM STIMULATE FAEE SECRETION

3H-18:1 added to HEP G2 cells for 12 hours

Cells rinsed & serum FAEE medium containing 100 mM ethanol and 100 µg/ml lipoprotein added for 9 hours

FAEE isolated & quantitated by scintillation counting

Ethyl Oleate (dpm/mg Cell Protein)

Serum FAEE Medium

VLDL

LDL

HDL

Cellular

Secreted
FAEE IN THE CYSTOSOLOF HEPG2 CELLS ARE BOUND PREDOMINANTLY TO A 13 - 15 kDa PROTEIN

HEPG2 cells + \(^{3}H\)-18:1 for 12 Hrs → Serum free medium containing 100 mM ethanol added for 12 Hrs → Cells harvested & fractionated by centrifugation → Cytosol fractionated by gel filtration

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Protein Concentration (μg/ml)</th>
<th>Ethyl Oleate (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 40 60 80 100 120 140 160</td>
<td>200 150 100 50 0</td>
<td>5000 4500 3000 1500 0</td>
</tr>
</tbody>
</table>

Protein Concentration (μg/ml) vs. FRACTION NUMBER

Ethyl Oleate (dpm) vs. FRACTION NUMBER
FAEE TRANSPORT
IN BLOOD
ALBUMIN

- A 66 kDa molecular weight protein
- Primary functions include:
  - building and transport of fatty acids, bilirubin, calcium and many other small molecules
  - maintenance of osmotic pressure in the blood
FREE FATTY ACIDS DISPLACE ETHYL [14C] OLEATE FROM ALBUMIN

Ethyl [14C] Oleate Recovered with Albumin as a Percent of Total

Ratio of free fatty acids to ethyl oleate

- 14:0
- 16:0
- 18:1
- 18:2
- 18:0
- 20:4
- 18:1
- 18:2
- 16:0
- 14:0
- 18:0
- 20:4
CONCLUSIONS: FAEE BINDING TO LDL AND PHOSPHOLIPID VESICLES

- Ethyl oleate binds to the core of lipoproteins
- FAEE align parallel to the fatty acid moieties of phospholipids in vesicles and are soluble up to 30 mole percent
- The transfer of ethyl oleate between phospholipid vesicles and LDL is a rapid bidirectional process
The percent of FAEE in lipoproteins correlates with serum FAEE concentrations in vitro.

Added ethyl oleate to serum, incubated at 37°C → Fractionated serum → Extracted and purified FAEE → Quantitated by GC-MS.

- Plot showing the relationship between percent of total ethyl oleate present in each fraction and serum ethyl oleate concentration (μM).
  - Red line: % in d > 1.21 g/ml, r = -0.78
  - Blue line: % in all lipoproteins, r = 0.78
FAEE DEGRADATION
METHOD

Delivered as oil directly into rat stomach

Radiolabeled triglyceride

0.5 M KOH in ethanol

FAEE Radiolabeled

Labeled FAEE mixed in oil of purified unlabeled FAEE

Blood collected at 5, 15, 30, 60, 90, and 120 minutes with sacrifice and harvest of organs

TLC to separate lipid classes

CE
FAEE
TG
FFA
PL
FATTY ACIDS FROM FAEE CAN BE ESTERIFIED INTO OTHER LIPIDS

FAEE

Fatty Acid + Ethanol

Phospholipid (PL)

Triglyceride (TG)

Cholesterol Ester (CE)
AFTER ADMINISTRATION OF FAEE INTO THE STOMACH, FAEE ARE EXTENSIVELY HYDROLYZED TO FREE FATTY ACIDS IN THE DUODENUM.

Portions of Harvested Organs Homogenized → Lipids Extracted → TLC to Separate the Different Lipid Classes

**[³H] Ethyl Oleate Metabolism**

- Stomach
- Duodenum
- Pancreas
- Liver
- Blood

**[³H] Ethyl EPA Metabolism**

- Stomach
- Duodenum
- Pancreas
- Liver
- Blood
IS THERE DEGRADATION OF ORALLY INGESTED FAEE PRIOR TO ENCOUNTER WITH TARGET ORGAN?
METHOD

- Radiolabeled triglyceride
- Injected into rat intra-arterially
- 0.5 M KOH in ethanol
- LDL core reconstituted with FAEE
- TLC to separate lipid classes

Blood collected at:
- 15 sec, 30 sec, 1,2,3,4,5 min
THE DEGRADATION OF FAEE IN THE BLOOD IS EXTREMELY RAPID WITH A HALF-LIFE OF 58 SECONDS

Human LDL reconstituted with $[^{3}\text{H}]$ ethyl oleate administered to rats intra-arterially

Blood collected at: 15 sec, 30 sec, 1,2,3,4,5 min

Lipids extracted and TLC performed to separate the different lipid classes
METHOD

- Radiolabeled triglyceride
- Platelet poor plasma
- Buffy coat
- Red blood Cells
- Platelet rich plasma
- LDL core reconstituted with FAEE
- Mixture incubated at 37º C for 2 hrs

0.5 M KOH in ethanol

FAEE Radiolabeled

TLC to isolate FAEE

CE
FAEE
TG
FFA
PL
RBC, WBC and platelets are all capable of hydrolyzing FAEE.

FAEE Hydrolysis

% of FAEE Remaining

- Plasma
- RBC: $7.5 \times 10^6$
- WBC: $6.6 \times 10^3$
- Platelets: $150 \times 10^3$
THE DEGRADATION OF FAEE IN THE BLOOD IS EXTREMELY RAPID WITH A HALF-LIFE OF 58 SECONDS

[³H] Ethyl Oleate Metabolism
UPTAKE OF FAEE BY HUMAN PLATELETS IS MAXIMAL BY 60 SECONDS

Isolation of platelets → Incubation with \(^3\text{H}-\text{E18:1}\) → Measurement of radioactivity in platelets

4 experiments, with \(n=2\) / timepoint in each experiment
HYDROLYSIS OF FAEE INCORPORATED INTO PLATELETS

3 experiments, with n=3 / lipid fraction in each experiment

FRACTIONS CONTAINING FATTY ACIDS DERIVED FROM FAEE
METHOD FOR ASSESSING FAEE EFFECT ON PLATELET AGGREGATION

- Platelet rich plasma was incubated with 25 µM ethyl oleate for one minute.

- At the end of the incubation, epinephrine (10 µM) was added to stimulate platelet aggregation.
5-25 μM FAEE REVERSES THE EFFECT OF IBUPROFEN ON PLATELET AGGREGATION IN RESPONSE TO ARACHIDONATE

Platelets obtained from 1 subject ingesting 400 mg ibuprofen
Platelets treated with FAEE have increased sensitivity to activation briefly after ethanol intake. At this point, platelets may be predisposed to aggregation. A loss of platelet granules in the brief activation may produce the hypofunctioning platelets reported to exist following ethanol intake.
FAEE AS MARKERS OF ETHANOL INTAKE
STATE MARKERS

Has there been recent intake of ethanol?
   *Ethanol*
   *Fatty Acid Ethyl Esters (FAEE)*

Is there evidence of chronic ethanol intake?
   *Fatty Acid Ethyl Esters*
   *Carbohydrate Deficient Transferrin (CDT)*
FAEE HAVE BEEN RECENTLY DISCOVERED IN SERUM AFTER ETHANOL INGESTION
STUDY DESIGN

**Time (minutes)**

-4 hr 0 15 30 45 60 75 90 105 120 135 150 180 210 240 300 360 420 480 24 hr

**Blood Drawn**

- ETOH Ingestion

**Low Fat Breakfast**

**Low Fat Lunch**

**Low Fat Supper**

## DEMOGRAPHICS: 7 STUDY PARTICIPANTS

<table>
<thead>
<tr>
<th>Physical Data</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Height (feet/inches)</td>
<td>6’2”</td>
<td>5’5”</td>
<td>5’9”</td>
<td>5’4”</td>
<td>5’6”</td>
<td>6’1”</td>
<td>5’6”</td>
</tr>
<tr>
<td>Weight (pounds)</td>
<td>214</td>
<td>165</td>
<td>142</td>
<td>123.5</td>
<td>133</td>
<td>204.5</td>
<td>136</td>
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<tr>
<td>% Body Fat - Skinfold</td>
<td>29</td>
<td>22.3</td>
<td>33.2</td>
<td>28.7</td>
<td>32.5</td>
<td>22.9</td>
<td>13.6</td>
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<tr>
<td>% Body Fat - Bioelectric</td>
<td>27</td>
<td>25</td>
<td>28</td>
<td>24</td>
<td>30</td>
<td>27</td>
<td>17</td>
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<tr>
<td>Basal Energy Expenditure</td>
<td>2318</td>
<td>1769</td>
<td>1491</td>
<td>1387</td>
<td>1428</td>
<td>2110</td>
<td>1608</td>
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## Alcohol History

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<th>7</th>
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</thead>
<tbody>
<tr>
<td># of beers per Month</td>
<td>28</td>
<td>16</td>
<td>24</td>
<td>16</td>
<td>6</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td># of glasses of wine per month</td>
<td>30</td>
<td>&lt;1</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td># of drinks of liquor per month</td>
<td>2</td>
<td>&lt;1</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1</td>
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## Laboratory Data

(Non Fasting)

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<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mg/dL</td>
<td>163</td>
<td>182</td>
<td>188</td>
<td>194</td>
<td>131</td>
<td>146</td>
<td>167</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>134</td>
<td>121</td>
<td>25</td>
<td>74</td>
<td>28</td>
<td>122</td>
<td>39</td>
</tr>
</tbody>
</table>

## Subjective Sense of Sobriety

<table>
<thead>
<tr>
<th>Subjective Sense of Sobriety</th>
<th>Intoxication</th>
<th>Intoxication</th>
<th>Intoxication</th>
<th>Intoxication</th>
<th>Highly Intoxicated</th>
<th>Highly Intoxicated</th>
<th>No Signs of Intoxication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intoxication</td>
<td>16&lt;1</td>
<td>18&lt;1</td>
<td>19&lt;1</td>
<td>14&lt;1</td>
<td>16&lt;1</td>
<td>16&lt;1</td>
<td>16&lt;1</td>
</tr>
</tbody>
</table>
FAEE ARE DETECTABLE IN SERUM UP TO 24 HOURS AFTER ETHANOL INGESTION
THERE IS A STRONG POSITIVE CORRELATION BETWEEN FAEE AND ETAOH CONCENTRATIONS IN SERUM FOLLOWING ETHANOL INGESTION

SUBJECT 1
\[ r = 0.929 \]

SUBJECT 2
\[ r = 0.968 \]

SUBJECT 3
\[ r = 0.975 \]

SUBJECT 4
\[ r = 0.889 \]
New Blood Test Confirms Alcohol Use

- New York (Reuters) - Fatty acid ethyl esters (FAEEs) are not a new rock group.

- They are highly sensitive biochemical markers for the presence of alcohol (ethanol) in the blood, which according to researchers can be used to confirm other blood tests for drunk driving.
8:00 am: Train derailment

11:00 am: Train conductor arrives at the hospital for evaluation and blood ethanol level determined

Result: Blood negative for ethanol

Was the train conductor alcohol-free at the time of accident?
SUBJECTS (n = 8)

- AGE 21 - 46
- SOCIAL DRINKERS
- NO DIAGNOSED MEDICAL CONDITION
- WOMEN WERE NOT PREGNANT

PROTOCOL

STUDY BEGINS

- No alcohol consumption
- 5 days prior to study

90 minute ethanol consumption period

Blood Sampling:

- Base line
- 48

STUDY ENDS

- No alcohol consumption
- Until 48 hour time point

Blood Sampling:

- 1.75
- 3.5
- 5.5
- 7.25
- 24

Hours after start of ethanol intake
RBC FAEE ACCOUNT FOR APPROXIMATELY 7% OF WHOLE BLOOD FAEE

Hours After Start of Ethanol Intake

% of Total Blood FAEE in RBC

12
10
8
6
4
2
0

1 2 3 4 5 6 7 8

Hours After Start of Ethanol Intake
Fatty acid ethyl esters remained detectable in plasma 48 hours post ethanol intake despite undetectable blood ethanol levels.
Is the Fatty Acid Composition in Fatty Acid Ethyl Esters from Plasma and Red Blood Cells the Same?
THE FATTY ACID COMPOSITION OF RBC & PLASMA FAEE ARE DIFFERENT

1.75 Hours after start of ethanol intake (n = 8)

FAEE Species in Plasma and RBC

3.5 Hours after start of ethanol intake (n = 8)

FAEE Species in Plasma and RBC

5.5 Hours after start of ethanol intake (n = 8)

FAEE Species in Plasma and RBC

7.25 Hours after start of ethanol intake

FAEE Species in Plasma and RBC
MEN HAVE SERUM FAEE LEVELS 2-FOLD HIGHER THAN WOMEN DESPITE COMPARABLE BLOOD ETHANOL LEVELS

Time course for serum FAEE concentrations and blood ethanol concentrations for men and women consuming alcohol to legal limits of intoxication

*indicates significant differences between sexes (p < 0.05) for a given time point
TYPE OF ALCOHOLIC BEVERAGE INGESTED HAS NO IMPACT ON PLASMA FAEE LEVELS

Plasma FAEE levels at two representative peak time points for blood ethanol in subjects who consumed equivalent amounts of ethanol adjusted for body weight.
## Detecting Chronic Alcoholism

<table>
<thead>
<tr>
<th>Never Drinks</th>
<th>Social Drinking</th>
<th>Light Drinking</th>
<th>Light-Moderate Drinking</th>
<th>Moderate Drinking</th>
<th>Occasional Binge</th>
<th>Chronic Alcoholic</th>
<th>Chronic Alcoholic / Binge</th>
<th>Never Stops Drinking</th>
</tr>
</thead>
</table>

- **The FAEE Test distinguishes between levels of drinking behavior**

**Because ethanol intake is at most moderate, distinguishing between the different levels of drinking behavior is not contributory to evaluation**

**Often clinically difficult to distinguish between chronic alcoholics and binge drinkers even with patient history, physical exam, and routine laboratory tests**

**Because ethanol intake is so great, distinguishing between the different levels of drinking behavior is not contributory to evaluation**
Ethyl Oleate as Percent of Total FAEE is Significantly Different in Chronic Alcoholics and Binge Drinkers Approximately 24 Hours after Ethanol Consumption was Discontinued

Sensitivity: 100 % and Specificity: 100 %

$p < 0.001$

Threshold for Chronic Alcoholism: Ethyl 18:1 > 52 % of Total FAEE
Absolute Difference between Ethyl Stearate and Ethyl Oleate Both as Percent of Total FAEE is Significantly Different in Chronic Alcoholics and Binge Drinkers Approximately 24 Hours after Ethanol Consumption was Discontinued

**Sensitivity: 100 % and Specificity: 100 %**

\[ p < 0.001 \]

Threshold for Chronic Alcoholism:

\[ [\text{Ethyl 18:0 } \% - \text{Ethyl 18:1 } \%] > 45 \]

- **Chronic Alcoholics**
  - \( n = 15 \)
  - Mean ± SEM: 71 ± 2

- **Binge Drinkers**
  - \( n = 13 \)
  - Mean ± SEM: 10 ± 3
31 cases from the Massachusetts Medical Examiner’s Office and the Department of Pathology at the Massachusetts General Hospital were evaluated.

- Postmortem intervals ranged from 5-29 hours (mean of 16 hours).
- In all cases blood ethanol concentrations were determined at autopsy.
- Liver and adipose tissue fatty acid ethyl esters were extracted and quantitated.
1. Detectable blood ethanol at the time of autopsy
2. Negative blood ethanol at the time of autopsy with a history of chronic alcoholism
3. Negative blood ethanol at the time of autopsy with a history of social drinking or abstinence
LIVER FATTY ACID ETHYL ESTERS FOR THE 3 GROUPS OF SUBJECTS

<table>
<thead>
<tr>
<th>Group</th>
<th>FAEE Total (pmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals with detectable blood ethanol at autopsy</td>
<td>1,235</td>
</tr>
<tr>
<td>Chronic alcoholics with negative blood ethanol at autopsy</td>
<td>5,315</td>
</tr>
<tr>
<td>Social drinkers with negative blood ethanol at autopsy</td>
<td>5,485</td>
</tr>
</tbody>
</table>

Sensitivity = 93%
Specificity = 100%
OUTLINE OF PRESENTATION

Background - FAEE
Toxicity of FAEE
FAEE Synthesis
FAEE Secretion
FAEE Transport in Blood
FAEE Degradation
FAEE as Markers of Ethanol Intake