Novel Strategies to Improve The Function of Steatotic Donor Livers

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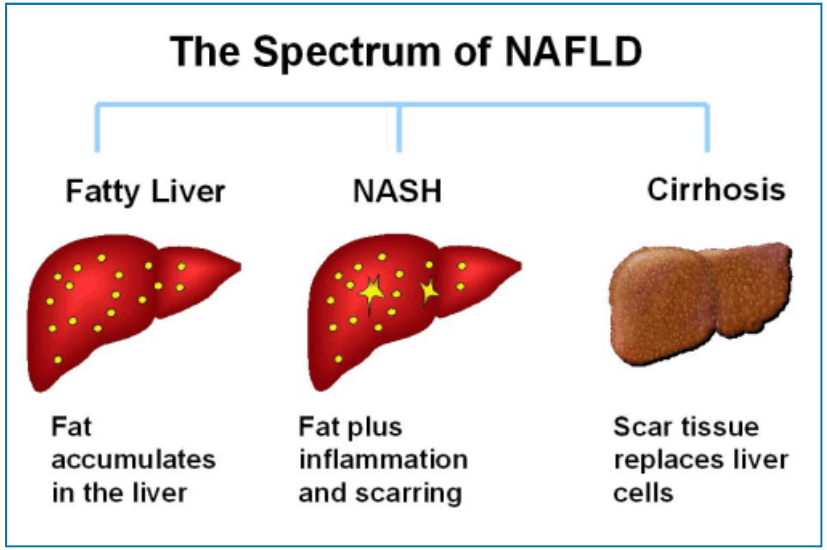
Conflict of Interest Disclosure

- "I have no relevant financial relationships to disclose."

The increasing incidence of NASH/NAFLD

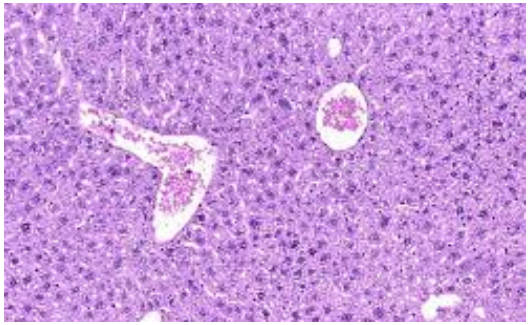
- Obesity and metabolic syndrome is on the rise in the US
- NASH is estimated to overtake HCV as biggest liver disease problem by 2020



NAFLD and NASH

Non-alcoholic Fatty Liver Disease

- 20-30% Prevalence
- 50% Prevalence by 2030

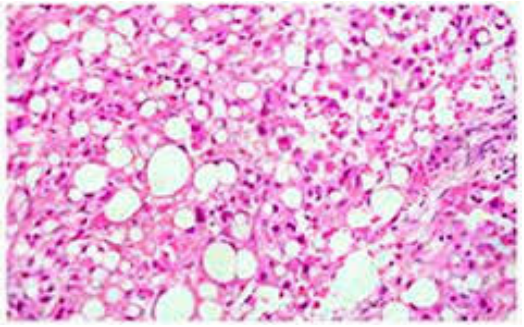


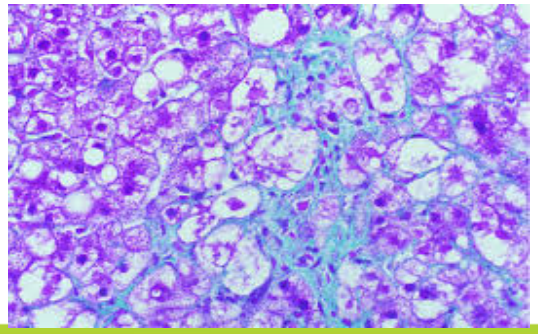
Normal Liver

Non-alcoholic steatohepatitis

- 3-5% Americans
- 3rd leading cause of end stage liver disease

NAFLD





NASH

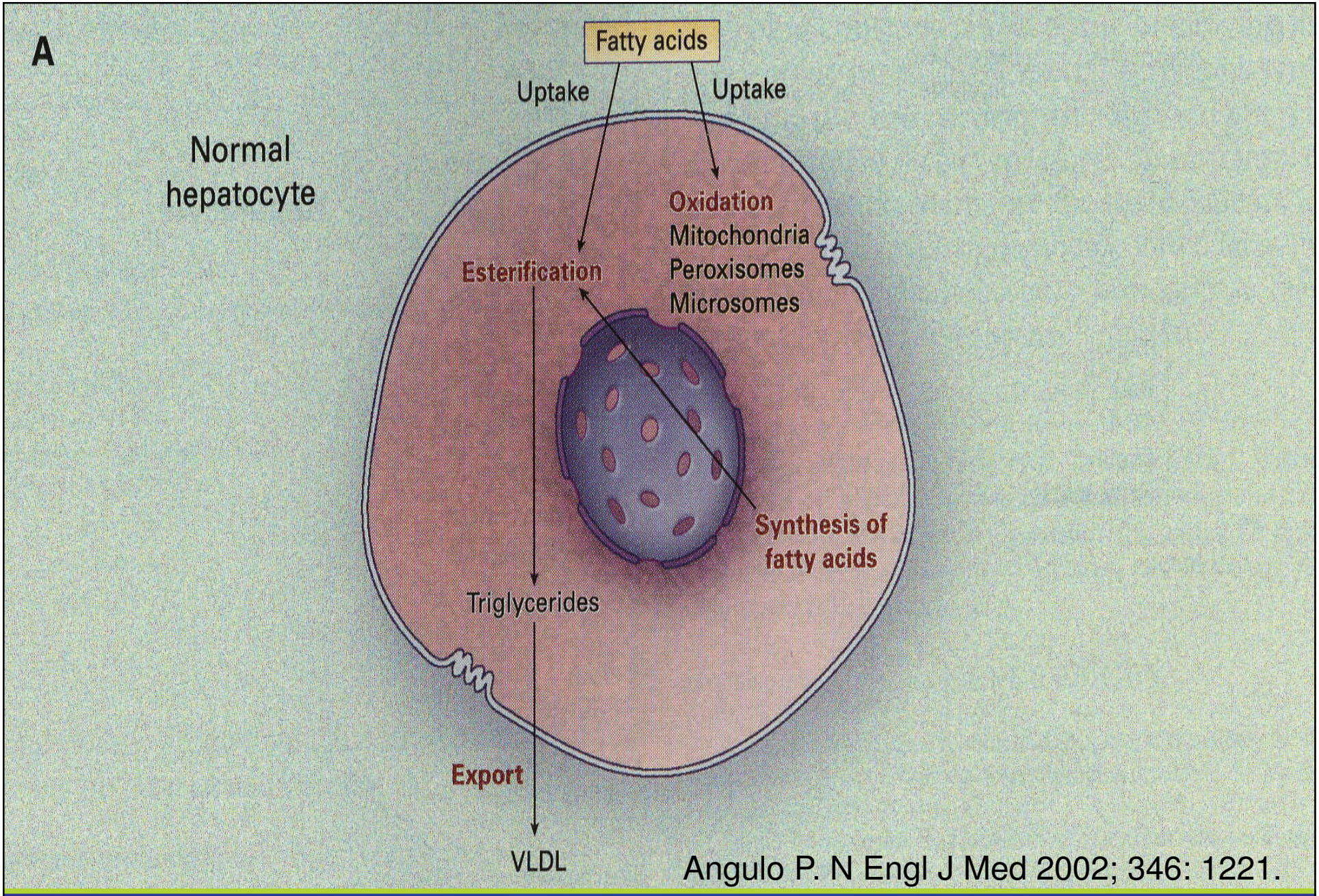
NAFLD -> NASH ?

Conditions Associated with Microvesicular Fatty Change

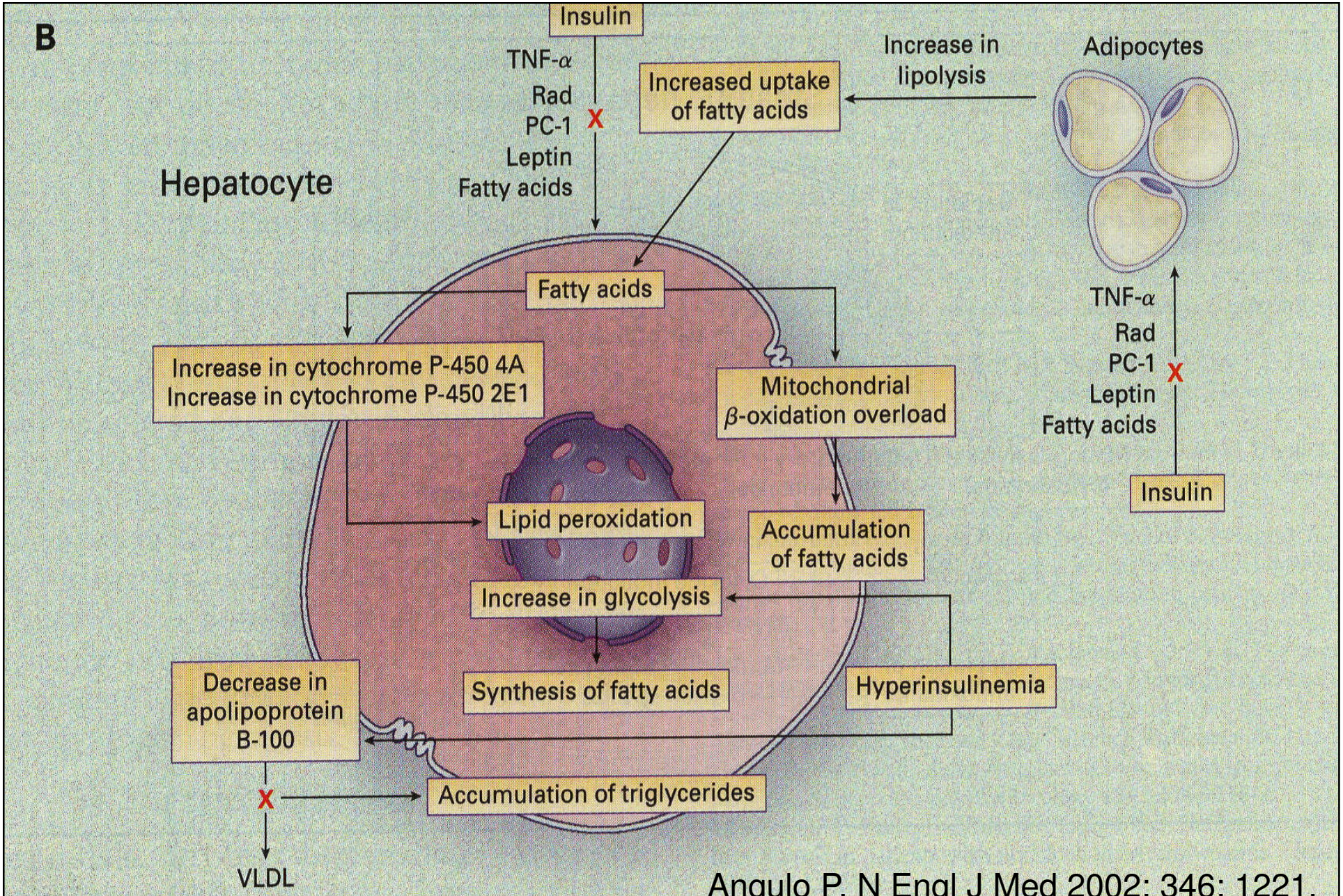
Acute fatty liver of pregnancy
Reye's syndrome
Alcoholic foamy degeneration
Drug- and toxin-induced injury (Chemo Therapies)
Valproic acid
Parenteral tetracycline
Salicylates
Hypoglycin A
Congenital metabolic conditions
Urea cycle disorders
Defects in fatty acid metabolism
Deficiencies in lysosomal acid lipases

Conditions Associated with Nonalcoholic Steatosis and Steatohepatitis

Obesity
Diabetes mellitus
Gastrointestinal surgery
Jejunoileal bypass
Extensive small bowel resection
Gastroplasty
Drug-related
Amiodarone
Perhexiline maleate
Glucocorticoids
Synthetic estrogens
Miscellaneous and idiopathic



Angulo P. N Engl J Med 2002; 346: 1221.



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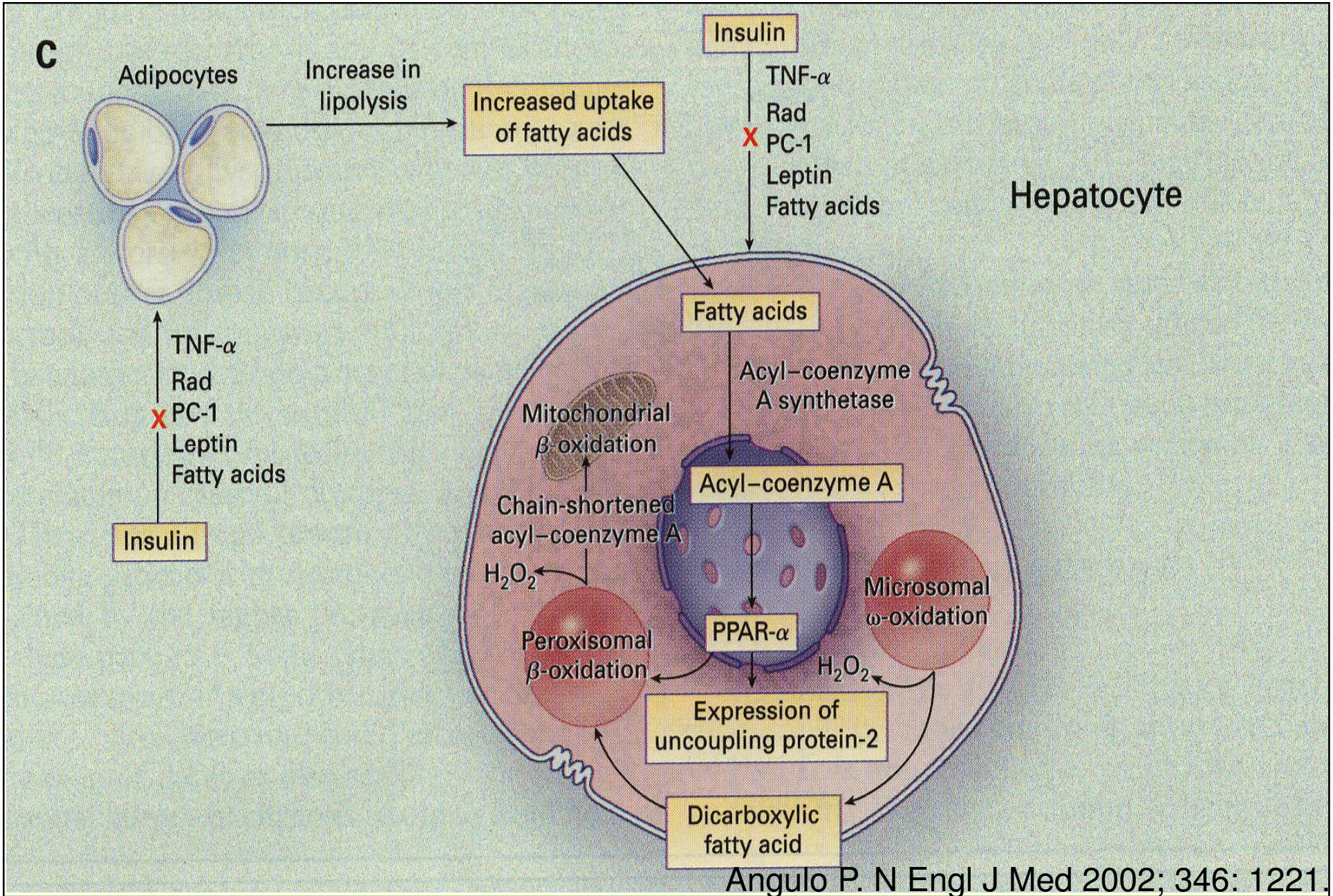

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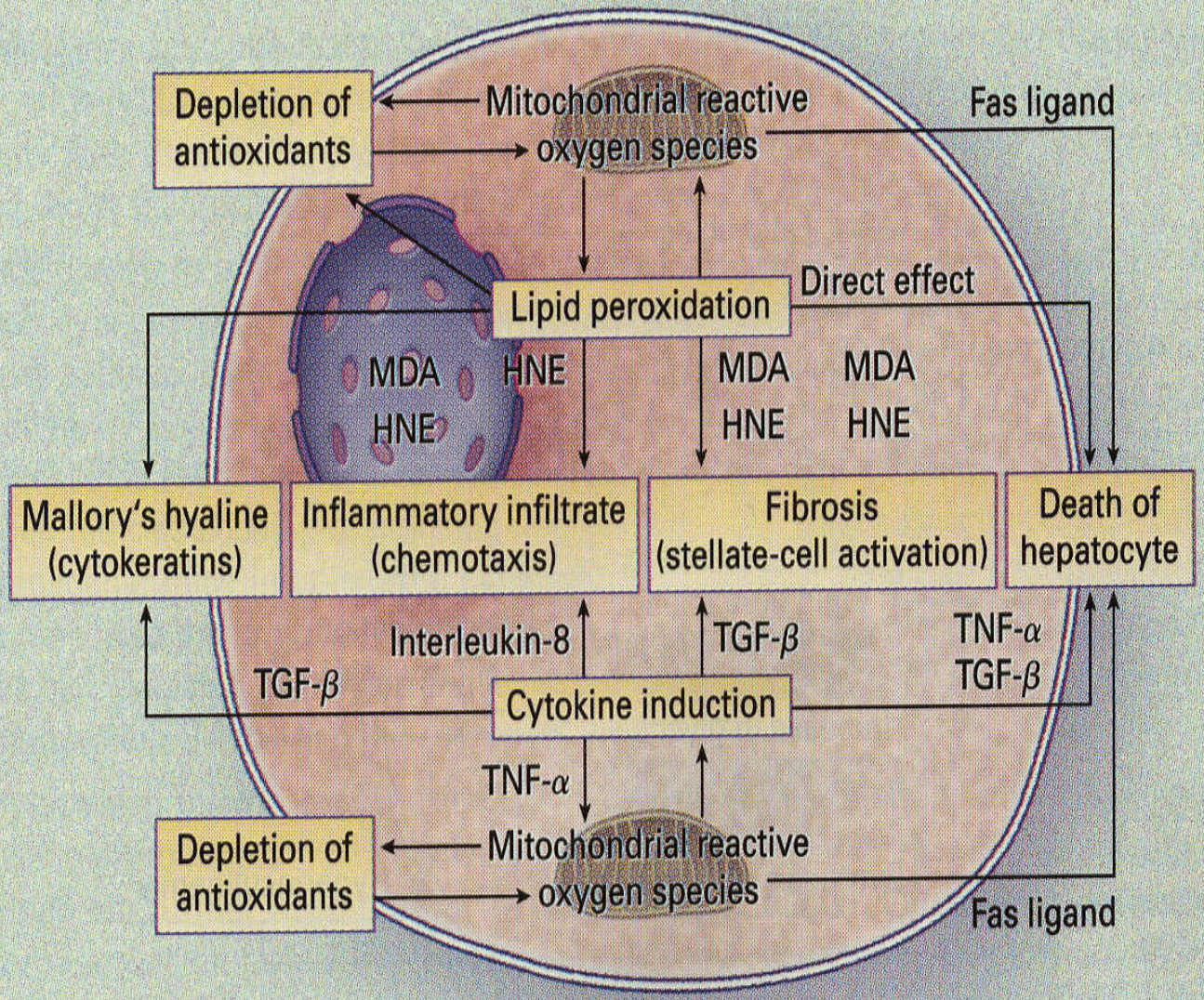


Angulo P. N Engl J Med 2002; 346: 1221.

Liver Transplantation

- >17,000 awaiting transplant in US
- 20% die prior to transplant
- Donors with steatosis unsuitable for transplant
- NAFLD and NASH
  - Increase Demand
  - Reduce supply

Expanded Criteria Donor

- Donor age >55yo
- Donor hospital stay >5 days
- Cold ischemia time >10 hours
- Warm Ischemia time >40 minutes

Cameron et al in Ann Surg. 2006 Jun; 243(6): 748–75.

Expanded Criteria Donor

TABLE 1. Multivariate Predictors of Graft and Recipient Survival

Variable	Level	Graft Failure	Mortality Ratio
Donor age (yr)	>55	1.2 ($P = 0.20$)	1.3 ($P = 0.07$)
Donor hospital stay (days)	>5	1.3 (0.03)	1.5 ($P = 0.002$)
Cold ischemia (hr)	>10	1.2 (0.08)	1.4 ($P = 0.006$)
Warm ischemia (m)	>40	1.8 (<0.0001)	1.7 ($P = 0.001$)
Recipient age (yr)	>55	1.5 (0.008)	1.5 ($P = 0.008$)
Recipient urgency	Yes vs. no	1.3 (0.008)	1.5 ($P = 0.0006$)

Cameron et al in Ann Surg. 2006 Jun; 243(6): 748–75.

Marginal Donors: Steatosis

Although organ from marginal donors may not be optimal, they are a viable alternative to dying while waiting for transplantation, and their use needs to be pursued

OPTN Annual Report 2003

Steatosis: Cadaveric Donors

- Multiple studies have demonstrated that > 30% steatosis carries a 25% rate of PNF
- Early graft dysfunction
- Increased susceptibility to IR injury
- Greater reduction in energy stores during cold preservation
- Decreased capacity to restore ATP levels after reperfusion

What factors go into the decision to use a steatotic liver for transplantation?

- D'Alessandro et al. and Adams et al. have proposed classifications schemes based on the degree of steatosis. Using these criteria PNF rates were reduced to 1.4%

Donor Risk Factors: Impact on Outcomes

Parameter	Relative Risk	Timing
↑vasopressor support	+	early
Long ICU stay	+	early
Na^+ >155mEq/L	+	early
Older age (>50)	++	early
Macrosteatosis (<30%)	++	early
Macrosteatosis (>30%)	++++	early
CIT (>12 hrs)	++/+	early/late

Adapted from Busuttil, RW, Tanaka K. Liver Transplantation 2003; 9: 651

Assessment of Graft Function

"The transplant surgeon still has to rely on a subjective interpretation of donor data and the macroscopic and microscopic appearance of the liver to decide whether to use the graft."

Melendez et al; Transplantation 70: 4, 2000.

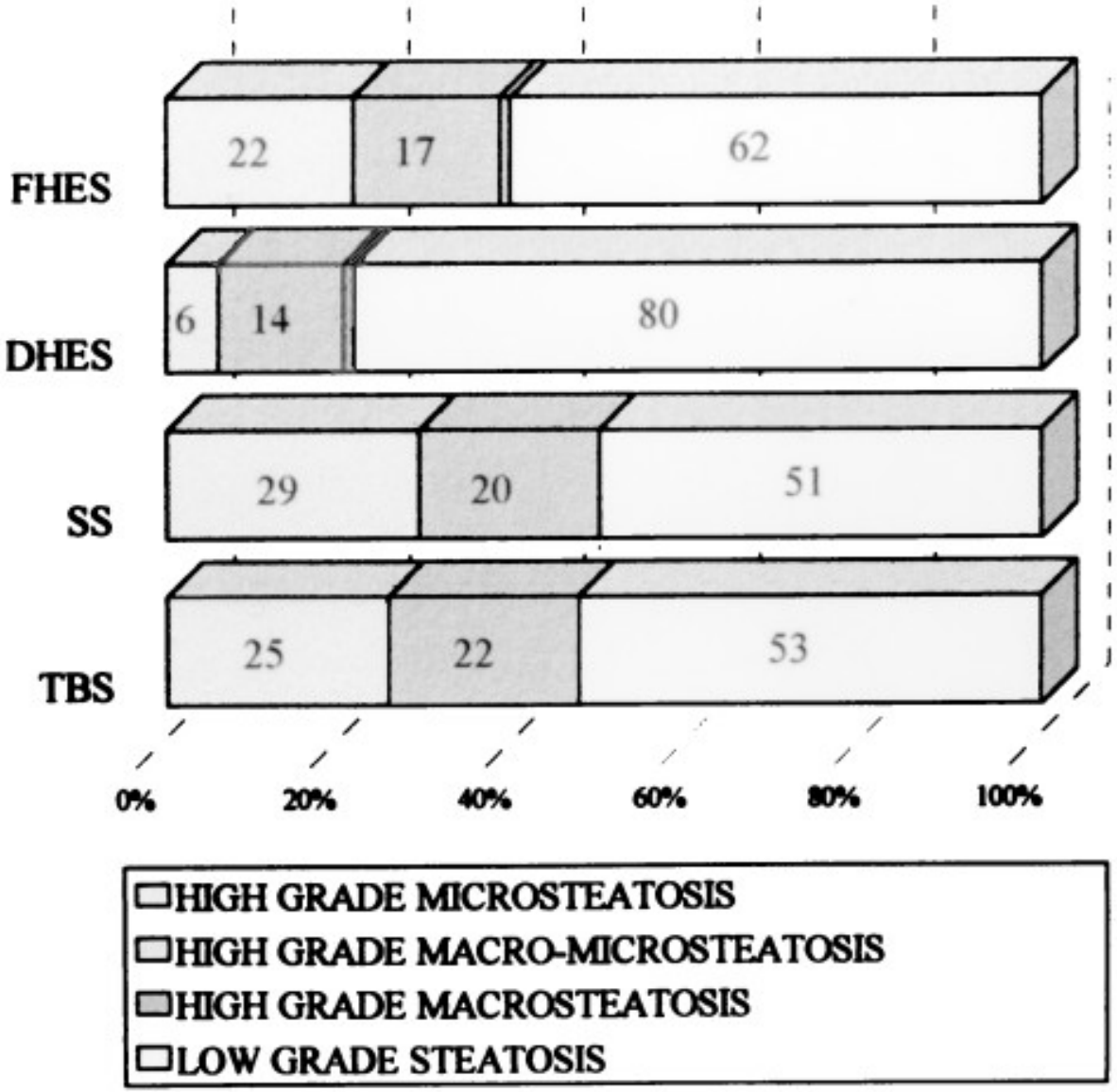
Does a Biopsy Help ?

Hepatic Steatosis in Donors

Published studies about prevalence of moderate to severe hepatic steatosis in liver transplant donors.

Study	No. of Grafts	Stain	Vacuole size	Prevalence (%)
Adam et al.,1991 [7]	390	?	?	17.0
D'Allesandro et. al., 1991 [6]	24[a]	Frozen H&E	?	17.0
Ploeg et al., 1993 [9]	323	?	?	9.0
Markin et al., 1993 [2]	385	Frozen H&E Oil red 0	Macrosteatosis Microsteatosis ?	13.2 0.7 51.3
Karayalcin et al., 1994 [18]	187 61	? H&E Sudan	?	18.0
Monsour et al., 1994 [22]	20[b]	Deparaffinated H&E	Macrosteatosis	20.0
Present Series	83	Thin Sections	Macromicrosteatosis Microsteatosis Total high-grade steatosis	21.6 25.3 46.9

?: Unknown [a]Prospective Study Data. [b]Living-Related Donors. Urena et al., World J Surg 1998; 22 (8).

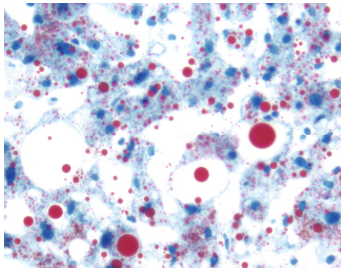
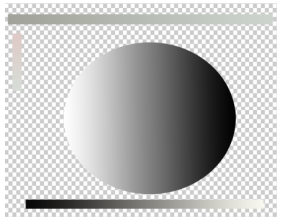


Urena et al., World J Surg 1998; 22 (8).

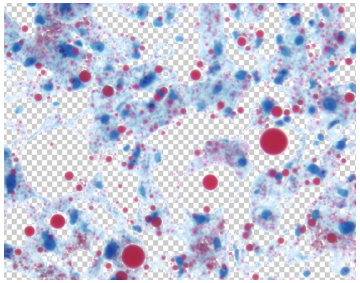
Development of an Unbiased Method for the Estimation of Liver Steatosis

Fiorini et al. 2004, Clinical Transplantation:18: 700-706

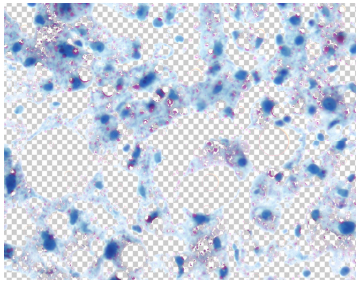
Methodology

1.Remove Artifact

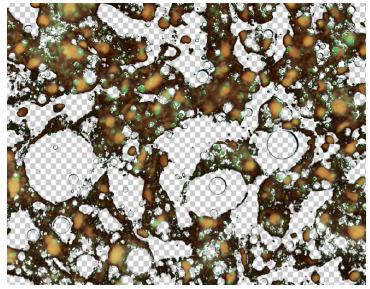



2.Remove Red Pixels



3.Invert Colors

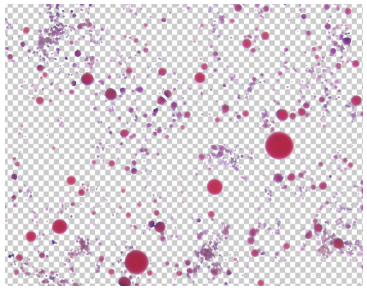
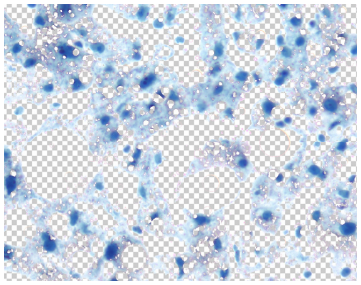
•This will allow us to better separate purples from blues



4. Remove Green Pixels

•This will separate purples (green) from blues (brown and yellow)

•Green pixels can now be returned to their original color and added to the red pixels

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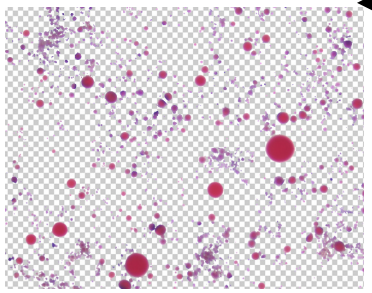
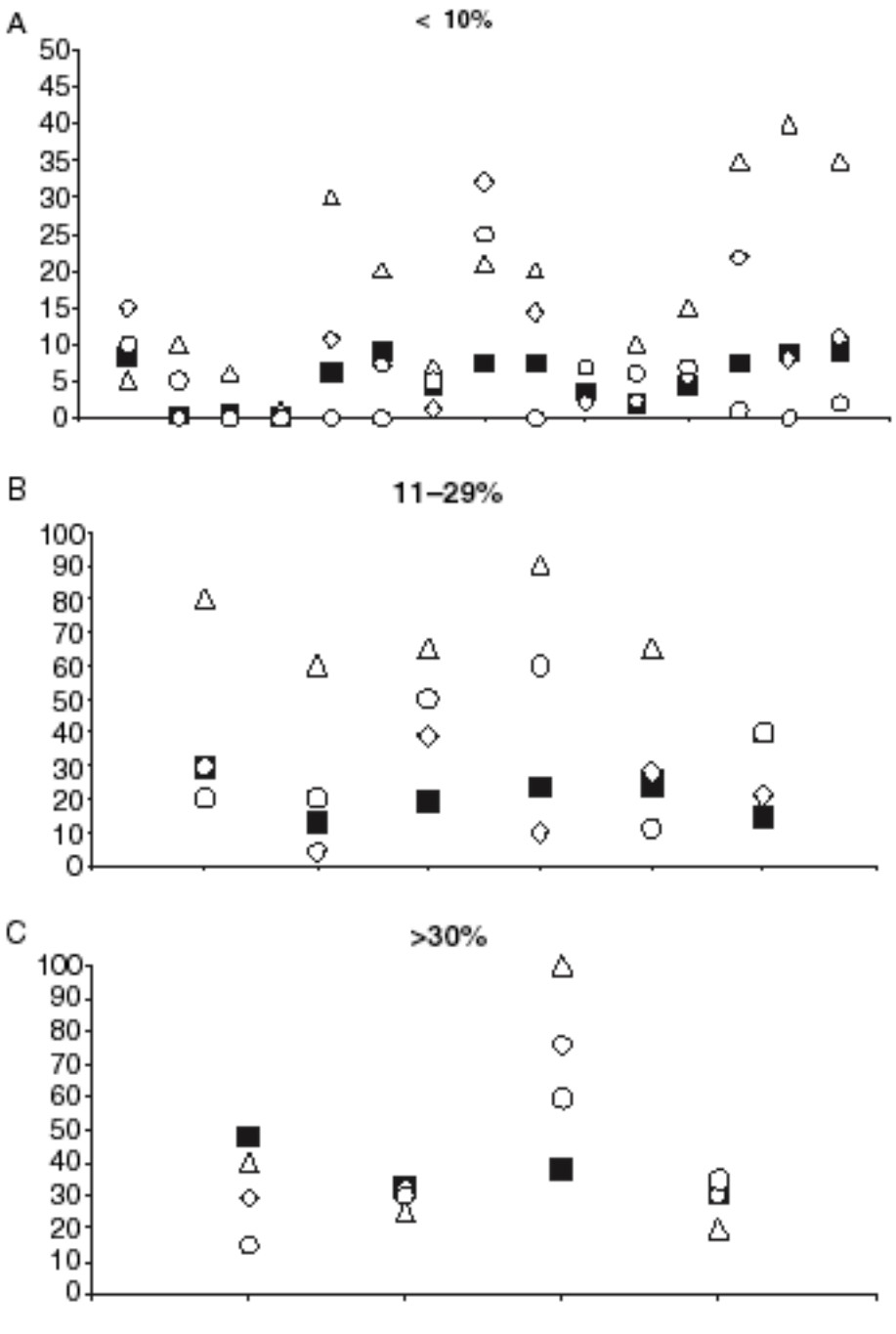


5. Determine Fat Content

•Use the Histogram Function to Sum the Number of Pixels in Each Layer

•# red pixels / (# red pixels + # blue pixels)



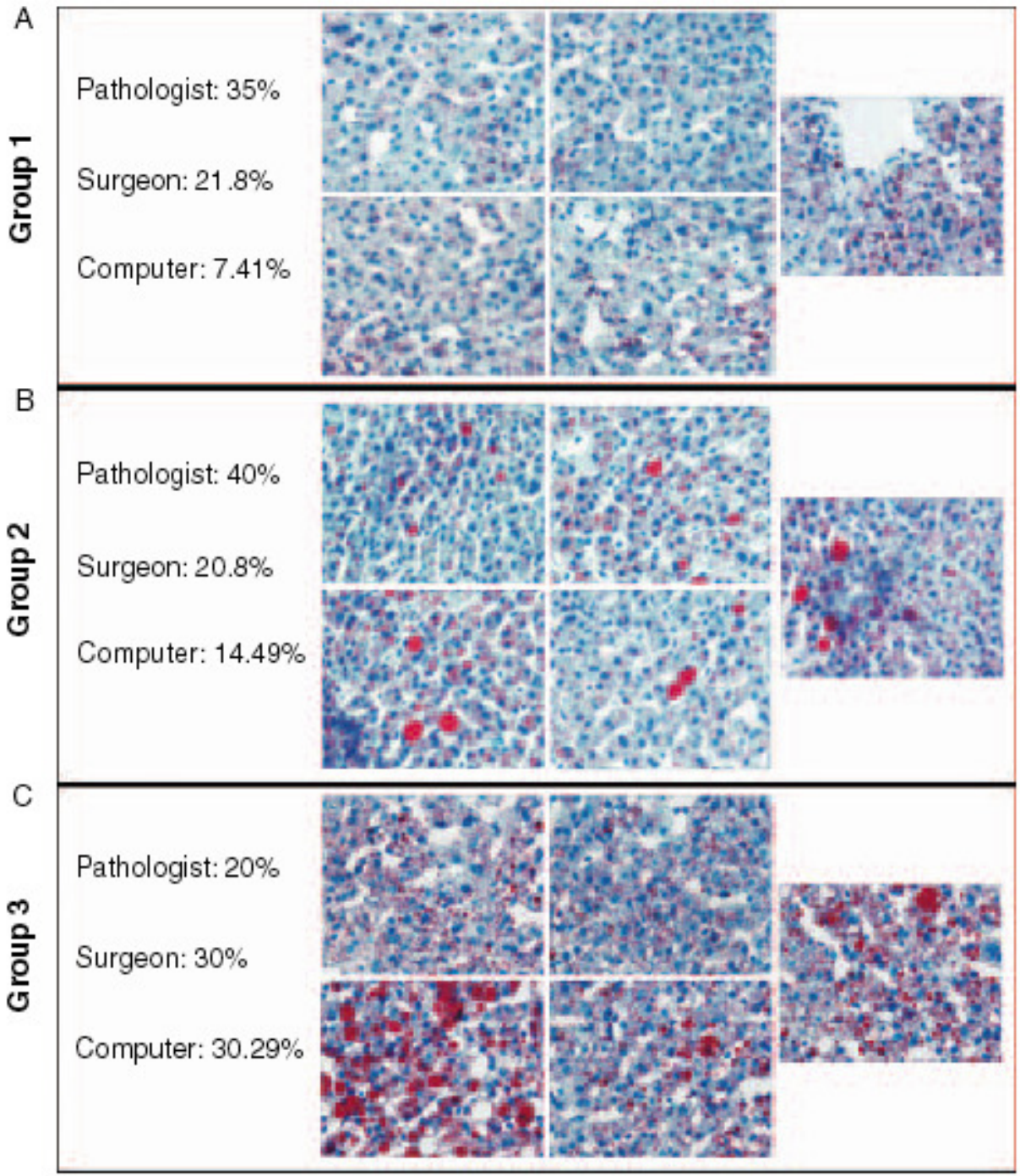
Computer Measurement

H&E Read by Pathologist

ORO Read by Pathologist

ORO Read by Surgeon

Fig. 2. Categorical analysis of percentage fat divided into three groups based on computer measurement. (A) Group I (< 10%) n = 15, (B) group II (11–29%) n = 6, and (C) group III (>30%) n = 4. (■) Computer measured values of percentage fat; (◇) transplant surgeon estimated percentage of fat by ORO stain; (△) pathologist estimated percentage of fat by ORO stain; (○) pathologist estimated percentage of fat by H&E stain. All patients in Table 1 plotted on graph in their respective groups.


A
Group 1
Pathologist: 35%
Surgeon: 21.8%
Computer: 7.41%
B
Group 2
Pathologist: 40%
Surgeon: 20.8%
Computer: 14.49%
C
Group 3
Pathologist: 20%
Surgeon: 30%
Computer: 30.29%

Micro vs Macro Steatosis

- What is the significance of each?

Clin Transplant 2013 DOI: 10.1111/ctr.12211

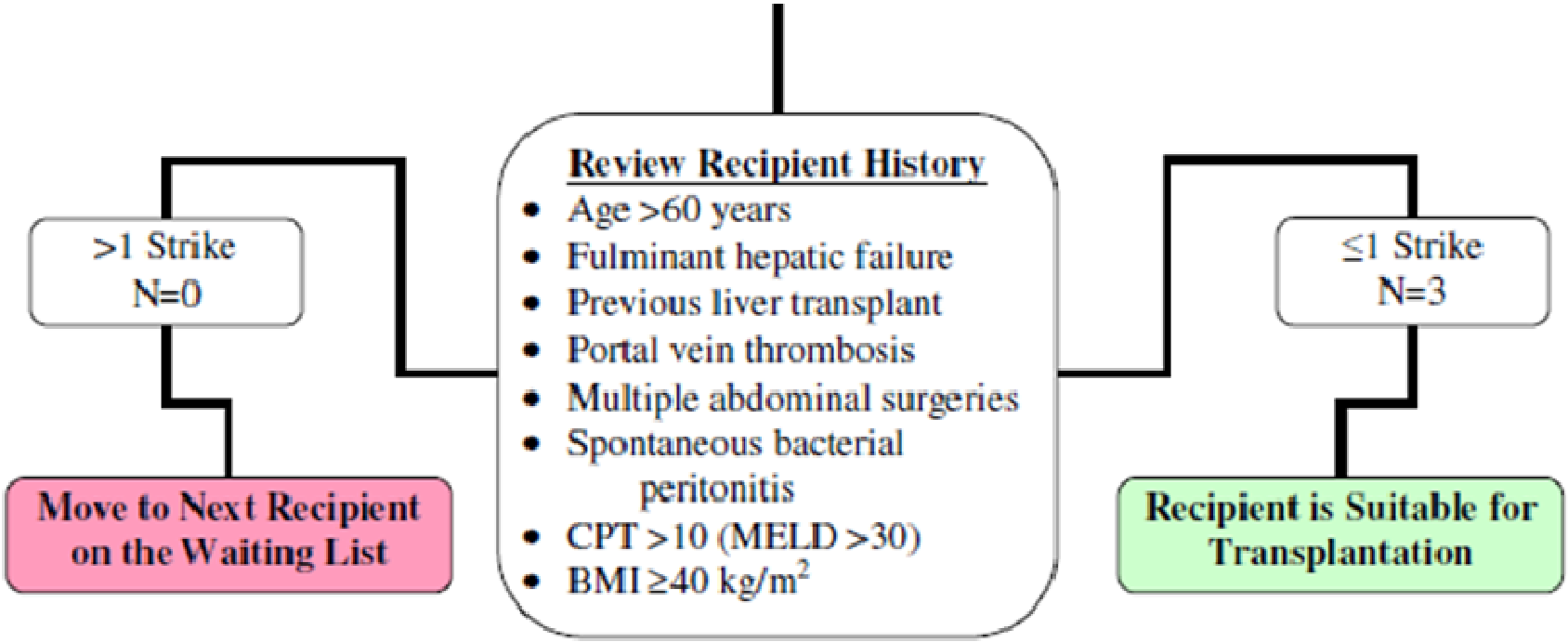
Clinical Transplantation

Safe use of highly steatotic livers by utilizing a donor/recipient clinical algorithm

Chavin KD, Taber DJ, Norcross M, Pilch NA, Crego H, McGillicuddy JW, Bratton CF, Lin A, Baliga PK.

Methods

- Prospective observational 10-year follow-up study
- Primary aim of determining patient and graft survival based on degree of donor liver steatosis
- Highly steatotic livers were utilized according to a detailed donor/recipient algorithm that guided the use of highly steatotic organs judiciously in low-risk recipients
- Patients were divided into three groups based on donor steatosis
  - Group 1: <30% steatosis
  - Group 2: 30-60% steatosis
  - Group 3: >60% steatosis



* Dopamine >10 mcg/kg/min, norephinenephrine, epinephrine, or phenylephrine infusion

** AST or ALT greater than 3 times the upper limit of normal

Methods: Intra-Operative

- Efforts to limit cold ischemia time to < 6 hours
- Infuse ~ 1600ml of portal flush (LR plus albumin 25%)
- Waste ~500cc of portal blood prior to reperfusion
- Simultaneously reperfuse with the hepatic artery and portal vein

Methods: Post-Operative

- Induction therapy with IL2R blockade
- Delayed initiation of calcineurin inhibitors
- Routine use of prostaglandin E-1
- Careful attention to detail
- Daily ultrasounds

Results

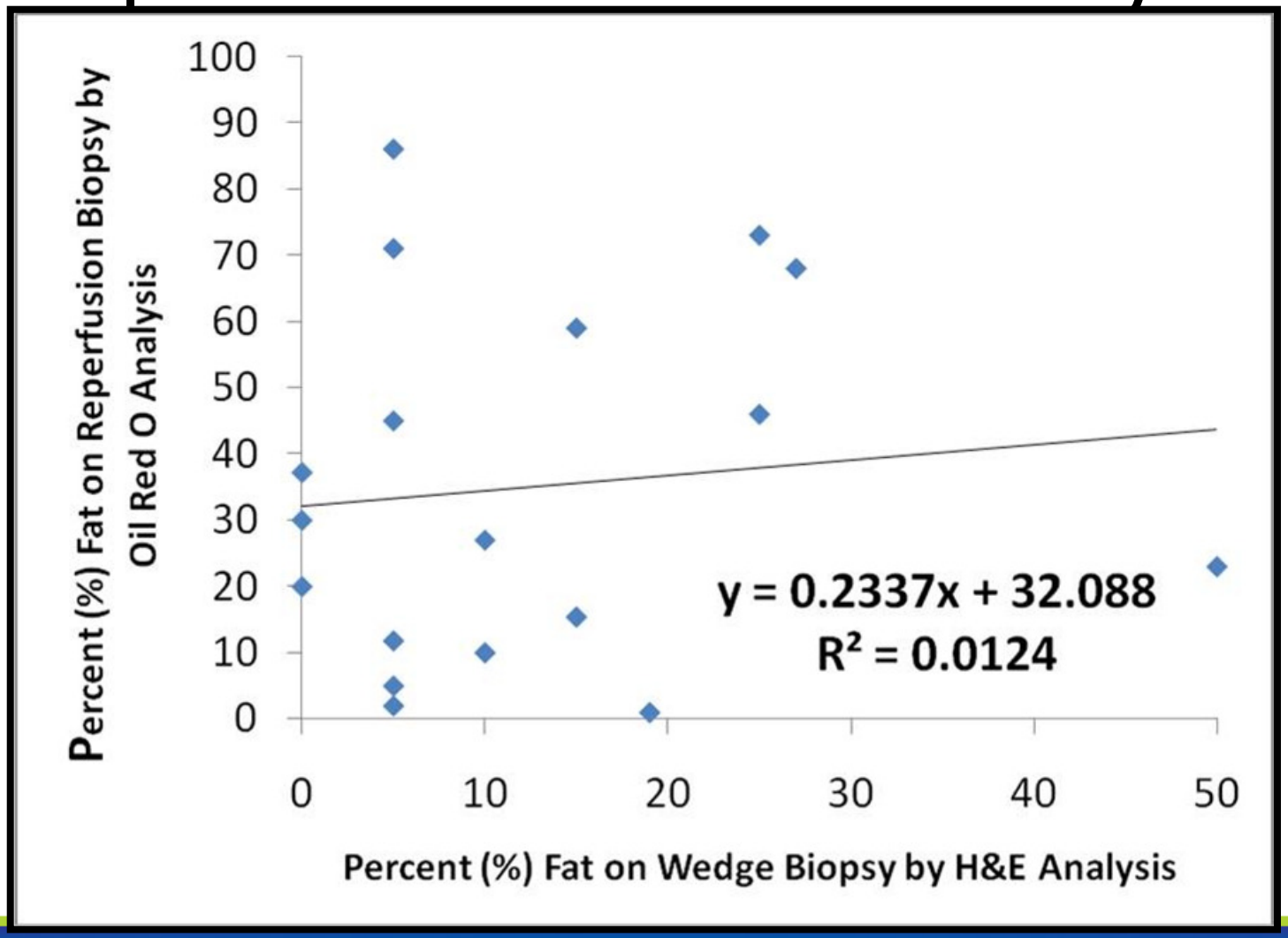
- From 6/1/99 to 12/31/01, 190 liver transplants performed at our institution
  - 49 patients were excluded from analysis (2 LRD, 12 multi-organ, 12 pediatric patients, 20 re-transplants, 2 split-liver and 1 highly steatotic import liver)
  - From the remaining 141 patients, 116 gave informed consent and were included in the analysis
- All patients included in this analysis were followed for at least 10 years post-transplant, or until graft loss or death

Baseline Characteristics	Normal <30% Fat (n=78)	Marginal 30-60% Fat (n=27)	High Marginal >60% (n=11)	P-value
Donor Age	34±17	38±16	26±10	0.12
Donor Gender (%)				0.64
Female	28 (36)	8 (30)	5 (45)	
Male	50 (64)	19 (70)	6 (55)	
Donor BMI	25±8	26±8	29±10	0.34
Donor Race (%)				0.10
African American	24 (31)	8 (30)	0 (0)	
Caucasian	54 (69)	19 (70)	11 (100)	
Donor Vasopressor Use (%)	22 (28)	11 (41)	4 (36)	0.46
Donor Peak Serum Sodium	154±10	153±8	154±10	0.92
Recipient Age	52±9	51±6	47±9	0.16
Recipient Gender (%)				0.11
Female	32 (41)	5 (19)	4 (36)	
Male	46 (59)	22 (82)	7 (64)	
Recipient Race (%)				0.43
African American	9 (12)	2 (7)	0 (0)	
Caucasian	69 (89)	25 (93)	11 (100)	
Cold Ischemic Time (min)	369±114	347±107	380±56	0.60
Warm Ischemic Time (min)	54±12	57±17	49±14	0.26
Recipient Baseline SrCr (mg/dL)	1.1±0.7	1.2±0.5	1.0±0.3	0.82

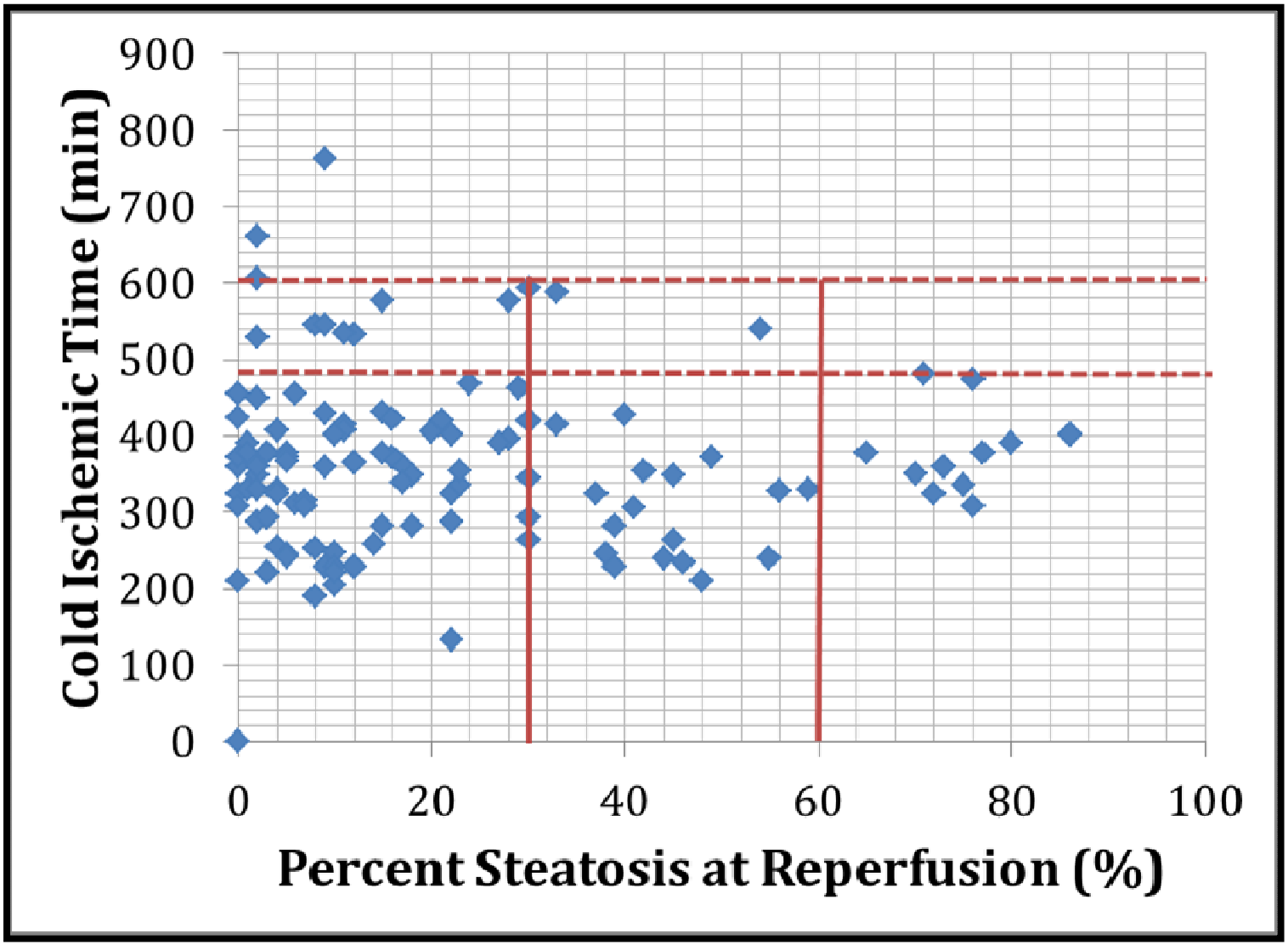
Clinical Outcomes	Normal (n=78)	Marginal (n=27)	High Marginal (n=11)	P-value
Mean ICU Stay (days)	4.7±3.8	3.6±2.6	4.6±4.5	0.36
Mean LOS (days)	10.9±6.6	10.3±6.6	8.6±4.3	0.92
Primary Graft Non-Function (%)	3 (3.8)	0 (0)	0 (0)	0.47
Hepatic Artery Thrombosis (%)	4 (5.1)	2 (7.4)	0 (0)	0.65
Biliary Complications (%)				
Leak	11 (14)	7 (26)	0 (0)	0.10
Stricture	11 (14)	3 (11)	2 (18	0.88
Graft Survival				0.62
30 day	79%	93%	82%	
1 year	71%	81%	82%	
3 year	64%	67%	82%	
5 year	54%	63%	73%	
10 year	41%	45%	45%	
Patient Survival				0.68
30 day	88%	96%	82%	
1 year	77%	85%	82%	
3 year	71%	70%	82%	
5 year	58%	70%	73%	
10 year	42%	49%	45%	

Example of Highly Steatotic Liver that was Successfully Utilized

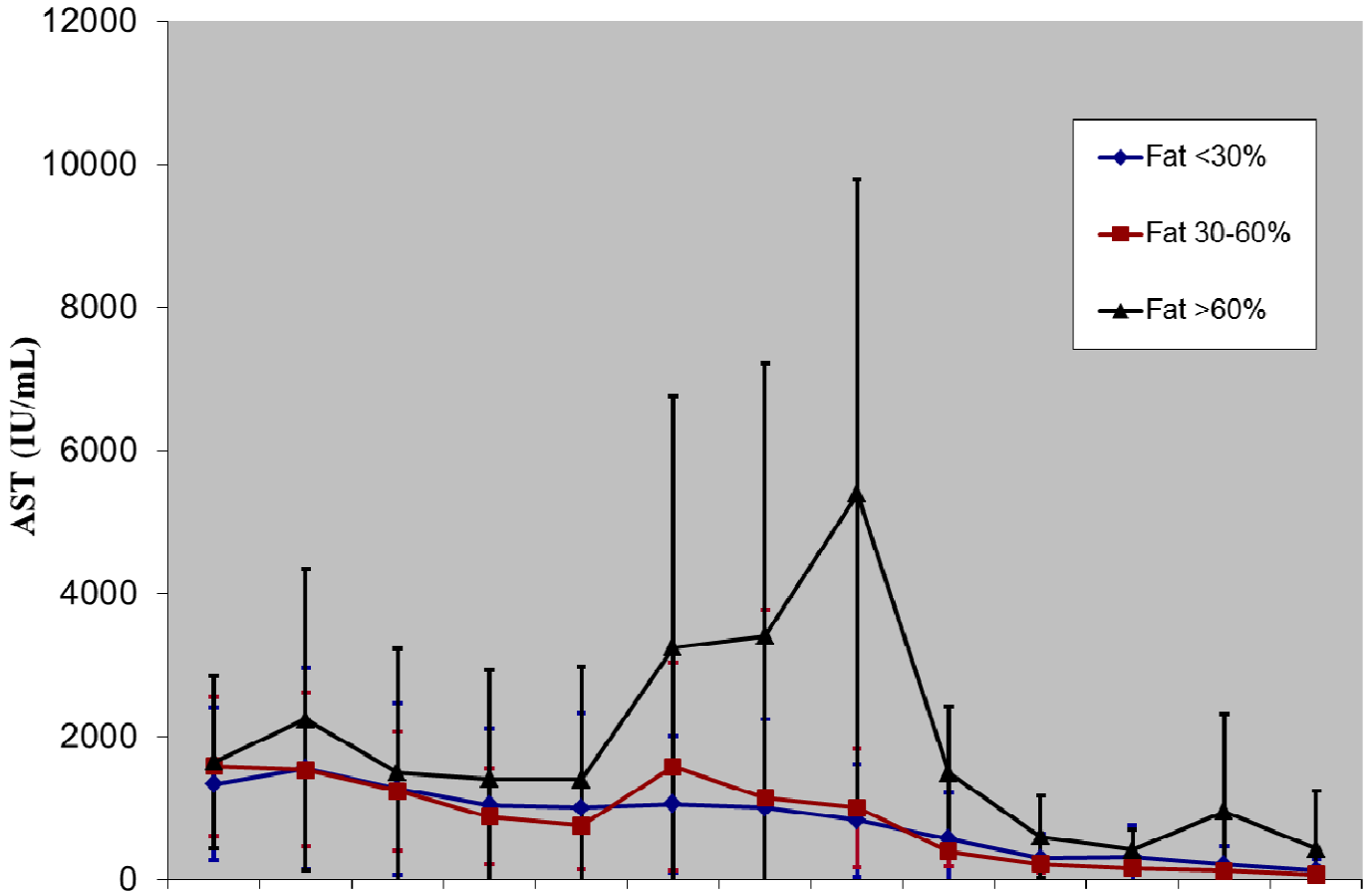
Comparison of Steatosis using H&E compared to Oil Red O Analysis



Comparison of Steatosis versus CIT

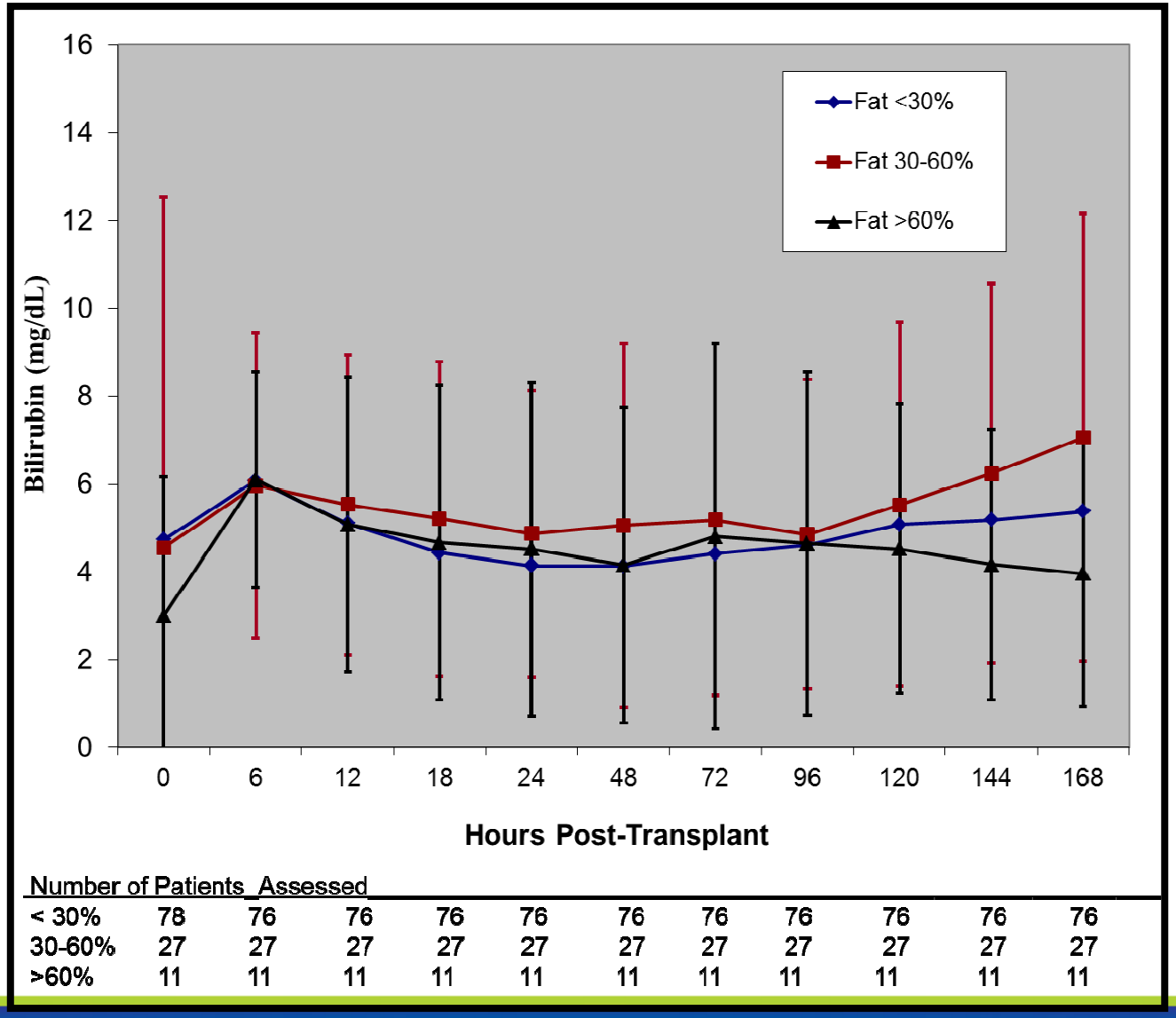


Biochemical Analysis - AST



Number of Patients Assessed													
< 30%	68	56	66	64	47	8	40	10	28	36	27	13	31
30-60%	26	22	27	25	19	4	16	3	7	17	7	7	7
>60%	9	8	9	9	6	2	4	1	4	5	3	2	5

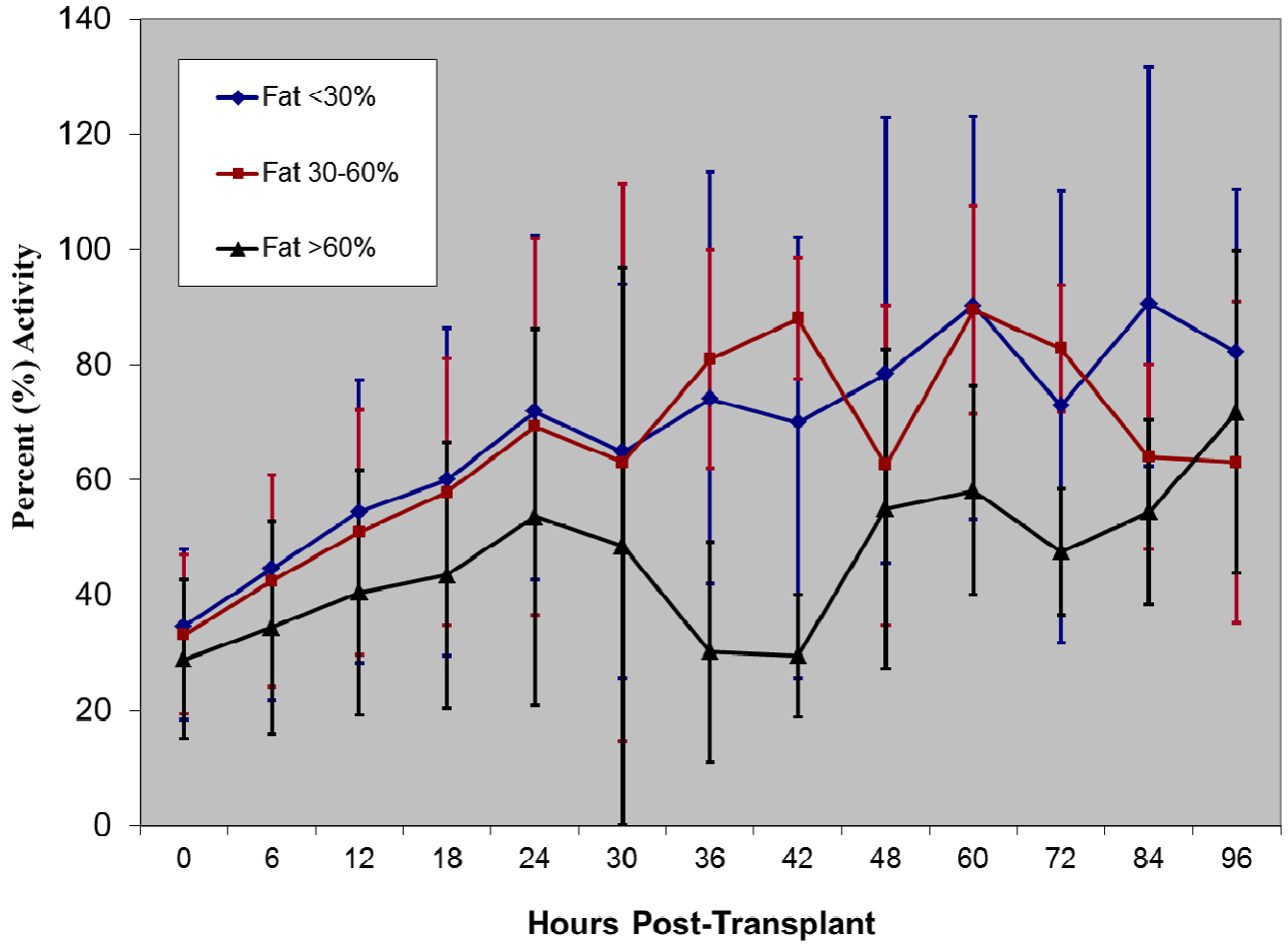
Biochemical Analysis - Bilirubin



Number of Patients Assessed

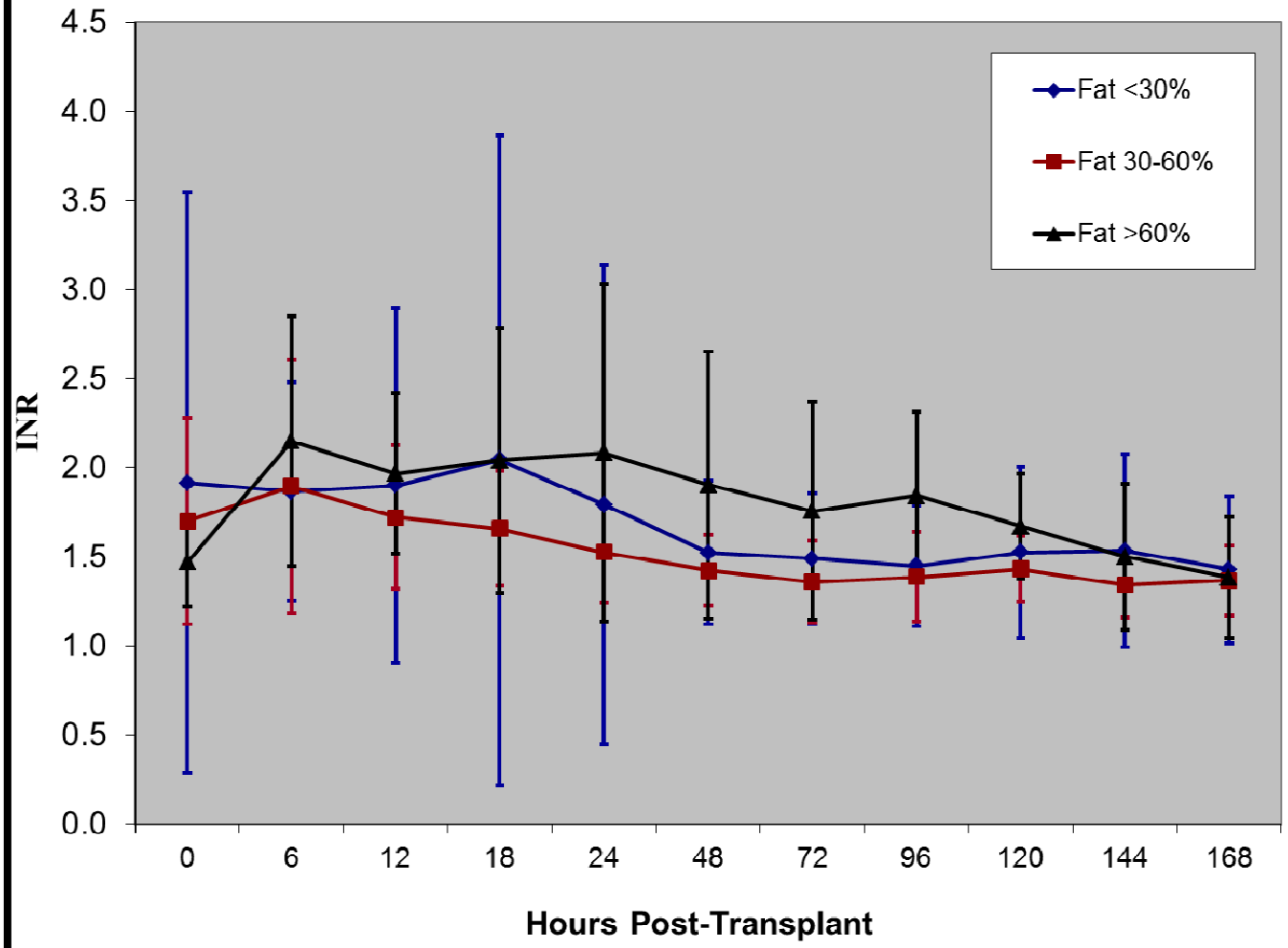
< 30%	78	76	76	76	76	76	76	76	76	76	76
30-60%	27	27	27	27	27	27	27	27	27	27	27
>60%	11	11	11	11	11	11	11	11	11	11	11

Biochemical Analysis – Factor V



Number of Patients Assessed													
< 30%	38	34	38	36	27	7	23	8	16	15	9	10	7
30-60%	19	16	19	19	16	2	10	2	5	10	5	3	3
>60%	6	5	7	7	3	2	4	1	4	3	2	1	1

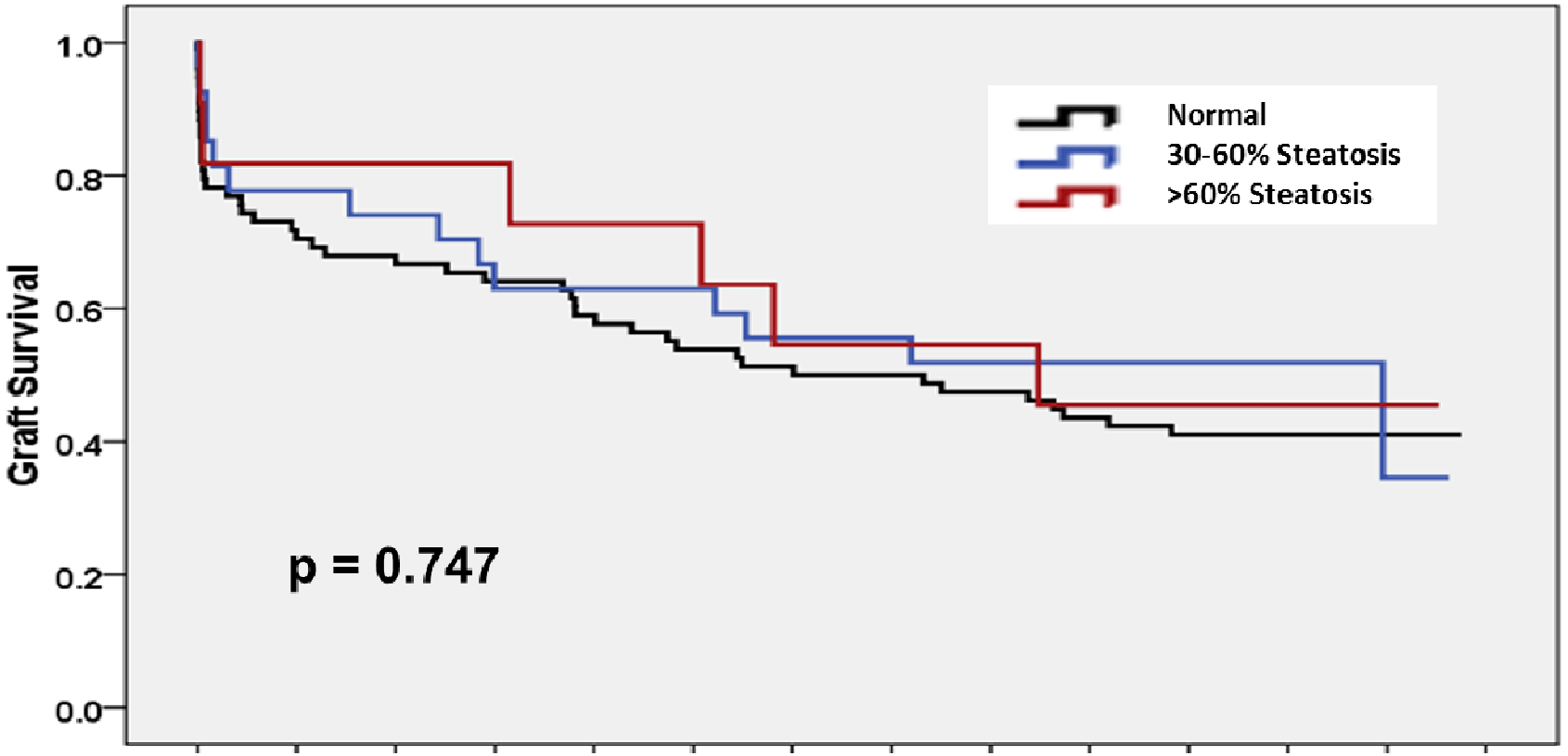
Biochemical Analysis - INR



Number of Patients Assessed

< 30%	78	76	76	76	74	67	53	43	36	33	32
30-60%	27	27	27	27	26	23	19	12	11	9	10
>60%	9	8	9	9	6	2	4	1	4	5	3

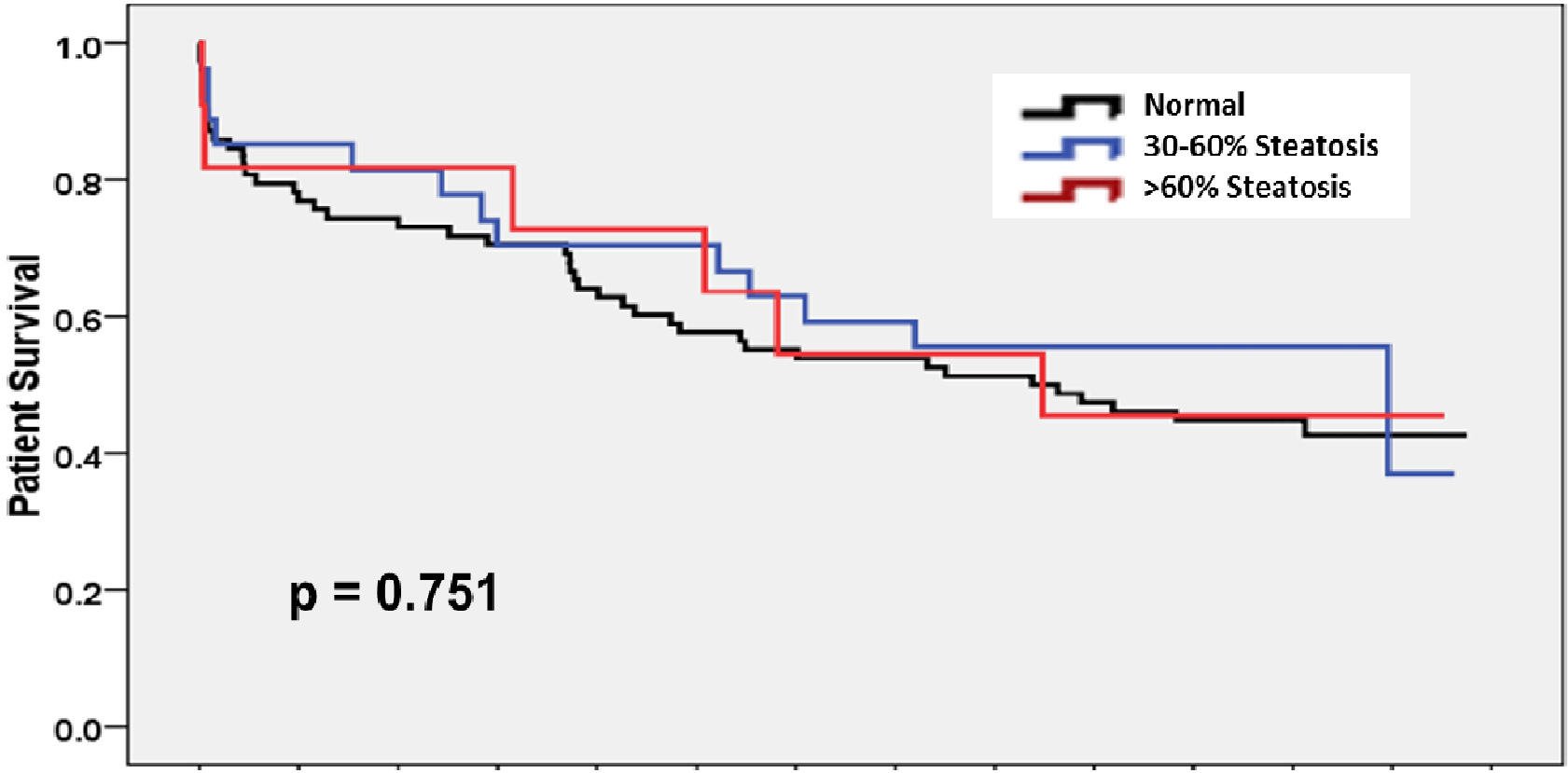
Graft Survival



Number at Risk

< 30%	78	55	53	50	46	42	40	39	37	34	25	13	4
30-60%	27	21	20	17	17	17	15	15	14	14	11	6	1
> 60%	11	9	9	9	8	8	6	6	6	5	4	2	1

Patient Survival



Number at Risk

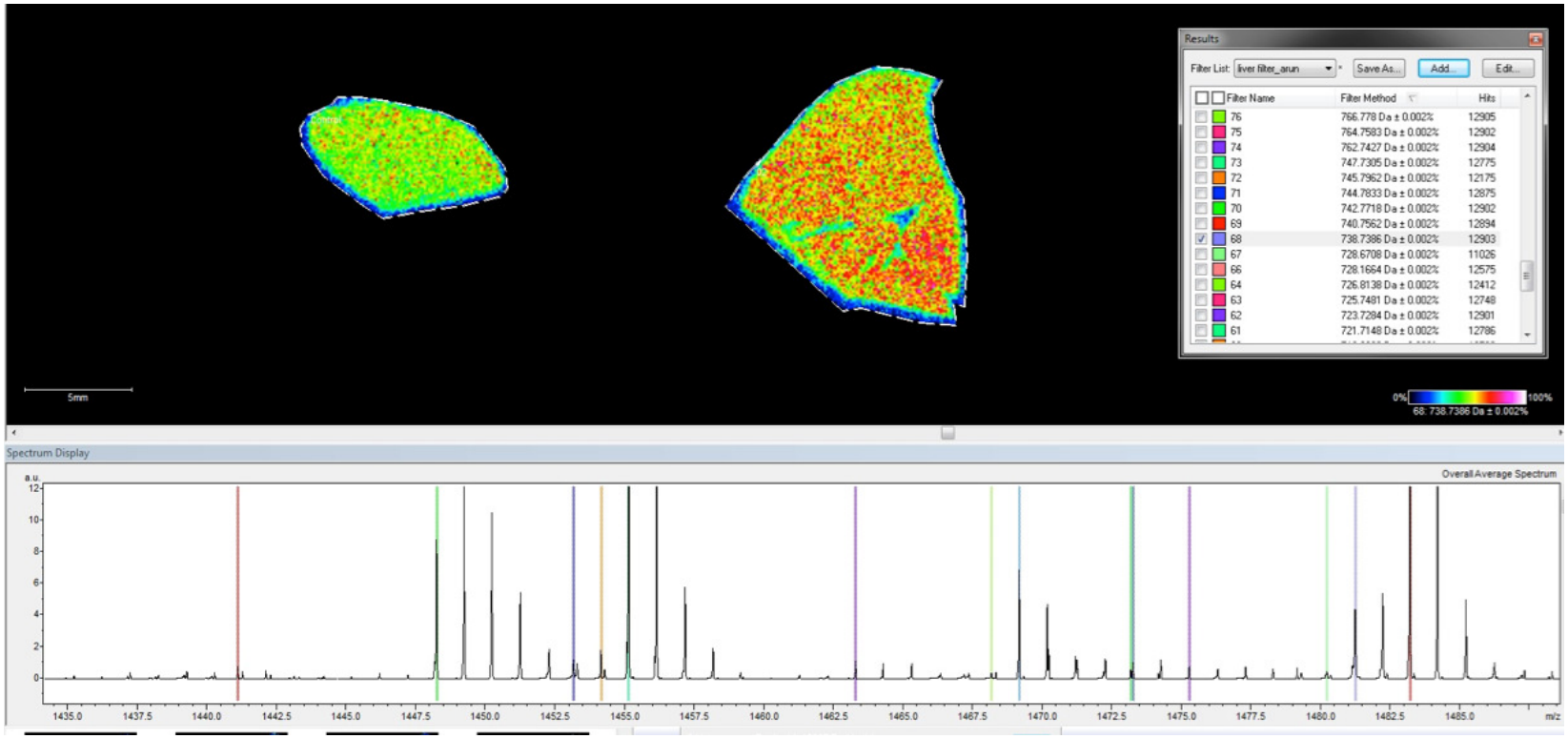
< 30%	78	60	58	55	50	45	43	42	40	37	28	15	4
30-60%	27	23	22	19	19	19	17	16	15	15	12	6	1
> 60%	11	9	9	9	8	8	6	6	6	5	4	2	1

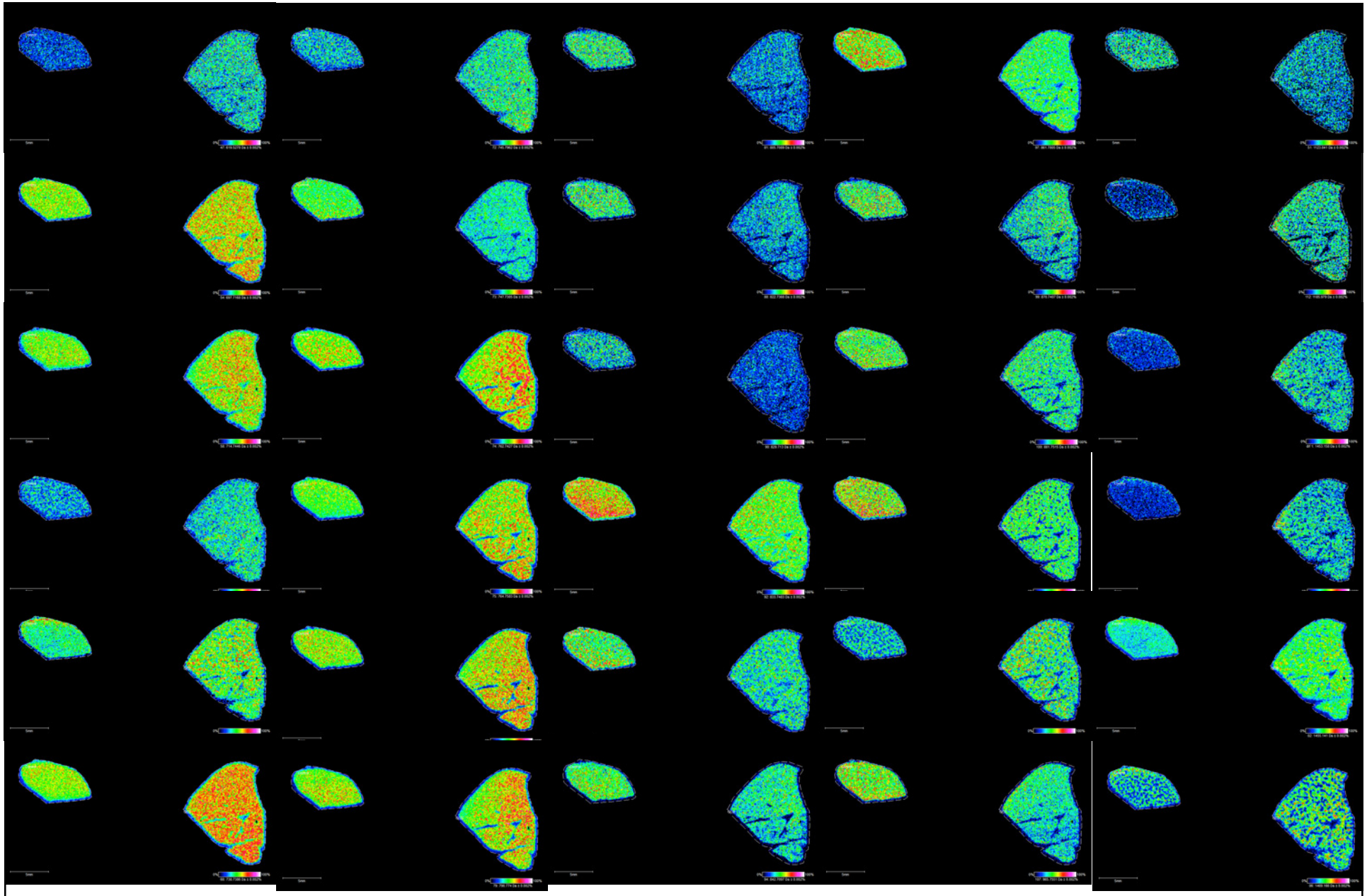
Conclusion

- The results of this study suggest that by minimizing other donor factors that determine marginality using a specific algorithm, moderate to severe steatotic livers can be successfully transplanted with similar short and long term patient and graft survival compared to non-steatotic livers

Future Areas of Investigation to Aid in the use of Steatotic Livers

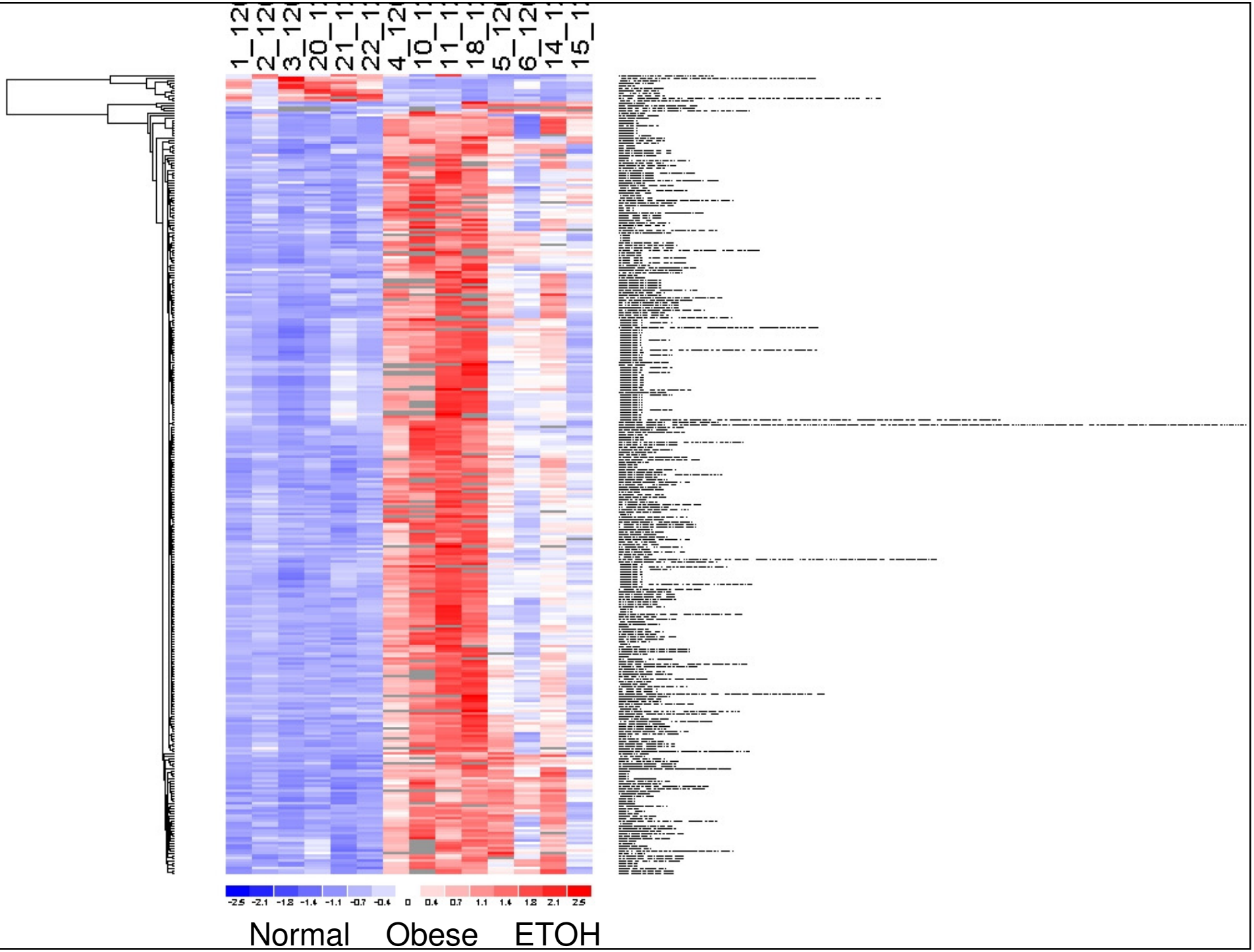
Lipidomics Analysis



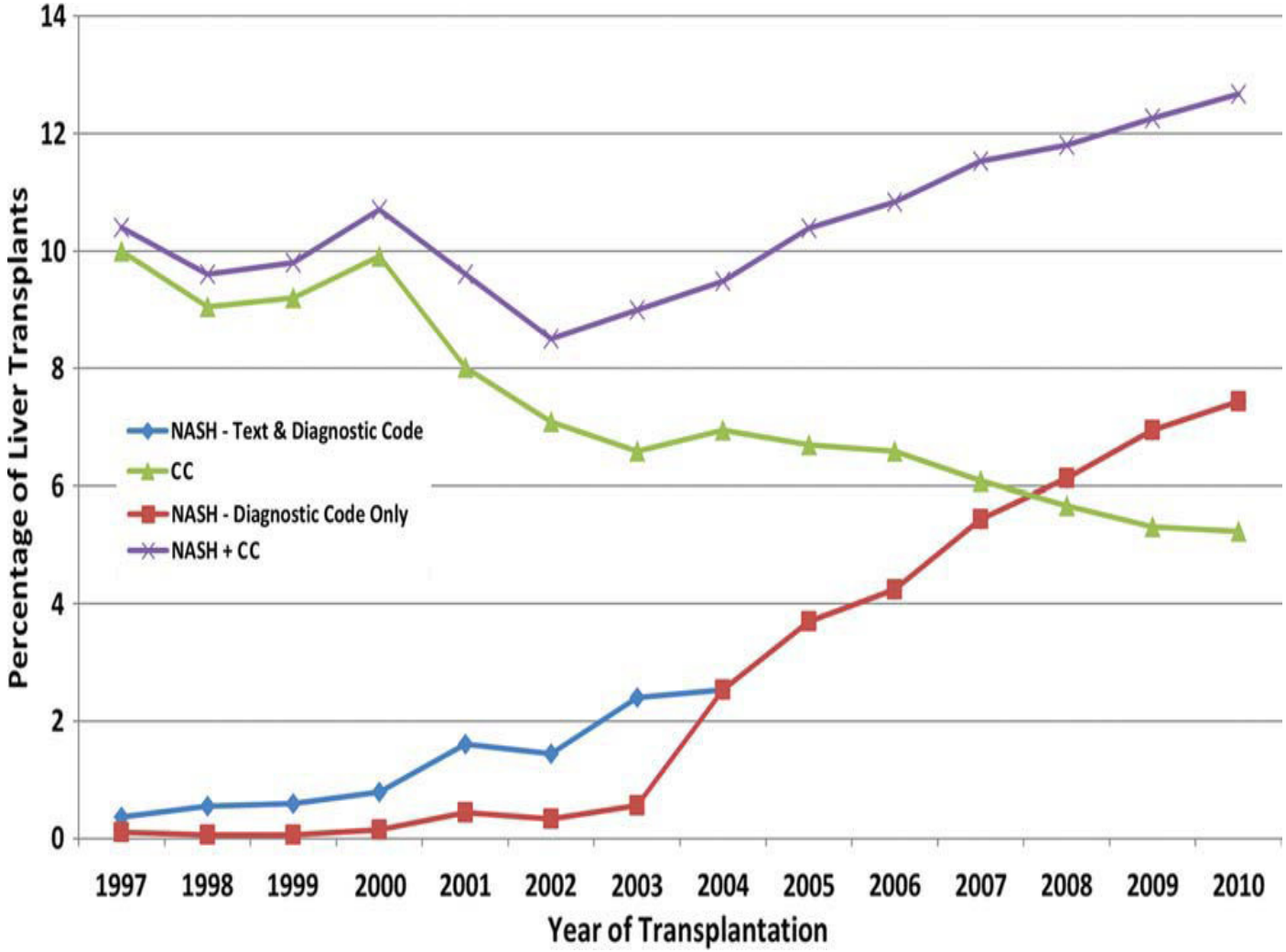


Lipidomics Analysis

PA(30:0) or LPG(26:2) or LPI(20:4)	619.5278 m/z	PE(36:1)	744.7833 m/z	PS(36:0) or PE(40:6)	790.774 m/z	PA(46:4) or PG(O-42:6) or PG(P-42:5) or PI(34:1)	835.7659 m/z	PA(P-52:1) or PG(46:4) or PA(52:9) or PI(40:6)	909.7811 m/z		1453.158 m/z
PA(36:3)	697.7168 m/z	PA(O-40:0)	745.7962 m/z	PA(O-44:0) or PG(38:2) or PA(44:7) or PI(32:4)	801.6818 m/z	PE(O-44:1) or PE(P-44:0) or PS(40:2) or PE44:8)	842.7097 m/z	PA(O-52:0) or PG(46:2) or PA(52:7) or PI(40:4)	913.8129 m/z	CL(72:5)	1454.169 m/z
PE(34:2)	714.7446 m/z	PG(34:1) or PA(40:6)	747.7305 m/z	PG(38:0) or PA(44:5) or PG(P-40:60 or PI(32:2)	805.7089 m/z	PA(O-48:0) or PG(42:2) or PA(48:7) or PI(36:4)	857.756 m/z	PG(50:4) or PA(56:9) or PI(44:6) or PIP(38:4)	965.7551 m/z		1455.141 m/z
PE(34:1)	716.7562 m/z	PS(34:0) or PE(38:6)	762.7427 m/z	PE(42:4) or PS(O-40:5) or PS(P-40:4) or SHexCer(t36:1)	822.7368 m/z	PG(42:0) or PA(48:5) or PG(P-44:6) or PI(36:2)	861.7805 m/z	SHexCer(d50:0) or PS(52:5) or PE(56:11)	1004.825 m/z	Cardiolipin 22:6/16:1	1469.166 m/z
PS(32:3)	728.1664 m/z	PE(38:5)	764.7583 m/z	PA(O-46:0) or PG(40:2) or PA(46:7) or PI(34:4)	829.713 m/z	PE(P-46:1) or PE(O-46:1) or PS(42:2) or PE(46:8)	870.7407 m/z	PG(62:9) or PIP(O-50:2) or PIP(P-50:1) or PI(56:11) or PA(66:0) or PIP(50:9)	1123.841 m/z		
PE(36:4)	738.7386 m/z	PE(O-40:0) or PS (36:1) or PE(40:7)	788.7604 m/z	PG(40:0) or PA(46:5) or PG(P-42:6) or PI(34:2)	833.7483 m/z	PA(O-50:2) or PA(P-50:1) or PG(44:4) or PA(50:9) o PI(38:6)	881.7515 m/z	PG(66:6) or PI(60:8) or PIP(54:6)	1185.979 m/z		


1_12
2_12
3_12
20_1
21_1
22_1
4_12
10_1
11_1
18_1
5_12
6_12
14_1
15_1
-2.5 -2.1 -1.8 -1.4 -1.1 -0.7 -0.4 0 0.4 0.7 1.1 1.4 1.8 2.1 2.5
Normal
Obese
ETOH

NASH Transplant Incidences



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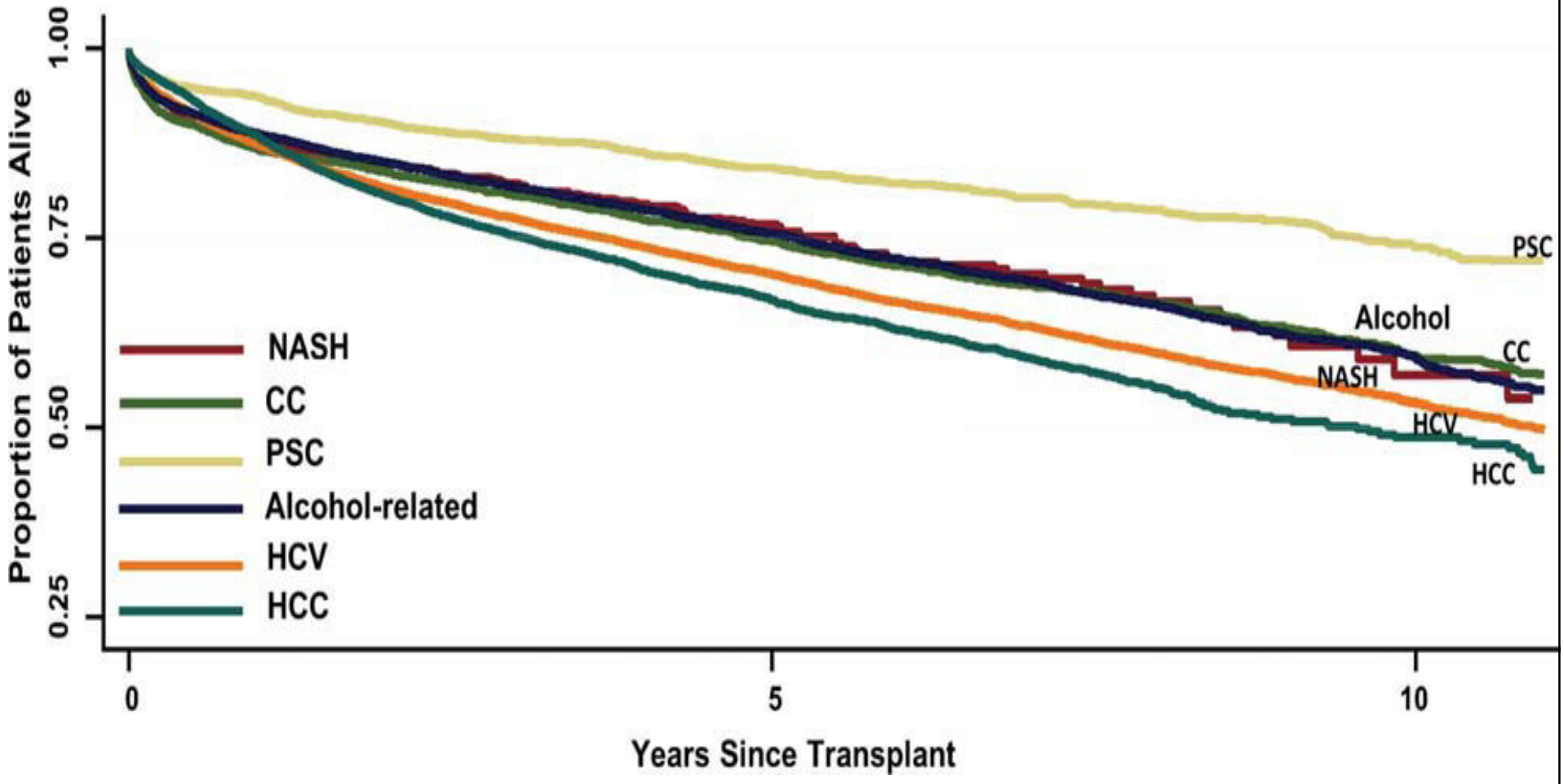
Kaplan-Meier Curves of Post Transplant Survival for Recipients with NASH or other Select Causes of Liver Disease from1997 to 2010



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Assessment of Liver Function

- "The Transplant surgeon still has to rely on a subjective interpretation of data and the macroscopic and microscopic appearance of the liver to decide whether to use the graft."

- "This decision will only be proved to have been right if the liver is resected safely or transplanted and the patient safely discharged from the hospital."

Melendez et al. Transplantation 70: 4, 2000.

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- Transplant Coordinators / Program Assistants
- Transplant Social work
- Transplant Dieticians
- Transplant Perfusion
- Interventional Radiology

Questions?

Alterations in UCP2 levels and mitochondrial function in ob/ob mice