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Menezes, Colin Nigel. (2006). The role of increased gastrointestinal alcohol production in patients with the metabolic syndrome: Implications for the pathogenesis of non-alcoholic fatty liver disease. [Master's dissertation, University of the Witwatersrand, Johannesburg]. WIReDSpace. http://hdl.handle.net/10539/2048

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THE ROLE OF INCREASED GASTROINTESTINAL ALCOHOL PRODUCTION IN PATIENTS WITH THE METABOLIC SYNDROME: IMPLICATIONS FOR THE PATHOGENESIS OF NON-ALCOHOLIC FATTY LIVER DISEASE.

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Student No: 0101826W, Staff No: 00300045

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the Degree of Master of Medicine in Internal Medicine.

Johannesburg 2006

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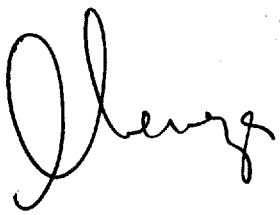
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DECLARATION

I declare that this dissertation is my own work. It is being submitted for the degree of Master of Medicine, in the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted for any degree or examination in any other University.



.....

Colin Nigel Menezes

.....day of 2006

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease with hepatic histology that resembles alcoholic liver disease. It is a frequent cause of chronic liver disease and is attracting increasing scientific attention worldwide. I explored the possibility that increased gastrointestinal alcohol production may have a role as a “second hit” in the pathogenesis of NAFLD in study subjects with the metabolic syndrome. In an attempt to investigate this hypothesis, this study looked at blood, urine and breath levels of alcohol in patients with the metabolic syndrome versus matched age and ethnic group healthy controls. Of the twenty study subjects, 80% had dyslipidaemia, 60% had hypertension and 70% had type 2 diabetes mellitus. Their mean BMI was 35.1±8.2 kg/m² (mean ± SD, $P < 0.0001$ versus controls). The serum aminotransferases were significantly elevated in the study subjects, their ALT levels being 57.4±44.79 U/L versus 17.4±4.60 U/L in the controls (95% CI 18.02 – 61.42, $P < 0.001$), and their AST levels 52.5±36.21 U/L versus 23.4±4.86 U/L in the controls (95% CI 11.99 – 46.20, $P < 0.01$). Seventy five percent of the study group had sonar features suggestive of fatty liver disease. Two adipocytokines, adiponectin and leptin, mediators of insulin resistance, an important factor in the

development and progression of NAFLD, were also measured. Adiponectin levels were significantly lower (6875 ng/L versus 15475 ng/L; median value, $P < 0.01$), and leptin concentration levels significantly higher (13.56 ng/L versus 3.05 ng/L; median value, $P < 0.05$) in the study subjects than in the control group.

Alcohol was detected in 60% of the study subjects, of which 35% tested positive for ethanol, 55% tested positive for methanol, and 30% tested positive for both ethanol and methanol. This was a statistically significant result, as none of the control group tested positive for any of the alcohols. The ethanol concentration in the study subjects' blood was 7.14±3.28 mg% (mean ± SD), in their urine 3.71± 12.87 mg% (mean ± SD) whilst none was detected in their breath. The methanol concentration in the study subjects' blood was 16.17±17.95 mg% (mean ± SD), in their urine 6.8± 13.58 mg% (mean ± SD) while their breath level was 2.05±3.19 mg (mean ± SD).

This study therefore suggests that endogenous alcohol production may be indeed be involved in the pathogenesis of NAFLD in subjects with the metabolic syndrome. Not only ethanol but also methanol was detected in the subjects tested. Endogenous alcohol may therefore be responsible for the 'second hit' theory in the pathogenesis of NAFLD, and it is likely that

formaldehyde, the metabolite of methanol may be a more potent toxin of hepatocyte injury as opposed to acetaldehyde, the metabolite of ethanol. The most likely source of the alcohol is from intestinal bacterial flora. These findings provide further insight into the pathogenesis of NALFD, suggesting other therapeutic alternatives such as the use of antibiotics and probiotics as a potential treatment strategy for NAFLD.

ACKNOWLEDGEMENTS

I wish to acknowledge the following people with heartfelt gratitude:

- My supervisors, Professor Derick Raal, Professor Ernie Song and Mr Ronnie Immelman for their invaluable assistance, guidance and support throughout the duration of my research.
- Sister Nancy Holden and Sister Nomsa Ramela of the Day Ward for their assistance with the collection of some specimens.
- The Research Incentive Fund and the Division of Endocrinology and Metabolism for the funding of this project.
- To all my loved ones, especially my parents, thank you for everything!

LIST OF ABBREVIATIONS

ADH	Alcohol dehydrogenase
ADIPO	Adiponectin
ALDH	Aldehyde dehydrogenase
ALT	Alanine aminotranferase
AST	Aspartate aminotransferase
ATP III	Adult Treatment Panel III
BL	Blood
BMI	Body Mass Index
BRE	Breath
CVD	Cardiovascular Disease
ETH	Ethanol
HbA1C	Glycated Haemoglobin
HDL-C	High Density Lipoprotein Cholesterol
hs-CRP	highly sensitive C-Reactive Protein
IDF	International Diabetes Federation
LDL-C	Low Density Lipoprotein Cholesterol
LEPT	Leptin

MET	Methanol
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NEFA	Non Esterified Fatty Acids
NIDDM	Non Insulin Dependent Diabetes Mellitus
PAI-1	Plasminogen activator inhibitor-1
TG	Triglycerides
WC	Waist circumference

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APPENDIX 1: CONTROL GROUP

PT NO.	AGE	SEX	RACE	WC	BMI	TC	TG	HDL	ALT	AST	hs-CRP	NEFA	ADIPO	LEPT
	yrs			cm	kg/m²	mmol/L	mmol/L	mmol/L	U/L	U/L	mg/L	µmol/L	ng/mL	ng/mL
1	25	male	White	87	24.5	3.7	0.3	1.4	16	22	3.34	158.0	4750	0
2	22	female	White	80	20.4	4.5	1	1.4	13	25	0.95	434.0	10960	2.02
3	43	female	Indian	76	23.9	6.2	2.2	1.6	15	18	0.29	258.0	16670	18.06
4	38	female	White	70	20.4	4.7	0.6	2.1	18	21	1.83	642.0	22070	4.48
5	30	male	White	92	24.5	4.1	0.8	1.1	23	22	0.61	365.0	7070	1.12
6	44	female	White	80	20.7	7.4	1	2.5	22	28	0.33	489.0	17180	2.78
7	37	male	White	87	20.6	5.4	1.4	1.3	21	24	2.93	383.0	14280	4.04
8	57	female	White	77	23.69	6.8	1	2.1	16	32	0.69	445.0	18710	1.22
9	31	male	White	81	22.78	5.8	1.3	1.4	15	26	0.23	460.0	9670	0.28
10	51	female	Colour	88	25	4.3	1.1	1.4	10	14	2.84	694.0	9930	6.52
11	39	male	Indian	94	20.6	4.7	0.5	1.4	28	28	0.39	350.0	9570	1.32
12	51	male	White	93	26	4.6	1.8	0.8	19	19	0.7	407.0	6990	1.68
13	53	female	White	72.5	24.26	5.4	1.1	1.3	25	20	0.17	663.0	8950	27.48
14	55	female	White	71	22.78	7	1.2	2.2	16	25	0.82	399.0	17220	1.84
15	77	female	Indian	93	24.5	6.5	2.8	1.3	15	30	4.85	348.0	14260	35.32
16	55	female	Black	77	26.7	4	0.8	1.6	17	22	2.62	293.0	18560	14.14
17	50	female	Black	73	23.72	4.4	0.8	1.3	22	26	1.56	182.0	22940	18.44
18	70	male	Indian	71	23.52	4.9	0.8	1.6	15	30	0.31	721.0	17120	1.36
19	79	female	White	83	20.6	6.4	1	2.2	11	16	16	615.0	25900	11.52
20	66	male	White	97.5	27	4.7	0.7	1.7	17	20	0.9	352.0	95200	3.32

APPENDIX 2: STUDY GROUP WITH FEATURES OF THE METABOLIC SYNDROME

PT NO.	AGE(YRS)	GENDER	RACE	WC(CMS)	BMI	T2DM	HPT	DYS
1	61	female	Chinese	90	30.71	No	Yes	Yes
2	30	male	White	99	29.34	No	No	Yes
3	65	male	White	110	27.7	No	No	Yes
4	60	female	White	101	28.01	No	Yes	Yes
5	76	female	White	96	22.7	IFG	No	Yes
6	65	male	Indian	101	25.22	Yes	Yes	No
7	35	male	White	130	42.7	Yes	Yes	Yes
8	56	female	White	112	33.01	Yes	No	Yes
9	67	female	Indian	112	33.8	Yes	Yes	No
10	40	male	Indian	108	32.35	Yes	No	Yes
11	52	female	Black	110	44.14	Yes	Yes	Yes
12	36	female	White	97	28	Yes	No	Yes
13	60	female	Black	116	47.7	Yes	Yes	Yes
14	51	male	White	94	27.4	No	No	Yes
15	25	male	White	109	33.4	Yes	No	No
16	22	female	White	115	41.5	No	Yes	Yes
17	45	female	Indian	115	41.6	Yes	Yes	Yes
18	45	female	White	123	39.8	Yes	Yes	Yes
19	32	male	White	157	52.3	Yes	Yes	Yes
20	46	female	Coloured	115	40.4	Yes	Yes	No

APPENDIX 3: STUDY GROUP-SONAR RESULTS AND BIOCHEMICAL MEASUREMENTS

PT NO.	AGE	GENDER	SONAR	TC	TG	HDL	ALT	AST	hs-CRP	NEFA	ADIPO	LEPT
	yrs			mmol/L	mmol/L	mmol/L	U/L	U/L	mg/L	µmol/L	ng/mL	ng/mL
1	61	female	Yes	6	2.8	1.4	42	29	2.05	213.0	6680	10.96
2	30	male	Yes	6.5	2.9	0.9	37	22	2.92	578.0	7070	0
3	65	male	Yes	3.41	0.81	1.21	38	38	2.43	799.0	8210	8.64
4	60	female	Yes	4.69	2.66	1.06	88	168	10.69	127.8	15510	48.92
5	76	female	Yes	5.1	3.05	1.58	45	46	1.14	696.0	4760	10.4
6	65	male	Yes	3.5	1.2	0.9	24	33	5.68	106.2	26500	16.16
7	35	male	Yes	3.5	5.1	0.6	61	39	7.19	128.8	1090	3.24
8	56	female	Yes	3.8	1.5	1.1	16	20	13.59	492.0	9160	2.16
9	67	female	Yes	3.5	0.8	1.8	29	28	8.02	651.0	5350	73.48
10	40	male	Yes	5.7	2	1.1	71	56	0.36	708.0	4690	26.84
11	52	female	No	4.2	1.3	1.7	16	20	141.8	154.7	29600	7.2
12	36	female	Yes	5.1	1.4	1.3	62	96	10.25	484.0	22800	18.75
13	60	female	No	6.2	4.9	1	15	21	23.9	196.6	9760	25.8
14	51	male	Yes	3.9	1.3	1.5	102	92	0.67	504.0	4200	0
15	25	male	No	6.6	2	1	204	83	0.46	110.2	8200	5.76
16	22	female	Yes	5.5	2.4	0.9	100	64	2.74	539.0	3050	37.74
17	45	female	No	5.1	2.2	1.4	59	63	28.35	378.0	19770	76.08
18	45	female	No	8.3	19.1	1.2	*	60	21.64	134.5	3440	0.68
19	32	male	Yes	18.4	84	2.1	21	23	0.76	126.7	3220	20.8
20	46	female	Yes	5.3	1.9	0.9	61	49	26	0.373	6250	23.48

APPENDIX 4: STUDY GROUP-ALCOHOL LEVELS

PT NO.	AGE	SEX	WC	BMI	ETH BL	ETH UR	ETH BRE	MET BL	MET UR	MET BRE
	yrs		cm	kg/m²	mg%	mg%	mg%	mg%	mg%	mg%
1	61	female	90	30.71	neg	neg	neg	neg	neg	neg
2	30	male	99	29.34	neg	neg	neg	neg	neg	neg
3	65	male	110	27.7	9	neg	neg	2	1.6	2.4
4	60	female	101	28.01	2	neg	neg	1.8	neg	neg
5	76	female	96	22.7	neg	neg	neg	neg	neg	neg
6	65	male	101	25.22	neg	neg	neg	50	neg	neg
7	35	male	130	42.7	8	44.6	neg	29	32.3	neg
8	56	female	112	33.01	8	neg	neg	2	neg	8
9	67	female	112	33.8	10	neg	neg	neg	neg	neg
10	40	male	108	32.35	10	neg	neg	21	neg	8
11	52	female	110	44.14	neg	neg	neg	7.1	neg	neg
12	36	female	97	28	neg	neg	neg	3	neg	neg
13	60	female	116	47.7	3	neg	neg	9	38	1
14	51	male	94	27.4	neg	neg	neg	7	neg	neg
15	25	male	109	33.4	neg	neg	neg	46	9.7	5.3
16	22	female	115	41.5	neg	neg	neg	neg	neg	neg
17	45	female	115	41.6	neg	neg	neg	neg	neg	neg
18	45	female	123	39.8	neg	neg	neg	neg	neg	neg
19	32	male	157	52.3	neg	neg	neg	neg	neg	neg
20	46	female	115	40.4	neg	neg	neg	neg	neg	neg

APPENDIX 5: DATA COLLECTION FORM

A STUDY ON THE ROLE OF INCREASED GASTROINTESTINAL ETHANOL PRODUCTION IN OBESE PATIENTS: IMPLICATIONS FOR THE PATHOGENESIS OF NON-ALCOHOLIC FATTY LIVER DISEASE.

Identification: (hospital sticker may be attached):

1) Clinic:…… ……

2) Date of birth: ……

3) Gender: ……

4) Ethnic group:……..

5) Hospital No:……

6) Contact Nos:……

Anthropometric measurements:

1) Weight:…….

2) Height:…….

3) Waist circumference:…….

4) BMI:…… Case < 25……. Control > 27…….

History:

1) Alcohol use (<72hrs): Yes…….. No…….

2) Smoking: Yes…….. No……..

3) Type 2 Diabetic: Yes…… No……
When diagnosed:……

4) Hypertension: Yes……. No…….
BP:……..

5) Other medical problems:

……
……
……
……
……
……

6) Treatment:

a) Oral:……
……..
……
b) Insulin:……
……..
……..
c) Others:……
……..
……..
……..
……..
……..
……..

7) Complications:

a) Microvascular:
   i) Retinopathy:……
   ii) Nephropathy: Microalbuminuria; Yes…… No……
   Proteinuria; Yes…… No……
   iii) Neuropathy:……

b) Macrovascular:
   i) CVA:……..
   ii) CAD:……..
   iii) PVD:……..

Lab results:

1) Lipid profile:
   a) Total cholesterol:……
   b) Triglycerides:……
   c) HDL:……
   d) LDL:……

2) Glycaemic levels:
   a) Fasting glucose:……. Yes…… No……
   b) HbA1C:……..

3) LFTS:
   a) ALT:…….
   b) AST:…….
   c) Alkaline phosphatase:…….
   d) GGT:…….

4) Iron studies:
   a) Ferritin levels:…….

5) Ethanol levels:
   a) Blood:……...
   b) Urine:…….
   c) Breath:…….

6) Spare tubes X2:……..

Ultrasound results:

APPENDIX 6:

SUBJECT INFORMATION SHEET AND INFORMED CONSENT FORM

Introduction:

Good day, my name is Dr Colin Menezes. I am a medical registrar at the Johannesburg General Hospital, in the department of Internal Medicine.

I would like to ask you to consider participating in a research study entitled "The role of increased gastrointestinal alcohol production in obese patients: implications for the pathogenesis of non-alcoholic fatty liver disease."

It is important that you read and understand the purpose of this study, procedures, benefits, risks and discomforts.

This information leaflet is to help you to decide if you would like to participate. If you have any questions, do not hesitate to ask me. It is important that you are truthful with me regarding your health history.

If you decide to take part, you will be asked to sign this document to confirm that you understand the study.

Purpose of the study:

Obesity is a chronic condition characterised by an excess of body fat. It is a health hazard because it is associated with numerous metabolic complications such high cholesterol, diabetes, heart disease and liver disease. The purpose of this study is to look at one possible cause of liver disease.

Procedures:

If you agree to take part, you will be asked a few questions, and examined to see if you qualify for this study. Your qualification will depend on your body mass index and medical history. You will be asked to sign the informed consent form. You may require an ultrasound. This is a painless procedure which will not cause you any harm. You will also have some blood drawn which will be about approximately 4 teaspoons and you will also have to provide a urine and breath sample.

Risks:

Drawing blood is normally done as a part of routine medical care and may present with a slight risk of discomfort. It may result in faintness, inflammation of the vein, pain, bruising or bleeding at the puncture site.

Benefits:

Your participation will contribute to a better understanding of this disease that may help identify future therapies.

Your rights:

Your participation is voluntary, and refusal to participate will result in no penalty or affect your access to medical care.

Contact:

If you have any questions, please do not hesitate to contact me on 083 995 3931 or Prof Raal at 011 488 3538 or Prof Song at 011 488 3627.

Confidentiality:

All information obtained in this study, including your hospital records, personal data and research data will be kept strictly confidential. Data that may be reported in scientific journals will not include any information that identifies you as a participant in this study.

Informed consent:

I, ……the undersigned hereby consent to the participation in this study, the nature, conduct, benefits and risks of which have been explained to me and which I understand, on myself/ my spouse/ the patient……
I have also read received, read and understand the above information regarding the clinical study.
Signature of the patient/ spouse/ near relative:

……..

Witness 1……

2……

Date ……

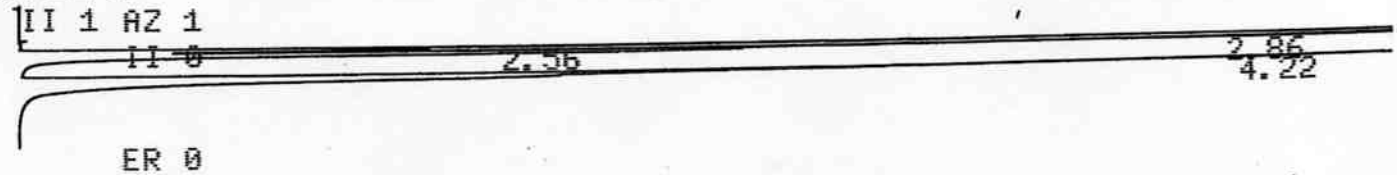
Time……

23.1.04 Pathinath Sharma

Std 0.1% each of CH_3OH, C_2H_5OH, C_3H_7OH - Int. Std.

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CHANNEL A INJECT 01/23/04 10:47:03



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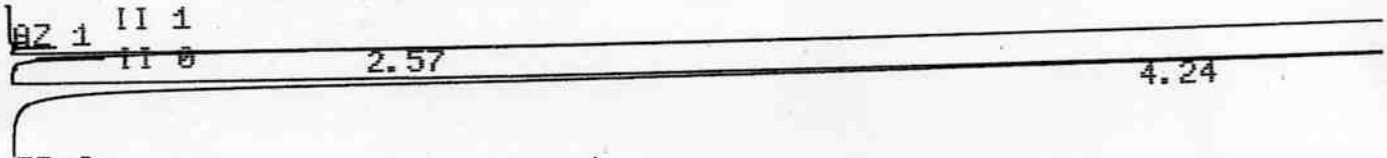
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2	33.825	2.86	38082 01	← C_2H_5OH
3	46.495	4.22	52347 01	← C_3H_7OH
TOTAL	100.		112587	

#2 CH_3

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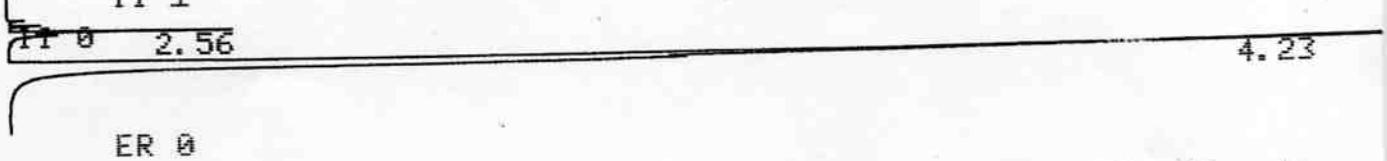
ER 0

ALCOHOL 01/23/04 11:12:12 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 12 INDEX 12

PEAK#	AREA%	RT	AREA BC	
1	13.095	2.57	18062 01	← CH_3OH
2	86.905	4.24	119871 01	← C_3H_7OH
TOTAL	100.		137933	

CHANNEL A INJECT 01/23/04 11:22:58



ER 0

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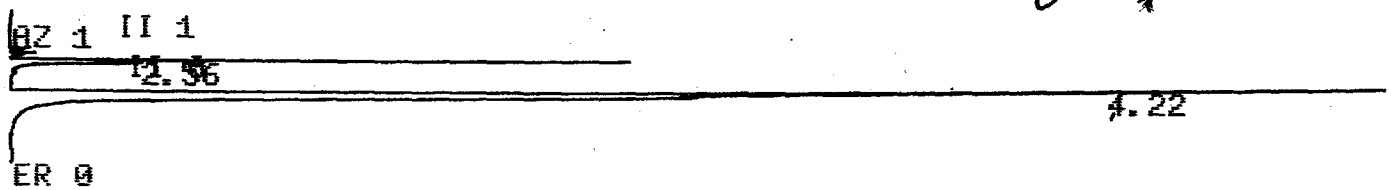
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PEAK#	AREA%	RT	AREA BC
1	2.476	2.56	1989 01

LE 1. METHOD 0. RUN 19 INDEX 19

AK#	AREA%	RT	AREA	BC
1	100.	4.29	38968	01
TAL	100.		38968	

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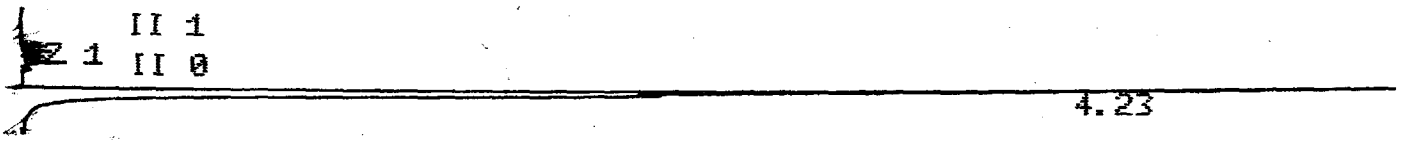


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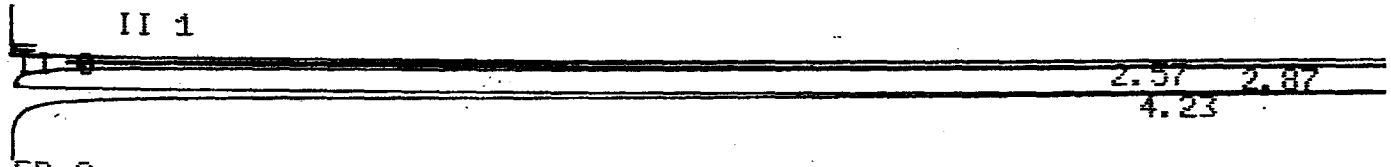


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LE 1. METHOD 0. RUN 21 INDEX 21

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TAL	100.		41337	

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3	43.689	4.23	139758	01
TAL	100.		319891	

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Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Menezes

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M03-09-25

PROJECT The Role of Increases Gastrointestinal Ethanol Production in Obese Patients: Implications for the Pathogenesis of Non-Alcoholic Fatty Liver Disease

INVESTIGATORS Dr CN Menezes

DEPARTMENT School of Clinical Medicine, Johannesburg hospital

DATE CONSIDERED 03-09-26

DECISION OF THE COMMITTEE Approved unconditionally

Unless otherwise specified the ethical clearance is valid for 5 years but may be renewed upon application
This ethical clearance will expire on 1 January 2008.

DATE 03-10-13 CHAIRMAN.....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof FJ Raal
Dept of School of Clinical Medicine, Johannesburg Hospital
Works2\lain0015\HumEth97.wdb\M 03-09-25

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress form. I/we agree to inform the Committee once the study is completed.

DATE 15/10/03 SIGNATURE .....

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 9: NORMAL BIOCHEMICAL VALUES [50, 76]

BIOCHEMISTRY	NORMAL VALUES
Total Cholesterol (mmol/L)	≤5
Triglycerides (mmol/L)	≤1.5
HDL-C (mmol/L)	≥1.2
LDL-C (mmol/L)	≤3.0
Alanine Aminotransferase (U/L)	5-40
Aspartate Aminotransferase (U/L)	5-40
hs-CRP (mg/L)	< 0.5

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CHAPTER 1

LITERATURE REVIEW

1.1 NON ALCOHOLIC FATTY LIVER DISEASE

1.1.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is an increasingly recognized as one of the commonest causes of chronic liver disease worldwide that may progress to end stage liver disease. The pathological picture resembles that of alcohol induced liver disease even though alcohol is not abused.[1, 2] Risk factors for NAFLD include obesity, type 2 diabetes, hyperlipidaemia, total parenteral nutrition, jejuno-ileal bypass surgery and the use of certain drugs.[3] A variety of other terms have been used to describe this entity such as fatty liver disease, diabetic hepatitis, alcohol like liver disease, and hepatic steatosis, a more serious subset of NAFLD.[3, 4]

1.1.2 Definition

NAFLD comprises a spectrum of liver disease, ranging from simple steatosis to steatohepatitis, advanced fibrosis and the potential to

progress to cirrhosis with its inherent complications of hepatic failure and hepatocellular carcinoma.[3, 4] Non-alcoholic steatohepatitis or NASH represents a stage within the spectrum of NAFLD and is defined pathologically by the presence of steatosis together with necro-inflammatory activity.[5]

1.1.3 Epidemiology

NAFLD affects 10% to 24% of the general population in various countries.[4] This prevalence increases to 57.5%[6] to 74%[7, 8] in obese persons. It is a common explanation for abnormal liver function tests in blood donors and a cause of asymptomatic elevation of liver enzymes in up to 90% of cases once other causes of liver diseases are excluded.[9] With the increasing prevalence of obesity, it is estimated that at least about a quarter of the American adult population currently has NAFLD. Approximately 20% to 30% of patients with NAFLD will develop fibrosing steatohepatitis that may progress to cirrhosis, end-stage liver disease, and hepatocellular carcinoma.[10] The prevalence of NAFLD in the different population groups of South Africa is unknown.

1.1.4 Characteristics

a) Clinical features

Patients with NAFLD are generally asymptomatic.[3, 4] It is often diagnosed after abnormalities are noted during “routine “laboratory testing.[11] Patients may report fatigue or malaise and a sensation of fullness or discomfort on the right side of the upper abdomen.[3, 4] Hepatomegaly may be the only physical sign.[3, 4] Acanthosis nigricans may be found in children with NAFLD.[12, 13] Other signs may include signs of chronic liver disease such gynaecomastia, spider naevi, testicular atrophy and palmar erythema.

b) Laboratory Abnormalities

Elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) or both are a common laboratory finding in patients with NAFLD. The ratio of AST to ALT in subjects with NAFLD is usually less than 1, but this ratio increases as fibrosis advances, leading to a loss of its diagnostic accuracy in patients with cirrhotic NAFLD.[14]

Serum alkaline phosphatase, gamma glutamyltransferase or both are often above the normal range in many patients.[15, 16, 17] Other laboratory abnormalities include hypoalbuminaemia, a prolonged prothrombin time, and hyperbilirubinaemia, but this is usually only found in end stage liver disease.[4]

c) Imaging Studies

Fatty infiltration of the liver produces a diffuse increase in echogenicity as compared with that of the kidneys on ultrasonography.[4] Regardless of the cause, cirrhosis has a similar appearance on ultrasonography.[4] Ultrasonography has a sensitivity of 89%, and a specificity of 93% in detecting steatosis. It has a sensitivity of 77% and a specificity of 89% in detecting fibrosis.[18] Fatty infiltration of the liver produces a low density hepatic parenchyma on computed tomographic scanning. Steatosis is generally diffuse however, occasionally it may be focal, and hence may be misinterpreted as showing malignant liver masses.[19] In such cases, magnetic resonance imaging can help distinguish the two.[20]

d) Histology

The histologic features of NAFLD are indistinguishable from those of alcohol-induced liver disease.[4] This includes steatosis (fatty liver), which is predominantly macrovesicular and generally diffusely distributed throughout the liver lobule. Microvesicular steatosis has been reported occasionally. It can progress to a hepatitis (parenchymal inflammation with or without accompanying focal necrosis) where there is lobular infiltration with both acute and chronic inflammatory cells and Mallory body formation, and it may result in variable degrees of perivenular and sinusoidal fibrosis progressing to cirrhosis.[1, 3, 16, 21-24]

1.1.5 Pathogenesis of NAFLD

The pathogenesis of NAFLD has remained poorly understood since the earliest description of the disease. A growing body of literature have documented the close relationship of NAFLD with the metabolic syndrome, which is characterized by abdominal obesity, insulin resistance with or without frank hyperglycaemia, dyslipidaemia, and hypertension.[10]

However, much of the current thinking remains hypothetical, since the mechanism or mechanisms remain uncertain.[4] A current concept in the pathogenesis of NAFLD involves a “two-hit” hypothesis in which an initial metabolic disturbance, namely insulin resistance causes steatosis, and a second pathogenic stimulus causes oxidative stress, reactive oxygen species generation, lipid peroxidation, and resultant steatohepatitis.[10] Another theory put forward is that NAFLD is almost certainly a polygenic disease affected by environmental factors and stressors, with disease pathogenesis related to “multiple hits.” Although the metabolic syndrome and insulin resistance are important risk factors for the development of NAFLD, our current knowledge of the genetic, biochemical, metabolic, inflammatory, endocrine, and environmental mechanisms responsible for the pathogenesis of NAFLD are poorly understood.[10]

It is also not fully understood why simple steatosis develops in some patients, whereas steatohepatitis and progressive disease develop in others.[4]

Differences in Body Mass Index (BMI) or antioxidant systems in the context of genetic predisposition may be an explanation. Retention of

lipids within hepatocytes, mostly in the form of triglycerides is a prerequisite for the development of NAFLD.[4] The primary metabolic abnormality leading to this, is not well understood. It is thought that it may be as a result of alterations in the pathways of uptake, synthesis, degradation or secretion in hepatic lipid metabolism resulting in insulin resistance which is a reproducible factor in the development of NAFLD.[25]

The molecular pathogenesis of insulin resistance is multifactorial, and several molecular targets involved in the inhibition of insulin action have been identified (Figure 1). These include Rad (Ras associated with diabetes, an enzyme, which is a member of the Rad-guanosine triphosphatase superfamily), [26] which interferes with several essential cell functions, such as growth, differentiation, vesicular transport and signal transduction; PC-1, a membrane glycoprotein that has a role in insulin resistance, [27] reduces insulin-stimulated tyrosine kinase activity; leptin, [28] which induces dephosphorylation of insulin-receptor substrate-1; fatty acids, [29] which inhibit insulin-stimulated peripheral glucose uptake; and tumour necrosis factor α, [30] which down regulates insulin-

induced phosphorylation of insulin receptor substrate-1 and reduces the expression of the insulin-dependent glucose-transport molecule Glut 4. Insulin resistance leads to fat accumulation in hepatocytes by two mechanisms: lipolysis and hyperinsulinemia. Significant amounts of dicarboxylic acids, which are potentially cytotoxic, can be formed by microsomal ω-oxidation. This pathway of fatty acid metabolism is closely related to mitochondrial β-oxidation and peroxisomal β-oxidation. Deficiency of the enzymes of peroxisomal β-oxidation has been recognised as an important cause of microvesicular steatosis and steatohepatitis.[31] Deficiency of acyl-coenzyme A oxidase disrupts the oxidation of very-long-chain fatty acids and dicarboxylic acids, leading to extensive microvesicular steatosis and steatohepatitis. Loss of this enzyme also causes sustained hyperactivation of peroxisome-proliferator-activated receptor-α (PPAR-α), leading to transcriptional up-regulation of PPAR-α regulated genes.[31] PPAR-α has been implicated in promoting hepatic synthesis of uncoupling protein-2, which is expressed in the liver of patients with NAFLD.[32]

Increased intrahepatic levels of fatty acids provide a source of oxidative

stress, which may in large part be responsible for the progression from steatosis to steatohepatitis to cirrhosis. Mitochondria are the main cellular source of reactive oxygen species, which may trigger steatohepatitis and fibrosis by three main mechanisms, a) lipid peroxidation, b) cytokine induction and c) induction of Fas ligand. Patients with steatohepatitis have ultrastructural mitochondrial lesions, [33] that is absent in patients with simple steatosis and in healthy subjects.[34] They also slowly resynthesize ATP in vivo after a fructose challenge, which usually causes acute hepatic ATP depletion.[35] This impaired ATP recovery may reflect the mitochondrial injury found in patients with steatohepatitis.[33, 34]

Although, symptoms of liver disease rarely develop in patients with the metabolic syndrome, the steatotic may be vulnerable to further injury when challenged by additional insults. This has lead to the presumption that progression from simple steatosis to steatohepatitis and advanced fibrosis results from two distinct events.[36]Firstly, insulin resistance leads to fat accumulation within hepatocytes and secondly, mitochondrial reactive oxygen species cause lipid peroxidation, cytokine oxidation and

the induction of Fas ligand (Figure 2). Lipid peroxidation results in end-products, malondialdehyde and 4-hydroxynonenal, which have proinflammatory and profibrogenic properties, may account for the typical histological features observed in NASH.[37]The correlation between the degree of lipid peroxidation and the amount of fat in the liver also provides an explanation for the association between the severity of steatosis and the risk of NASH.[38]

In summary, the two metabolic abnormalities most strongly associated with NAFLD are insulin resistance and an increased supply of fatty acids to the liver. These advances in the understanding of the pathogenesis of NAFLD have lead to the rational design of therapy for this disorder.

Figure 1: The First Hit for NAFLD: [Adapted from Ref 4]

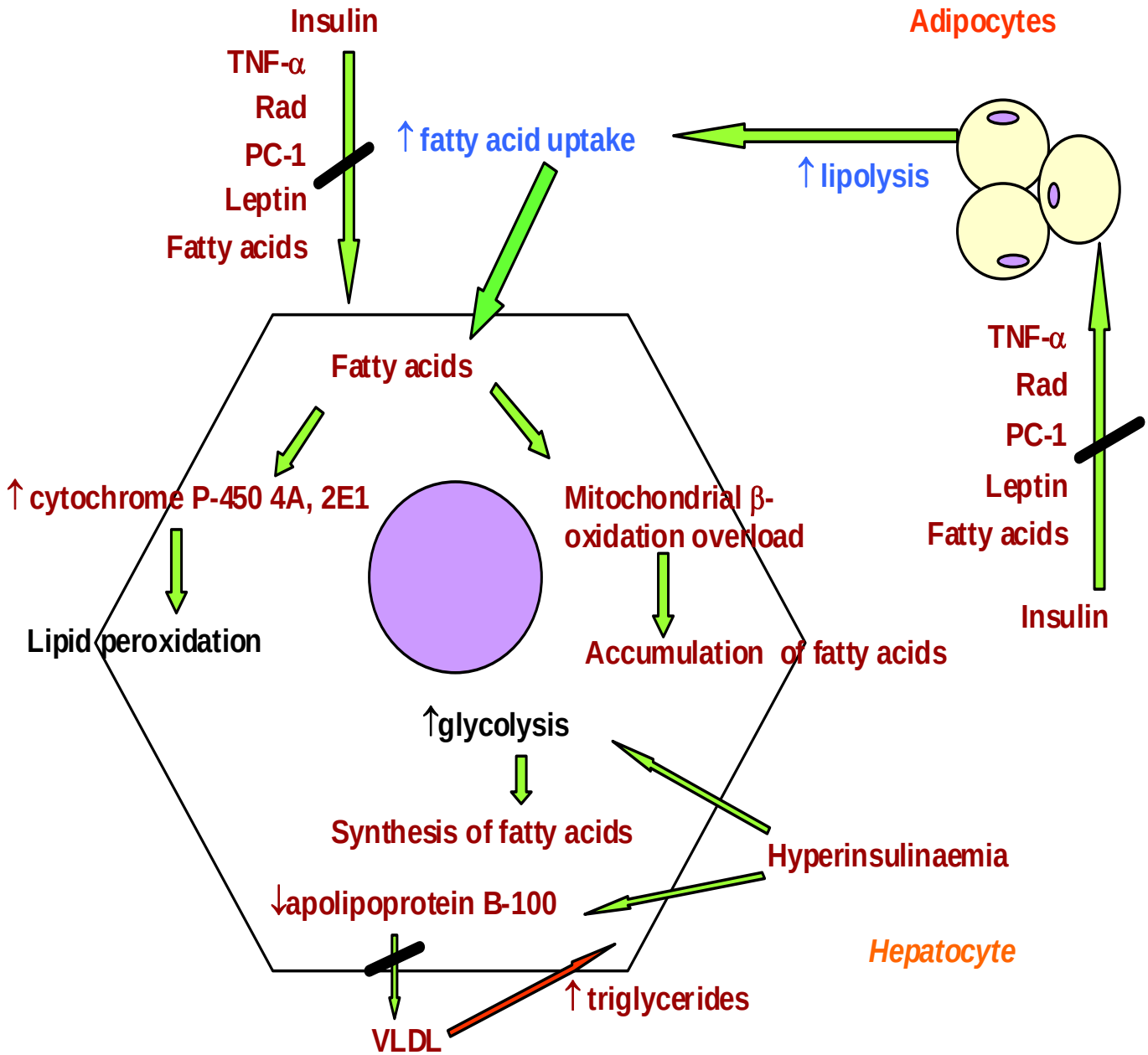
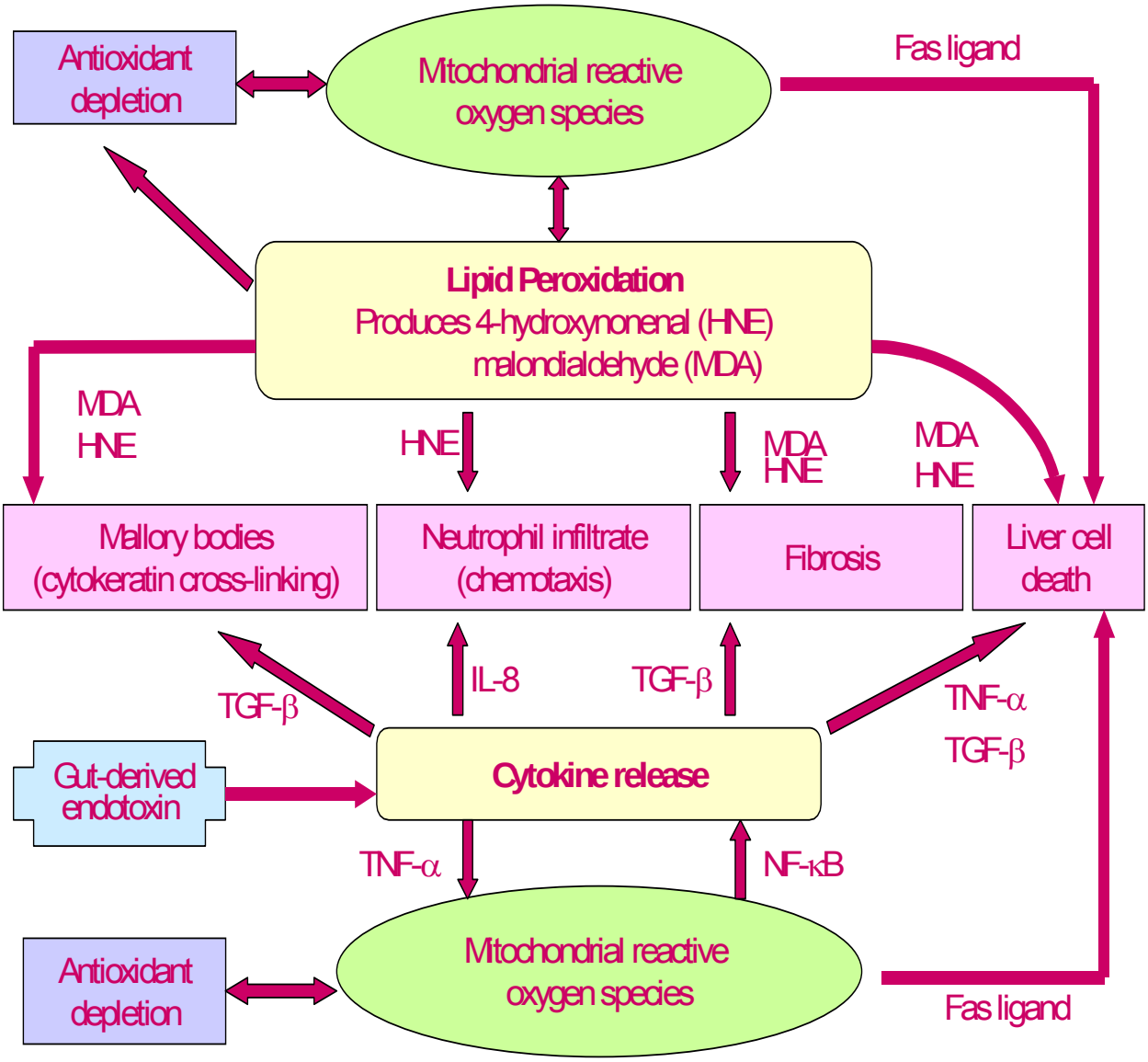


Figure 2: The Second Hit for NAFLD: [Adapted from Ref 4]



1.1.6 Diagnosis of NAFLD

NAFLD is usually suspected in persons with asymptomatic elevation of aminotranferase levels, radiologic findings of fatty liver, or unexplained persistent hepatomegaly.[4] Histology of a liver biopsy specimen is generally the gold standard. Whilst the typical features on liver biopsy are well described, given the anticipated high numbers of patients having NAFLD in the context of a high prevalence of both obesity and diabetes, it could be argued that the number of patients likely to warrant a biopsy would be enormous.[40]

However, a liver biopsy may be considered when other hepatic pathologies need to be excluded and it may also be helpful in ascertaining the extent of inflammation so as to separate simple steatosis from NASH, which may have important prognostic implications. A biopsy is also expensive and invasive, and it is associated with the risk of complications. It has therefore been argued that although there is promise of new treatments, the only effective therapy at present for the obese patient is weight loss, and why subject these patients to a liver biopsy? [40] There is also the opportunity for sampling errors. Histologic lesions of NASH are unevenly distributed throughout the liver parenchyma, therefore, sampling error of liver biopsy

can result in substantial misdiagnosis and staging inaccuracies.[41]Therefore, each case has to be assessed individually. Recently, measurements of serum concentration of adipocytokines have been used as an assessment for separating patients with bland steatosis from NASH, suggesting that liver biopsies may become unnecessary in the majority of patients with suspected NAFLD. Recent studies have found that serum levels of leptin and resistin were significantly higher in patients with NASH as compared to those with simple steatosis, whereas serum levels of adiponectin were significantly lower. This suggests that these adipocytokines may play a role in the pathogenesis of NAFLD.[42-44] Leptin is a mediator of hepatic inflammation and fibrosis in NAFLD, and resistin and adiponectin are important modulators of insulin resistance, a central factor in the pathogenesis of NAFLD.[42]

1.1.7 Role of Obesity and Alcohol

Obesity increases the risk of both fatty liver and cirrhosis in patients who consume alcohol, the mechanism of which remains obscure. The pathological picture of NASH resembles that of alcohol induced liver injury, but it occurs in patients who do not abuse alcohol as stated earlier.[45]

Obesity increases hepatic exposure to alcohol; this is because ingested alcohol accumulates in the excessive adipose tissue of obese individuals due to its lipid solubility, prolonging the alcohol exposure from social drinking.[45] Long-term alcohol intake can also alter enteric flora, resulting in the overgrowth of gram negative bacteria. Increased permeability produces endotoxaemia, which activates Kupffer cells to up-regulate lipopolysaccharide receptors and mediators such as macrophage inflammatory protein 2, prostaglandin E2, tumour necrosis factor-α, and α-hydroxyethyl. This can result in steatohepatitis. Subsequently, fibrogenesis results from the activation of hepatic stellate cells, which are responsible for the overproduction of the extracellular matrix that produces the fibrous scar.[47]

It is important to note that even small increases in gut derived ethanol might increase portal blood alcohol levels enough to induce hepatic steatosis, given evidence that alcohol produces obligatory redox changes that promote the accumulation of triglycerides in hepatocytes.[45]

Evidence has also suggested that obesity is associated with alterations in gut motility, because of decrease in sensitivity to neuropeptides such as cholecystokinin and bombesin.[48] This may favour bacterial overgrowth.

Therefore, there is a possibility that obese individuals, who do not consume alcohol, are capable of producing and metabolising alcohol endogenously, and this possibility may play a role in the pathogenesis of obesity related fatty liver disease and merits further consideration.[45]

A recent study showed that breath collected from genetically obese mice, ob/ob mice showed a higher alcohol content than their lean littermates. Hence, even in the absence of alcohol ingestion, alcohol could be detected in exhaled breath, reflecting an increase production of alcohol by intestinal bacteria and hence probably contributing to the pathogenesis of obesity related fatty liver disease.[45]

Another study in human subjects found that the breath alcohol levels in obese individuals where higher as compared to lean controls.[46]

Of note, because of efficient hepatic metabolism of alcohol, this eliminates most of the toxin before it reaches the systemic circulation, resulting in blood alcohol determinations which underestimate the actual production of alcohol in the gut.[45] Hence it is important to look at urine and breath levels of alcohol, apart from just blood levels.

1.1.8 Alcohol Metabolism and the Liver

Normal liver function is essential for life. Alcohol-induced liver damage disrupts the body's metabolism, eventually impairing the function of other organs. Most of the alcohol that a person imbibes is eventually broken down by the liver. Alcohol is readily absorbed from the gut.[49]

The metabolism of ethanol occurs in the cytosol via hepatic alcohol dehydrogenase (ADH) and certain microsomal enzymes. Alcohol cannot be stored and must be metabolised. which produces acetaldehyde, this is then rapidly biotransformed by aldehyde dehydrogenase (ALDH) in the cytosol and mitochondria to acetoacetate.[49]

These by-products are toxic to the liver and other organs. Methanol is metabolized primarily in the liver through similar sequential oxidative steps to formaldehyde, formic acid and carbon dioxide. The initial step involves oxidation to formaldehyde by hepatic ALDH. Formaldehyde is then oxidized by formaldehyde dehydrogenase to formic acid/or formate depending on the pH. In the final step, formic acid is detoxified to carbon dioxide by folate-dependent reactions.[49]

If methanol is co-ingested with a significant amount of alcohol, the

methanol conversion is temporarily blocked since ethanol has nine times greater affinity for alcohol dehydrogenase than does methanol. This allows elimination of methanol from the blood via the urine and exhaled air before conversion to formaldehyde.[49]

The by-products of alcohol are more toxic than alcohol itself. These metabolites accumulate within cells and interfere with normal cellular function, sometimes without ever reaching levels that are detectable in body fluids or tissues, and affecting virtually every system in the body, especially the liver.[49]

1.2 METABOLIC SYNDROME

1.2.1 Introduction

The metabolic syndrome is a metabolic disorder that is becoming more common with the increased prevalence of obesity.[50] The disorder is defined in various ways, and a new definition was recently proposed by the International Diabetes Federation (IDF). [51] In my study, the latest National Cholesterol Education Program's Adult Treatment Panel III (NCEP: ATP III) criteria [53] were used.

The NCEP: ATP III identified the metabolic syndrome as a multiplex risk factor for cardiovascular disease (CVD). However, according to the new stricter IDF definition, for a person to be defined as having the metabolic syndrome they must have central obesity plus any two other factors as listed in figure 4.

The concept of the metabolic syndrome has existed for at least 80 years. In 1988, Reaven resurrected the syndrome and noted that several risk factors, in particular dyslipidaemia, hypertension, hyperglycaemia, commonly cluster together in what he called Syndrome X.[54]

Even though CVD is a primary clinical outcome of the metabolic syndrome, most people with this syndrome have insulin resistance, which increases the risk for type 2 diabetes.[52] Other associations of the metabolic syndrome include hyperuricaemia, gout, gallbladder disease, polycystic ovary syndrome, hypertension, sleep disturbances, asthma, some forms of cancer as well as NAFLD.[52]

1.2.2 Components of the Metabolic Syndrome

Six components of the metabolic syndrome that relate to CVD, have been Identified by the ATP III: [52,53]

a) Abdominal obesity as measured by waist circumference
b) Atherogenic dyslipidaemia, manifested by raised concentrations of serum triglycerides and low concentrations of HDL cholesterol.
c) Hypertension, which is strongly associated with obesity and insulin resistance.
d) Glucose intolerance, that may later evolve into frank diabetes mellitus, constituting a major independent risk factor for CVD.
e) A proinflammatory state, manifests clinically by a raised hs C-reactive protein (hs CRP)
f) A prothrombotic state, recognized by an elevation in the levels of plasminogen activator inhibitor-1 (PAI-1) and fibrinogen.

1.2.3 Pathogenesis of the Metabolic Syndrome

There are several proposed aetiological factors. These include the following:

a) Obesity and Abdominal Body Fat Distribution

Obesity is a chronic condition characterised by an excess of body fat. It is most often defined by the Body Mass Index (BMI), a mathematical formula

that is highly correlated with body fat. BMI is the weight in kilograms divided by the height in metres squared (kg/m²). The World Health Organization recognises the following classification system for health consequences of obesity using the BMI. A BMI of more than or equal to 25kg/m² is defined as overweight, which is further classified as pre-obese with index between 25.0 and 29.9 kg/m², and a BMI above 30 kg/m² is categorised as obese.[55] Those with a BMI of more than and equal 40.0 kg/m² are morbidly obese.[56] Obesity has reached epidemic proportions in America, effecting 25% to 30% of the population.[10] The epidemic however, is no longer confined to the USA. In South Africa, the prevalence of obesity is about 19%.[57] These figures parallel the rise in insulin-resistant states such as the metabolic syndrome and type 2 diabetes.[10] Obesity contributes to hypertension, high serum cholesterol, low HDL cholesterol and hyperglycaemia and is associated with a higher CVD risk. Abdominal obesity is strongly associated with the other metabolic risk factors. Excess adipose tissue releases several by-products which aggravate the risk. These include nonesterified fatty acids (NEFA), which overload muscle with lipids that are later diverted to the liver enhancing insulin resistance and promoting fatty liver. Other products include, high interleukin 6, resistin, TNF α and CRP

levels signifying a proinflammatory state and an elevated PAI-1 contributing to a prothrombotic state. Low adiponectin levels on the other hand correlate inversely with the degree of obesity.

b) Insulin Resistance:

It is thought that insulin resistance plays a greater role than obesity in the pathogenesis of the metabolic syndrome, where it directly causes other metabolic risk factors.[54, 58] However, trying to assign a unique role for insulin resistance is complicated by the fact that it is linked to obesity making the identification of the cause and effects difficult.

Insulin resistance rises with an increasing BMI, yet a broad range of insulin sensitivities exist at any given BMI.[59] Insulin resistance may exist even with BMIs of less than 25 kg/m² such as in the South Asians. In fact, it may contribute to a high prevalence of type 2 diabetes and premature CVD in this population group. They can be said to have primary insulin resistance. However, even with primary insulin resistance, weight gain may worsen insulin resistance and the metabolic syndrome. Thus, the two appear to be closely linked and are not easily dissociated.[52]

c) Other Independent and Contributing Factors that mediate specific components of the Metabolic syndrome:

Each risk factor of the metabolic syndrome is subject to its own regulation by both genetic and acquired factors and, as a result, there is a variation in their expression. For example, lipoprotein metabolism is modulated by genetic variation, hence the expression of dyslipidaemias in response to obesity and/or insulin resistance varies considerably. Glucose levels depend on insulin secretory capacity as well as insulin sensitivity. Endocrine factors, advancing age and proinflammatory states have also been implicated.[52, 60]

1.2.4 Diagnosis of the Metabolic Syndrome

Using the ATP III criteria for the diagnosis of the metabolic syndrome, the clinical signs of the metabolic syndrome can be easily detected (Figure 3). It is critical to ensure proper diagnosis, dissemination of information to patients regarding their risks and appropriate therapies, and initiation of appropriate therapies.

The evaluation of patients who present features suggestive of metabolic syndrome should include the following:

a) Measurement of vital signs including blood pressure, and body weight;

b) Measurement of waist circumference;

c) Measurement of fasting blood sugar; and

d) A fasting lipid profile (total cholesterol, HDL-C, TG, and a calculated LDL-C)

According to the ATP III definition, a subject has the metabolic syndrome if 3 or more of the criteria listed in Figure 3 are present. These are similar to the Guidelines for Type 2 Diabetes at Primary Care in 2003 published by the Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA). [61]

Figure 3. The ATP III Diagnostic Criteria for the Metabolic Syndrome [53]

1. Abdominal obesity * (waist circumference > 102 cm [40 in] in men, > 88 cm [35 in] in women)
2. Hypertriglyceridaemia (>/=1.7mmol/L [>/= 150 mg/dL]) or drug treatment for elevated TG).
3. Low HDL-C (<1.03mmol/L [< 40 mg/dL] in men, <1.3mmol/L [< 50 mg/dL] in women or drug treatment for reduced HDL-C).
4. High blood pressure (>/= 130/85 mm Hg or drug treatment for hypertension).
5. High fasting glucose (>/=5.6mmol/L [>/= 100 mg/dL] or drug treatment for elevated glucose)

*These cut offs are lower for Europids, >/= 94 cm in men and >/= 80 cm in women, and even lower for South Asians >/= 90 cm in men and >/= 80 cms

in women. For South Africa (Sub-Saharan Africans), the Europid cut offs should probably be used, and for Asians, the South Asian cut offs. [51]

Figure 4. The IDF Diagnostic Criteria for the Metabolic Syndrome [51]

According to the new IDF definition, for a person to be defined as having the metabolic syndrome they must have:

Central obesity (waist circumference >/= 94 cm in men, >/= 80 cm in women)*

Plus any two of the following four factors:

1. Hypertriglyceridaemia (>/=1.7 mmol/L [>/= 150 mg/dL]), or specific treatment for this lipid abnormality
2. Low HDL-C (<1.03mmol/L [< 40 mg/dL] in men, <1.3 mmol/L [< 50 mg/dL] in women), or specific treatment for this lipid abnormality
3. High blood pressure (systolic BP >/= 130 or diastolic BP >/= 85 mm Hg), or treatment of previously diagnosed hypertension
4. High fasting glucose (>/=5.6 mmol/L [>/= 100 mg/dL]), or previously diagnosed type 2 diabetes

* Using the Europid cutoffs

CHAPTER 2

THE ROLE OF INCREASED GASTROINTESTINAL ALCOHOL PRODUCTION IN PATIENTS WITH THE METABOLIC SYNDROME

2.1 AIMS OF THE STUDY

It has been shown that small increases in gut derived alcohol might increase portal blood alcohol levels which are enough to induce hepatic steatosis. Animal studies have demonstrated the presence of increased gut alcohol production by intestinal bacteria which has probably lead to the pathogenesis of NAFLD.

The main aim of this study was to evaluate patients with the metabolic syndrome who had clinical features of non-alcoholic fatty liver disease (NAFLD) for the presence of increased intestinal production of alcohol. The study was done in an attempt to increase our level of understanding of the aetiology and pathogenesis of NALFD, and thereby possibly identify new approaches to the management of the disease.

2.2 SUBJECTS UNDER STUDY

The study group included 20 patients attending both state and private

Endocrine and Liver clinics at the Johannesburg Hospital. The control group included 20 healthy hospital staff members or their relatives. Informed consent was obtained from every subject before enrollment into the study. Ethics Committee clearance was obtained from the University of the Witwatersrand (M03-09-25, see Appendices).

Inclusion criteria included:

1. **NCEP:ATP III criteria for the diagnosis of the metabolic syndrome.**
2. No history of alcohol use.
3. Deranged liver function tests.
4. The presence of fatty liver disease on abdominal ultrasound.

The inclusion criteria for the control group were:

1. **Absence of the metabolic syndrome as defined by the NCEP: ATP III criteria.**
2. No history of alcohol use/ abuse.
3. Normal liver function tests.
4. No history of medical illness.

Fasting blood, breath, and urine samples were obtained from the study group and the control group to assess the levels of alcohol in blood, breath and urine.

2.3 DATA COLLECTION

2.3.1 Data

Data were collected from patients' records. This included details of risk factors for NAFLD such as type 2 diabetes, hypertension and dyslipidaemia. Diabetes mellitus was defined as a fasting blood glucose >/= to 5.6 mmol/L or a previous diagnosis of diabetes. Hypertension was defined as a blood pressure of >/= to 130/85 or on antihypertensive therapy. Dyslipidaemia was defined as a fasting total-cholesterol of > 5 mmol/l, a triglyceride of > 1.5 mmol/l, a HDL-cholesterol of < 1.2 mmol/l and/or a LDL-cholesterol of > 3 mmol/l, or was assumed to be present if the subject was on lipid modifying therapy (Appendix 9).

Details of current and previous drug as well as supplement medication was also taken.

They were all physically examined, and anthropometric measurements

included weight, height and waist circumference. BMI was calculated using weight in kilograms divided by height in metres squared. All subjects were also examined for complications of their medical problems. Blood tests included the total cholesterol, HDL-C, TG, calculated LDL-C, HbA1C levels, fasting glucose levels, AST as well as ALT levels.

Ultrasound scanning of the liver was also performed to assess the degree of steatosis. Healthy control subjects were age, sex and race matched. Blood samples were obtained for liver enzymes, a fasting lipogram and a fasting glucose. Both the study and control subjects were screened to exclude other causes of chronic liver disease. This included screening for Hepatitis B and C infection.

2.4 SPECIMEN ANALYSIS

2.4.1 Alcohol Analysis

Blood, urine and breath samples were collected and analysed for alcohol levels. This was performed using Gas Chromatography (GC), which has been accepted as a worldwide standard for forensically estimating blood alcohol concentrations.

The columns used for alcohol GC analyses were

mainly supplied by Sigma-Aldrich being one of them. Their recommendations (Sigma-Aldrich catalogue, 2004/2005, page 318) were adapted to suit the experimental procedures and sensitivity required. Sources of error were greatly reduced by limiting the number of steps in the protocol for the sample procedure. For this reason the direct injection method was used.[62]

Most samples were analysed on the same day. If not possible, the samples were stored at –20ºC and thawed slowly at ambient room temperature and mixed well before analysis. In order to verify the stability of these preserved samples, the samples that were analysed on the day of collection were aliquoted and frozen. After 30 days, these samples were thawed to room temperature and the alcohol concentration estimated by GC. This was a standard practice in the estimation of volatile substances such as alcohol.

Samples were measured using a Hewlett Packard 5890A GC, Spectra Physics 4820 Integrator, Supelco Carbowax Capillary column (30 metre x 0.32 mm ID), Hamilton 5 µL syringe, Amican Ultra- MC centrifugal filter.

Laboratory pure ethanol, methanol and n-Propanol standards were used for calibration.The carbowax column was inserted into the GC as per the

manufacturer’s instructions.

Nitrogen was used as the carrier gas and set to a flow rate of approximately 1.0mL/min. The oven was controlled at 45ºC. Stock standard solutions of methanol, ethanol and n-propanol were made to 2% in distilled water.

A 190µL aliquot was placed in a Millipore filter with a cut-off of 10kD and 10µL of the internal standard of 2% n-propanol was added. This was then centrifuged in a microfuge at 12rpm (xG) for 30 minutes.

Four microlitres of the filtrate was then injected into the GC and the integrator activated at the same time. Both methanol and ethanol were quantified by preparing a range of concentrations between 0.01%-0. 1% and including n-propanol at a fixed concentration of 0.1% for each standard.

The calculation of the ratios of the integrated area of each alcohol to the area of the 0.1% n-propanol gave a figure that could be plotted on a graph, the vertical y-axis being the ratio and the horizontal x-axis, the alcohol concentration. The graph thus depicted yielded a straight line indicating that the ratio of alcohol to n-propanol was linear, confirming a directly propotional relationship. Incorporation of the 0.1% n-propanol in

each unknown sample permitted the direct calculation of the alcohol being sought.

The carbowax column separated the alcohols presented according to their chain length, it therefore followed that methanol was the first to be eluted, this was followed by ethanol and then the n-propanol standard. Once the ratio to n-propanol to either the methanol or the ethanol standards was established, one was able to directly calculate the concentration of the alcohol eluted. Since methanol had a molecular mass of only 32, it became fairly difficult to establish its presence using mass spectrometry. However, by fitting a less polar capillary column (DB24) in the GC, the elution profile of the different alcohols did vary with time. This was definitive in identifying the alcohols eluted when compared with the known alcohol standards. Analytes that appeared to be methanol were identified and confirmed using this technique.

Of note, urine and breath samples were injected directly onto the GC column without preparation, however serum samples have a more complex matrix and the proteins present could form deposits on the capillary column in the GC thus affecting the efficiency of the column. It was therefore deemed necessary to remove most of the protein prior to

analysis by employing Millipore cut-off filters that excluded any molecules greater than 10kD. Alcohols have a molecular masses much lower than the cut-off and would therefore pass freely through the filter. This was verified by incorporating the propanol as the internal standard into the serum samples to be tested. The propanol from these serum samples gave results comparable to the aqueous propanol standard.

2.4.2 Analysis of Blood

Blood samples were collected from the study and control subjects for liver enzymes, a fasting lipogram and a fasting glucose. Hepatitis B and C serologies were also done.

Blood was assessed for adiponectin, leptin, NEFA, and hsCRP levels. Blood specimens were centrifuged and their supernatants (serum) were dispensed into 5ml labelled plastic tubes and stored at -70°C for batch analysis at a later stage.

Serum NEFA analysis was performed manually using the Half-micro test (Roche), an enzymatic colorimetric assay. Leptin analysis was performed using the Quantikine® Human Leptin Immmunoassay (R&D systems) while

Adiponectin analysis was done using the Quantikine® Adiponectin Immunoassay (R&D systems). These assays employed the quantitative sandwich enzyme immunoassay technique.

Immunoturbidimetric analysis for the in vitro quantitative determination of hs-CRP in serum was carried out using the Tina-quant (Roche) C-reactive (latex) high sensitive assay.

All tests were performed following the manufacturer's instructions and the concentrations were calculated as per instructions. To avoid inter-assay variability, all assays were performed as single batch assays. All assays had an inter-assay variability of less than 5%.

2.5 STATISTICAL ANALYSIS

Data was analysed using the GB- Stat v4 program (Dynamic Microsystems, Inc. Silver Springs, MD 20904). Quantitative or continuous data were either expressed as the mean ± SD, with data that was normally distributed, or as median with a range for data that was non-parametric. Categorical variables which were expressed as percentages were evaluated using the Fisher's 2-tailed exact test. Continuous variables were analysed with the unpaired Student's t test. All reported P values were considered as significant at a level of $P< 0.05$.

CHAPTER 3

RESULTS

3.1 Table1: Anthropometric Data

The study group and control group were comparable in terms of age, gender and ethnicity. All the study subjects were already on treatment for their medical conditions.

Although 45% of the control group were found to have dyslipidaemia this was mild in all cases (TC < 7.5 mmol/l), and none were on lipid modifying treatment.

There was a significant difference between the two groups in the waist circumference and body mass index ($p < 0.0001$).

	Study Group (n=20)	Control Group (n=20)	P value
Gender (M/F)	8/12	8/12	NS
Age (years)	48±15.3	48±16.2	NS
WC (cm)	111±14.9	82.±8.8	<0.0001
BMI (kg/m²)	35.1±8.2	23.3±2.1	<0.0001
Hypertension	12/20 (60%)	None	<0.01
Dyslipidaemia	16/20 (80%)	9/20 (45%)	<0.01
Diabetes/IFG	14/20 (70%)	None	<0.01
Ethnicity:			
Black	2/20 (10%)	2/20 (10%)	NS
White	12/20 (60%)	12/20 (60%)	NS
Indian	4/20 (20%)	4/20 (20%)	NS
Chinese	1/20 (5%)	1/20 (5%)	NS
Coloured	1/20 (5%)	1/20 (5%)	NS

Values are mean ± SD. NS – Not Significant

3.2 Table 2: Fasting Lipid Profiles

HDL levels were significantly lower and NEFA concentrations significantly higher in the study group versus the control group. Total cholesterol and triglyceride levels were also higher in the study group but this was not significant.

	Study Group (n=20)	Control Group (n=20)	P value
Total Cholesterol (mmol/L)	5.71±3.25	5.27±1.10	NS
Triglycerides (mmol/L)	7.16±18.50	1.11±0.58	NS
HDL C (mmol/L)	1.23±0.36	1.58±0.42	<0.01
NEFA (µmol/L)	863.5±0.46	432.9±0.16	<0.001

Values are mean ± SD. NS – Not Significant

3.3 Table 3: Serum Aminotransferases

The differences between the study and control groups were significant with the study groups' ALT levels being 57.4±44.79 U/L versus 17.4 ±4.60 U/L in the control group (95% CI 18.02 - 61.42, P < 0.01) and the study groups'AST levels being 52.5 ±36.21 U/L versus 23.4 ±4.86 U/L in the

control group (95% CI 11.99 – 46.20, P < 0.01).

	Study Group (n=19)	Control Group (n=20)	P value
ALT (U/L)	57.4±44.79	17.7±4.60	<0.001
AST (U/L)	52.5±36.21	23.4±4.86	<0.01
AST:ALT ratio	1.01	1.37	<0.01

Values are mean ± SD

3.4 Table 4: Ultrasound Results

Seventy five percent had features of fatty liver disease on sonar.

Five of the study subjects had liver biopsies confirming the diagnosis of NAFLD. These were done prior to inclusion into the study.

	Study Group (n=20)	Control Group (n=20)
Yes	15(75%)	ND
No	5(25%)	ND

ND – Not Done

3.5 Table 5: Alcohol Measurements

Of the twenty patients with NAFLD screened for any alcohol present, 60% tested positive. Fifty five percent tested positive for methanol, while 35% were positive for ethanol. Of note, no alcohol was detected in the control subjects.

Alcohol	**Study Group (n=20)**	**Control Group (n=20)**	**P value**
Ethanol Present	7 (35%)	0 (0%)	=0.0084*
Methanol present	11(55%)	0 (0%)	=0.0000*
Ethanol and Methanol present	6 (30%)	0 (0%)	=0.0202*
Any alcohol present	12 (60%)	0 (0%)	=0.0000*

*2 tailed Fisher exact test.

3.6 Table 6: Study Subjects with detectable Alcohol concentration levels

The methanol concentration was highest in the blood and significantly higher when compared to that in the urine and breath.

Case No.	**Ethanol (mg %)**			**Methanol (mg %)**		
	Blood	**Urine**	**Breath**	**Blood**	**Urine**	**Breath**
3	9	ND	ND	2	1.6	2.4
4	2	ND	ND	1.8	ND	ND
6	ND	ND	ND	50	ND	ND
7	8	44.6	ND	29	32.3	ND
8	8	ND	ND	2	ND	8
9	10	ND	ND	ND	ND	ND
10	10	ND	ND	21	ND	8
11	ND	ND	ND	7.1	ND	ND
12	ND	ND	ND	3	ND	ND
13	3	ND	ND	9	38	1
14	ND	ND	ND	7	ND	ND
15	ND	ND	ND	46	9.7	5.3
Mean ± SD	7.14± 3.28	3.71± 12.87	0	16.17± 17.95	6.8± 13.58	2.05± 3.19

ND - Not Detected.

3.7 Table 7: Adipocytokines

There was a significant difference in the adiponectin and leptin concentrations between the two groups, the adiponectin levels being significantly lower and the leptin levels being significantly higher in the study group than in the controls.

	Study Group (n=20)	**Control Group (n=20)**	**P value**
Adiponectin (ng/L)	6875 (1090-29600)	15475 (4750-95200)	<0.01
Leptin (ng/L)	13.56 (0-76.1)	3.05 (0-35.3)	<0.05

Values are the median with the minimum-maximum range.

3.8 Table 8: Inflammatory Markers

The hs-CRP levels were significantly higher in the study group as compared to the control.

	Study Group (n=20)	**Control Group (n=20)**	**P value**
hs-CRP (mg/L)	6.46 (0.36-141.8)	0.86 (0.17-16)	<0.01

Values are the median with minimum-maximum range.

CHAPTER 4

DISCUSSION

NAFLD is considered to represent the hepatic expression of the Metabolic Syndrome. NAFLD can lead to NASH and subsequent chronic liver disease and cirrhosis. With the increasing prevalence of obesity, coupled with diabetes, dyslipidaemia and hypertension, a very large segment of the population is at risk for impending liver disease in the coming decades. It still remains an active area of research and a better understanding of the pathophysiology of NAFLD would help develop future therapies.

The study examined the possibility that endogenous production of alcohol may be responsible as a part of the 'second hit' theory for the pathogenesis of NAFLD. The fact that alcohol was detected in blood, urine and breath is important evidence for its pathogenesis in the development of NAFLD. This was in keeping with previous animal and human studies. [45,46]

Contrary to previous studies, methanol was also detected. [45, 46] A significantly higher percentage of patients had detectable levels of methanol than ethanol, 55% compared to 35%. No alcohol was detected in the controls, in keeping with the reported non consumption of

alcohol by our patients during their interviews. Of note, no ethanol was detected in the breath of the study subjects, which is not consistent with previous studies. [45, 46] This may be explained by the fact that the intestinally derived ethanol indeed underwent hepatic metabolism, and could not reach the lungs. However, methanol was detected in all body fluids measured. The methanol concentration was highest in blood and significantly higher when compared to that in urine and breath. It is a natural constituent of body fluids. [64] It is available in the diet and sourced principally from vegetables, fresh fruits and fruit juices. Other sources include diet drinks, in which the artificial sweetener aspartame is widely used, and when hydrolysed is partly converted to methanol. The amount of methanol ingested from fruit juices or diet beverages is debatable, but litres would have to be ingested before even being considered to be toxic.[64]

Intestinal bacterial overgrowth has been implicated to explain the increased production of endogenous ethanol. Alterations in gut motility associated with obesity, and complications associated with diabetes, such as visceral autonomic neuropathy, may be responsible for an increase in the intestinal bacterial overgrowth.

This study suggests that importance of endogenous alcohol production may be important in the pathogenesis of NAFLD in patients with the metabolic syndrome. The origin of the alcohol is uncertain, but the importance of intestinal bacterial flora and the role of foods, may be important in the pathology of this disease. If so, it may be that therapeutic alternatives such as the use of antibiotics and probiotics as new treatment strategies may have a role as been alluded to by recent studies.[64]

Rapid weight loss and long lasting fasting periods should be avoided, since they lead to an increase in the flow of NEFA to the liver. A gradual weight reduction is suggested as it is associated with improvement of hepatic lesions, including fibrosis.[65-69]

Control of diabetes, dyslipidaemia and hypertension with diet and drugs, with simultaneous treatment of obesity as suggested above, is of paramount importance.

Oral antibiotics such as metronidazole (flagyl) may be efficacious in reverting steatosis, and in some cases, inflammation and fibrosis. [70, 71] Doses may have to be reduced in severe liver disease. However, side effects such as gastrointestinal intolerance and an unpleasant taste, may affect long term compliance.

Prolong use may also cause a reversible peripheral neuropathy and it is contraindicated in the first trimester during pregnancy.

Probiotics may be a better alternative. They have shown promising results in recent studies. [72-74] They may interfere with the development of NAFLD at various levels: 1) decrease proinflammatory cytokines, 2) alter the inflammatory effects of pathogenic strains of intestinal bacteria, through changes in cytokine signaling, 3) improve epithelial barrier function and thereby avoid excessive exposure of the liver to cytokines and bacterial alcohol. Probiotics have shown to improve liver enzymes in treated patients.

Several cytoprotective agents and antioxidants have shown promising results. These include ursodeoxycholic acid, vitamin E, lecitin, B carotene, selenium, S-adenosyl-methionine, metadoxine and silimarin.[63]

In conclusion, this study therefore suggests that endogenous alcohol production may indeed be responsible for the ‘second hit’ theory in the pathogenesis of NAFLD.

CHAPTER 5

LIMITATIONS

The sample size may have been to small, and further larger studies are required to confirm these results. Eight control subjects have a high total cholesterol. However, none of them had severe hyperlipidaemia (defined as a TC > 7.5 mmol/L or TG > 5). This may have limited the overall result of the study.

Even though the waist circumference and BMI are considered to be reliable anthropometric indices of abdominal visceral adipose tissue accumulation, MRI or CT scans would precisely identify the proportion of abdominal adipose tissue, but these are expensive and surrogate measures of visceral fat had to be used instead, these being the waist circumference and the BMI.

No liver biopsies were performed. It has been argued that although a fatty liver may be readily detected on ultrasonography, a liver biopsy is necessary for the diagnosis of NASH, especially since imaging techniques do not detect hepatic fibrosis or inflammation easily in the

presence of fat. Since the only effective therapy at the moment is weight loss, it can be argued that these patients should not be subjected to liver biopsy and its associated risks. Only five patients had confirmed biopsies done prior to recruitment in to this study. No permission is granted generally by the Ethics Committee for invasive procedures. The use of other radiological studies such as the MRI or CT scans are expensive, and maybe nonspecific. We therefore made use of alternative surrogate markers such as hepatic enzymes, abdominal ultrasounds and adipocytokines as markers of steatosis.

In terms of alcohol levels detected, it would be a better to analyse portal blood for alcohol rather then peripheral blood. This is to avoid the first pass effect of metabolism of the liver, where most the body's toxins are metabolised, but this would be invasive and not practical. There are other factors also playing a role in the levels of alcohol detected such as the amount of adipose tissue present as this is a storage site. However, analysis of portal blood may be difficult and would require sophisticated techniques, but they should be considered.

Four patients had higher than normal levels of methanol, however.

This could have been due to porto-systemic shunting, explaining that some alcohol may have escaped directly into the systemic circulation escaping the portal blood, but this was not in keeping with their clinical picture as all the patients were clinically well.

REFERENCES

1. Lugwig J, Viggiano T, McGill D and Oh B. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980; **55**: 434-8.
2. Schaffner F and Thaler H. Nonalcoholic fatty liver disease. Prog Liver Dis 1986; **8**: 283-98.
3. Diehl AM. Non-alcoholic steatohepatitis. Seminars in Liver Disease. 1999; **19**:221-229.
4. Angulo P. Non-alcoholic fatty liver disease. NEJM 2002; **346**:1221-1231.
5. Alba LM et al. Non alcoholic fatty liver disease. Alimentary Pharmacology and Therapeutics 2003; **17**:977.
6. Nomura H, Kashiwagi S, Hayashi J, Kajiyama W, Tani S and Goto M. Prevalence of fatty liver in a general population of Okinawa, Japan. Jpn J Med 1988; **27**: 142-9.
7. Bellentani S, Saccoccio G, Masutti F et al. Prevalence of, and risk factors for, hepatic steatosis in northern Italy. Ann Intern Med 2000; **132**:112-7.

8. Luyckx F, Desaive C, Thiry A et al. Liver abnormalities in severely obese subjects: effect of drastic weight loss after gastroplasty. Int J Obes Related Metab Disord 1998; **22**: 222-6.
9. Daniel S, Ben-Menachem T, Vasudevan G, Ma CK and Blumenkehl M. Prospective evaluation of unexplained chronic liver transminase abnormalities in asymptomatic and symptomatic patients. Am J Gastroenterology 1999; **94**: 3010-4.
10. Green RM. NASH-hepatic metabolism and not simply the metabolic syndrome. Hepatology 2003; **38**: 14-17.
11. Sheth S, Gordon F and Chopar S. Nonalcoholic steatohepatitis. Ann Intern Med 1997; **126**: 137-145.
12. Rashid M and Roberts E. Nonalcoholic steatohepatitis in children. Journal of Pediatrics Gastroenterology Nutrition 2000; **30**: 48-53.
13. Manton N et al. Non-alcoholic steatohepatitis in children and adolescents. Med J Aust 2000; **173**: 476-9.
14. Angulo P, Keach J, Batts K and Lindor K. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999; **30**: 1356-62.

15. Itoh S, Yougel T and Kawagoe K. Comparison between non-alcoholic steatohepatitis and alcoholic hepatitis. Am J Gastroenterology 1987; **82**: 650-4.
16. Diehl A, Goodman Z and Ishak K. Alcohol-like liver disease in nonalcoholics: a clinical and histologic comparison with alcohol-induced liver disease. Gastroenterology 1989; **95**: 1056-62.
17. Pinto H, Baptista A, Camilo M, Valente A, Saragoca A, de Moura MC. Non-alcoholic steatohepatitis: clinicopathological comparison with alcoholic hepatitis in ambulatory and hospitalized patients. Dig Dis Sci 1996; **41**: 172-9.
18. Joseph A, Saverymuttu S, al-Sam S, Cook M and Maxwell J. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. Clin Radio 1991; **43**:26-31.
19. Debaere C, Rigauts H and Laukens P. Transient focal fatty liver infiltration mimicking liver metastasis. J Belge Radiol 1998; **81**: 174-5.
20. Mitchell D. Focal manifestations of diffuse liver disease at MR imaging. Radiology 1992; **185**:1-11.

21. Itoh S, Igarashi M, Tsukada T et al. Non-alcoholic fatty liver with alcoholic hyaline after long term glucocorticoid therapy. Acta Hepatogastoenterol 1977; **24**:415-419.
22. Lee R. Non-alcoholic steatohepatitis: Tightening the morphological screws on a hepatic rambler. Hepatology 1995; **21**:1742-1744.
23. Falchuk K, Fiske S, Hagit R et al. Pericentral hepatic fibrosis and intracellular hyalin in diabetes mellitus. Am J Gastroenterol 1980; **78**: 535-540.
24. Silverman J, O'Brien K, Long S et al. Liver pathology in morbidly obese patients with and without diabetes. Am J Gastroenterol 1990; **85**: 1345-1355.
25. Marchesini G, Brizi M, Morselli-Labate A et al. Association of non-alcoholic fatty liver disease with insulin resistance. Am J Med 1999; **107**:450-5.
26. Reynet C and Kahn C. Rad: A member of the Ras family overexpressed in muscle of type II diabetic humans. Science 1993; **262**:1441-4.
27. Maddux B, Sbraccia P, Kumakura S et al.Membrane glycoprotein

PC-1 and insulin resistance in non-insulin dependent diabetes mellitus. Nature 1995; **373**; 448-51.

28. Cohen B, Novick D and Rubinstein M. Modulation of insulin activities by leptin. Science 1996; **274**: 1185-8.
29. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 1997; **46**: 3-10. (Erratum, Diabetes 1997; 46:536)
30. Hotamisligil G, Peraldi S, Budvari A, Ellis R, White M and Spiegelman B. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF alpha and obesity-induced insulin resistance. Science 1996; **271**: 665-8.
31. Fan C, Pan J, Usuda N, Yeldandi A, Rao M and Reddy J. Steatohepatitis, spontaneous peroxisome proliferation and liver tumours in mice lacking peroxisomal fatty acyl-CoA oxidase: implications for peroxisome proliferator-activated receptor alpha natural ligand metabolism. J Biol Chem 1998; **273**:15639-45.
32. Chavin K, Yang S, Lin H et al. Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. J Bio Chem 1999; **274**: 5692-700.

33. Caldwell S, Swerdlow R, Khan E et al. Mitochondrial abnormalities in non-alcoholic steatohepatitis. J Hepatol 1999; **31**: 430-4.

34. Sanyal A, Campbell-Sargent C, Mirshani F et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001; **120**: 1183-92.

35. Cortez-Pinto H, Chatham J, Chacko V, Arnold C, Rashid A and Diehl A. Alterations in liver ATP homeostasis in human non-alcoholic steatohepatitis: a pilot study. JAMA 1999; **282**:1659-64.

36. Day C and James O. Steatohepatitis: a tale of two “hits”? Gastroenterology 1998; **114**: 842-5.

37. James O and Day C. Commentary: Non-alcoholic steatohepatitis: another disease of affluence. The Lancet. 1999; **353**: 1634-1636.

38. Wanless I and Lentz J. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology 1990; **12**: 1106-10.

39. McNair A. Non-alcoholic steatohepatitis (NASH): why biopsy? Gut

2002; **51**(6): 898-899.

40. Ratziu V, Charlotte F, Heurtier A et al. Sampling variability of liver biopsy in Non-alcoholic fatty liver disease. Gastroenterology 2005; **128**(7): 1898-906.

43. Gawrieh S, Rosdo B, Lindor M and Charlton M. Adipokine levels are predictive of histology in patients with non-alcoholic fatty liver disease. Hepatology. 2004; **40**: 237A.

44. Uygun A, Kadayifci A, Yesilova Z, Erdil A, Yaman H et al. Serum leptin levels in patients with non-alcoholic steatohepatitis. Am J Gastroenterology 2000; **95**(12): 3584-9.

45. Vuppalanchi R, Marri S, Kolwankar D, Considine R and Chalasani N. Is adiponectin involved in the pathogenesis of non-alcoholic steatohepatitis? A preliminary human study. J Clin Gastroenterology 2005; **39**(3): 237-42.

46. Cope K, Terence R and Diehl AM. Increased gastrointestinal ethanol production in obese mice: Implications for fatty liver disease pathogenesis. Gastroenterology 2000; **119**:1340-1347.

47. Nair S, Cope K, Terence R and Diehl AM. Obesity and female

gender increase breath ethanol concentration: Potential implications for the pathogenesis of non alcoholic steatohepatitis. Am J Gastroenterol 2001; **96**: 1200-1204.

48. Friedman S. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 2000; **275**: 2247-2250.

49. Bansil R, Stanley P and LaMont J. Mucin biophysics. Annu Rev Physiol 1995; **57**: 635-657.

50. The Merck Manual of Diagnosis and Therapy. Liver disease due to alcohol. 16th Edition; Chap 67: 890-93.

51. Eckel R, Grundy S and Zimmet P. The metabolic syndrome. The Lancet. 2005; **365**: 1415-1428.

52. The metabolic syndrome- a new world-wide definition. The Lancet 2005; **366**: 1059-62.

53. Grundy S et al. Definition of the metabolic syndrome. Circulation. 2004; **109**: 433-438.

54. Grundy et al. Diagnosis and Management of the Metabolic Syndrome. Circulation 2005; **112**: e285-e290.

55. Reaven G. Banting lecture 1988: Role of insulin resistance in

human disease. Diabetes. 1988; **37**: 1595-1607.

56. Arterburn D and Noel P H. Extracts from “Clinical Evidence”: obesity. BMJ 2000; **322**: 1406-1409.

57. Despres JP, Lemieux I and Prud’homme. Treatment of obesity: Need to focus on high-risk abdominally obese patients. BMJ 2001; **322**: 716-720.

58. Data from South Africa’s first National Demographic and Health Survey, 1998: Guidelines for the prevention and management of overweight and obesity in South Africa. SA Society for the Study of Obesity; Appendix A, Table A1.

59. Ferrannini E, Haffner S, Mitchell B et al. Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. Diabetologia. 1991; **34**: 416-422.

60. Abbasi F, Brown B, Lamendola C et al. Relationship between obesity, insulin resistance and coronary heart disease risk. J Am Coll Cardiol. 2002; **40**: 937-943.

61. Ford E, Giles W and Dietz W. Prevalence of the metabolic syndrome among US adults: Findings from the third National Health and Nutrition Examination Survey. JAMA. 2002; **287**: 356-

359.

62. Guidelines for Type 2 diabetes at primary care in 2003. Society for Endocrinology, Metabolism and Diabetes of South Africa.
63. Tangerman A. The high sensitive gas chromatographic analysis of ethanol in whole blood, serum, urine and faecal supernatant by the direct injection method. Clinical Chemistry. 1997; **43**(6): 1003-1009.
64. Medina J, Fernandez-Salazar L, Garcia-Buey L and Moreno-Otero R. Approach to the pathogenesis and treatment of non-alcoholic steatohepatitis. Diabetes Care. 2004; **27**: 2057-2066.
65. Environmental Health Criteria 196: Methanol pp. 1-9 (1997) by The International Programme on Chemical Safety (IPCS) under the joint sponsorship of the UN Environment Programme, the ILO and the WHO.
66. Kugelsmas M et al. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. Hepatology 2003; **38**: 413-419.
67. Ueno T et al. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. J Hepatol 1997; **27**: 103-107.

68. Palmer M and Schaffner F: Effect of weight reduction on hepatic abnormalities in overweight patients. Gastroenterology 1990; **99**: 1408-1413.

69. Drenick E et al. Effect on hepatic morphology of treatment of obesity by fasting, reducing diets and small-bowel bypass. N Engl J Med 1970; **282**: 829-834.

70. Neuschwander-Tetri B and Bacon E. Nonalcoholic steatohepatitis. Med Clin North Am 1996; **80**: 1147-1166.

71. Sheth S et al. Non-alcoholic steatohepatitis. Ann Intern Med 1997; **126**; 137-145.

72. Lugwig J et al. Review: Non-alcoholic steatohepatitis. J Gastroenterol Hepatol 1997; **12**: 398-403.

73. Li Z et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve non-alcoholic fatty liver disease. Hepatology 2003; **37**: 343-350.

74. Solga S and Diehl. Non-alcoholic fatty liver disease: lumen-liver interactions and possible role for probiotics. J Hepatol 2003; **38**: 681-687.

75. Loguercio C et al. Gut-liver axis: a new point of attack to treat

chronic liver damage? Am J Gastroenterol 2002; **97**: 2144-2146.

76. Diagnosis, management and prevention of the common dyslipidaemias in South Africa. Clinical Guideline 2000. S Afr Med J 2000; **90**: 164-178.

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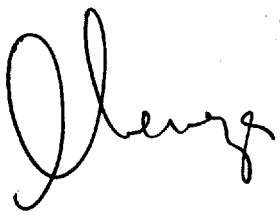
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DECLARATION

I declare that this dissertation is my own work. It is being submitted for the degree of Master of Medicine, in the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted for any degree or examination in any other University.



.....

Colin Nigel Menezes

.....day of 2006

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease with hepatic histology that resembles alcoholic liver disease. It is a frequent cause of chronic liver disease and is attracting increasing scientific attention worldwide. I explored the possibility that increased gastrointestinal alcohol production may have a role as a “second hit” in the pathogenesis of NAFLD in study subjects with the metabolic syndrome. In an attempt to investigate this hypothesis, this study looked at blood, urine and breath levels of alcohol in patients with the metabolic syndrome versus matched age and ethnic group healthy controls. Of the twenty study subjects, 80% had dyslipidaemia, 60% had hypertension and 70% had type 2 diabetes mellitus. Their mean BMI was 35.1±8.2 kg/m² (mean ± SD, $P < 0.0001$ versus controls). The serum aminotransferases were significantly elevated in the study subjects, their ALT levels being 57.4±44.79 U/L versus 17.4±4.60 U/L in the controls (95% CI 18.02 – 61.42, $P < 0.001$), and their AST levels 52.5±36.21 U/L versus 23.4±4.86 U/L in the controls (95% CI 11.99 – 46.20, $P < 0.01$). Seventy five percent of the study group had sonar features suggestive of fatty liver disease. Two adipocytokines, adiponectin and leptin, mediators of insulin resistance, an important factor in the

development and progression of NAFLD, were also measured. Adiponectin levels were significantly lower (6875 ng/L versus 15475 ng/L; median value, $P < 0.01$), and leptin concentration levels significantly higher (13.56 ng/L versus 3.05 ng/L; median value, $P < 0.05$) in the study subjects than in the control group.

Alcohol was detected in 60% of the study subjects, of which 35% tested positive for ethanol, 55% tested positive for methanol, and 30% tested positive for both ethanol and methanol. This was a statistically significant result, as none of the control group tested positive for any of the alcohols. The ethanol concentration in the study subjects' blood was 7.14±3.28 mg% (mean ± SD), in their urine 3.71± 12.87 mg% (mean ± SD) whilst none was detected in their breath. The methanol concentration in the study subjects' blood was 16.17±17.95 mg% (mean ± SD), in their urine 6.8± 13.58 mg% (mean ± SD) while their breath level was 2.05±3.19 mg (mean ± SD).

This study therefore suggests that endogenous alcohol production may be indeed be involved in the pathogenesis of NAFLD in subjects with the metabolic syndrome. Not only ethanol but also methanol was detected in the subjects tested. Endogenous alcohol may therefore be responsible for the 'second hit' theory in the pathogenesis of NAFLD, and it is likely that

formaldehyde, the metabolite of methanol may be a more potent toxin of hepatocyte injury as opposed to acetaldehyde, the metabolite of ethanol. The most likely source of the alcohol is from intestinal bacterial flora. These findings provide further insight into the pathogenesis of NALFD, suggesting other therapeutic alternatives such as the use of antibiotics and probiotics as a potential treatment strategy for NAFLD.

ACKNOWLEDGEMENTS

I wish to acknowledge the following people with heartfelt gratitude:

- My supervisors, Professor Derick Raal, Professor Ernie Song and Mr Ronnie Immelman for their invaluable assistance, guidance and support throughout the duration of my research.
- Sister Nancy Holden and Sister Nomsa Ramela of the Day Ward for their assistance with the collection of some specimens.
- The Research Incentive Fund and the Division of Endocrinology and Metabolism for the funding of this project.
- To all my loved ones, especially my parents, thank you for everything!

LIST OF ABBREVIATIONS

ADH	Alcohol dehydrogenase
ADIPO	Adiponectin
ALDH	Aldehyde dehydrogenase
ALT	Alanine aminotranferase
AST	Aspartate aminotransferase
ATP III	Adult Treatment Panel III
BL	Blood
BMI	Body Mass Index
BRE	Breath
CVD	Cardiovascular Disease
ETH	Ethanol
HbA1C	Glycated Haemoglobin
HDL-C	High Density Lipoprotein Cholesterol
hs-CRP	highly sensitive C-Reactive Protein
IDF	International Diabetes Federation
LDL-C	Low Density Lipoprotein Cholesterol
LEPT	Leptin

MET	Methanol
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NEFA	Non Esterified Fatty Acids
NIDDM	Non Insulin Dependent Diabetes Mellitus
PAI-1	Plasminogen activator inhibitor-1
TG	Triglycerides
WC	Waist circumference

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APPENDIX 1: CONTROL GROUP

PT NO.	AGE	SEX	RACE	WC	BMI	TC	TG	HDL	ALT	AST	hs-CRP	NEFA	ADIPO	LEPT
	yrs			cm	kg/m²	mmol/L	mmol/L	mmol/L	U/L	U/L	mg/L	µmol/L	ng/mL	ng/mL
1	25	male	White	87	24.5	3.7	0.3	1.4	16	22	3.34	158.0	4750	0
2	22	female	White	80	20.4	4.5	1	1.4	13	25	0.95	434.0	10960	2.02
3	43	female	Indian	76	23.9	6.2	2.2	1.6	15	18	0.29	258.0	16670	18.06
4	38	female	White	70	20.4	4.7	0.6	2.1	18	21	1.83	642.0	22070	4.48
5	30	male	White	92	24.5	4.1	0.8	1.1	23	22	0.61	365.0	7070	1.12
6	44	female	White	80	20.7	7.4	1	2.5	22	28	0.33	489.0	17180	2.78
7	37	male	White	87	20.6	5.4	1.4	1.3	21	24	2.93	383.0	14280	4.04
8	57	female	White	77	23.69	6.8	1	2.1	16	32	0.69	445.0	18710	1.22
9	31	male	White	81	22.78	5.8	1.3	1.4	15	26	0.23	460.0	9670	0.28
10	51	female	Colour	88	25	4.3	1.1	1.4	10	14	2.84	694.0	9930	6.52
11	39	male	Indian	94	20.6	4.7	0.5	1.4	28	28	0.39	350.0	9570	1.32
12	51	male	White	93	26	4.6	1.8	0.8	19	19	0.7	407.0	6990	1.68
13	53	female	White	72.5	24.26	5.4	1.1	1.3	25	20	0.17	663.0	8950	27.48
14	55	female	White	71	22.78	7	1.2	2.2	16	25	0.82	399.0	17220	1.84
15	77	female	Indian	93	24.5	6.5	2.8	1.3	15	30	4.85	348.0	14260	35.32
16	55	female	Black	77	26.7	4	0.8	1.6	17	22	2.62	293.0	18560	14.14
17	50	female	Black	73	23.72	4.4	0.8	1.3	22	26	1.56	182.0	22940	18.44
18	70	male	Indian	71	23.52	4.9	0.8	1.6	15	30	0.31	721.0	17120	1.36
19	79	female	White	83	20.6	6.4	1	2.2	11	16	16	615.0	25900	11.52
20	66	male	White	97.5	27	4.7	0.7	1.7	17	20	0.9	352.0	95200	3.32

APPENDIX 2: STUDY GROUP WITH FEATURES OF THE METABOLIC SYNDROME

PT NO.	AGE(YRS)	GENDER	RACE	WC(CMS)	BMI	T2DM	HPT	DYS
1	61	female	Chinese	90	30.71	No	Yes	Yes
2	30	male	White	99	29.34	No	No	Yes
3	65	male	White	110	27.7	No	No	Yes
4	60	female	White	101	28.01	No	Yes	Yes
5	76	female	White	96	22.7	IFG	No	Yes
6	65	male	Indian	101	25.22	Yes	Yes	No
7	35	male	White	130	42.7	Yes	Yes	Yes
8	56	female	White	112	33.01	Yes	No	Yes
9	67	female	Indian	112	33.8	Yes	Yes	No
10	40	male	Indian	108	32.35	Yes	No	Yes
11	52	female	Black	110	44.14	Yes	Yes	Yes
12	36	female	White	97	28	Yes	No	Yes
13	60	female	Black	116	47.7	Yes	Yes	Yes
14	51	male	White	94	27.4	No	No	Yes
15	25	male	White	109	33.4	Yes	No	No
16	22	female	White	115	41.5	No	Yes	Yes
17	45	female	Indian	115	41.6	Yes	Yes	Yes
18	45	female	White	123	39.8	Yes	Yes	Yes
19	32	male	White	157	52.3	Yes	Yes	Yes
20	46	female	Coloured	115	40.4	Yes	Yes	No

APPENDIX 3: STUDY GROUP-SONAR RESULTS AND BIOCHEMICAL MEASUREMENTS

PT NO.	AGE	GENDER	SONAR	TC	TG	HDL	ALT	AST	hs-CRP	NEFA	ADIPO	LEPT
	yrs			mmol/L	mmol/L	mmol/L	U/L	U/L	mg/L	µmol/L	ng/mL	ng/mL
1	61	female	Yes	6	2.8	1.4	42	29	2.05	213.0	6680	10.96
2	30	male	Yes	6.5	2.9	0.9	37	22	2.92	578.0	7070	0
3	65	male	Yes	3.41	0.81	1.21	38	38	2.43	799.0	8210	8.64
4	60	female	Yes	4.69	2.66	1.06	88	168	10.69	127.8	15510	48.92
5	76	female	Yes	5.1	3.05	1.58	45	46	1.14	696.0	4760	10.4
6	65	male	Yes	3.5	1.2	0.9	24	33	5.68	106.2	26500	16.16
7	35	male	Yes	3.5	5.1	0.6	61	39	7.19	128.8	1090	3.24
8	56	female	Yes	3.8	1.5	1.1	16	20	13.59	492.0	9160	2.16
9	67	female	Yes	3.5	0.8	1.8	29	28	8.02	651.0	5350	73.48
10	40	male	Yes	5.7	2	1.1	71	56	0.36	708.0	4690	26.84
11	52	female	No	4.2	1.3	1.7	16	20	141.8	154.7	29600	7.2
12	36	female	Yes	5.1	1.4	1.3	62	96	10.25	484.0	22800	18.75
13	60	female	No	6.2	4.9	1	15	21	23.9	196.6	9760	25.8
14	51	male	Yes	3.9	1.3	1.5	102	92	0.67	504.0	4200	0
15	25	male	No	6.6	2	1	204	83	0.46	110.2	8200	5.76
16	22	female	Yes	5.5	2.4	0.9	100	64	2.74	539.0	3050	37.74
17	45	female	No	5.1	2.2	1.4	59	63	28.35	378.0	19770	76.08
18	45	female	No	8.3	19.1	1.2	*	60	21.64	134.5	3440	0.68
19	32	male	Yes	18.4	84	2.1	21	23	0.76	126.7	3220	20.8
20	46	female	Yes	5.3	1.9	0.9	61	49	26	0.373	6250	23.48

APPENDIX 4: STUDY GROUP-ALCOHOL LEVELS

PT NO.	AGE	SEX	WC	BMI	ETH BL	ETH UR	ETH BRE	MET BL	MET UR	MET BRE
	yrs		cm	kg/m²	mg%	mg%	mg%	mg%	mg%	mg%
1	61	female	90	30.71	neg	neg	neg	neg	neg	neg
2	30	male	99	29.34	neg	neg	neg	neg	neg	neg
3	65	male	110	27.7	9	neg	neg	2	1.6	2.4
4	60	female	101	28.01	2	neg	neg	1.8	neg	neg
5	76	female	96	22.7	neg	neg	neg	neg	neg	neg
6	65	male	101	25.22	neg	neg	neg	50	neg	neg
7	35	male	130	42.7	8	44.6	neg	29	32.3	neg
8	56	female	112	33.01	8	neg	neg	2	neg	8
9	67	female	112	33.8	10	neg	neg	neg	neg	neg
10	40	male	108	32.35	10	neg	neg	21	neg	8
11	52	female	110	44.14	neg	neg	neg	7.1	neg	neg
12	36	female	97	28	neg	neg	neg	3	neg	neg
13	60	female	116	47.7	3	neg	neg	9	38	1
14	51	male	94	27.4	neg	neg	neg	7	neg	neg
15	25	male	109	33.4	neg	neg	neg	46	9.7	5.3
16	22	female	115	41.5	neg	neg	neg	neg	neg	neg
17	45	female	115	41.6	neg	neg	neg	neg	neg	neg
18	45	female	123	39.8	neg	neg	neg	neg	neg	neg
19	32	male	157	52.3	neg	neg	neg	neg	neg	neg
20	46	female	115	40.4	neg	neg	neg	neg	neg	neg

APPENDIX 5: DATA COLLECTION FORM

A STUDY ON THE ROLE OF INCREASED GASTROINTESTINAL ETHANOL PRODUCTION IN OBESE PATIENTS: IMPLICATIONS FOR THE PATHOGENESIS OF NON-ALCOHOLIC FATTY LIVER DISEASE.

Identification: (hospital sticker may be attached):

1) Clinic:…… ……

2) Date of birth: ……

3) Gender: ……

4) Ethnic group:……..

5) Hospital No:……

6) Contact Nos:……

Anthropometric measurements:

1) Weight:…….

2) Height:…….

3) Waist circumference:…….

4) BMI:…… Case < 25……. Control > 27…….

History:

1) Alcohol use (<72hrs): Yes…….. No…….

2) Smoking: Yes…….. No……..

3) Type 2 Diabetic: Yes…… No……
When diagnosed:……

4) Hypertension: Yes……. No…….
BP:……..

5) Other medical problems:

……
……
……
……
……
……

6) Treatment:

a) Oral:……
……..
……
b) Insulin:……
……..
……..
c) Others:……
……..
……..
……..
……..
……..
……..

7) Complications:

a) Microvascular:
   i) Retinopathy:……
   ii) Nephropathy: Microalbuminuria; Yes…… No……
   Proteinuria; Yes…… No……
   iii) Neuropathy:……

b) Macrovascular:
   i) CVA:……..
   ii) CAD:……..
   iii) PVD:……..

Lab results:

1) Lipid profile:
   a) Total cholesterol:……
   b) Triglycerides:……
   c) HDL:……
   d) LDL:……

2) Glycaemic levels:
   a) Fasting glucose:……. Yes…… No……
   b) HbA1C:……..

3) LFTS:
   a) ALT:…….
   b) AST:…….
   c) Alkaline phosphatase:…….
   d) GGT:…….

4) Iron studies:
   a) Ferritin levels:…….

5) Ethanol levels:
   a) Blood:……...
   b) Urine:…….
   c) Breath:…….

6) Spare tubes X2:……..

Ultrasound results:

APPENDIX 6:

SUBJECT INFORMATION SHEET AND INFORMED CONSENT FORM

Introduction:

Good day, my name is Dr Colin Menezes. I am a medical registrar at the Johannesburg General Hospital, in the department of Internal Medicine.

I would like to ask you to consider participating in a research study entitled "The role of increased gastrointestinal alcohol production in obese patients: implications for the pathogenesis of non-alcoholic fatty liver disease."

It is important that you read and understand the purpose of this study, procedures, benefits, risks and discomforts.

This information leaflet is to help you to decide if you would like to participate. If you have any questions, do not hesitate to ask me. It is important that you are truthful with me regarding your health history.

If you decide to take part, you will be asked to sign this document to confirm that you understand the study.

Purpose of the study:

Obesity is a chronic condition characterised by an excess of body fat. It is a health hazard because it is associated with numerous metabolic complications such high cholesterol, diabetes, heart disease and liver disease. The purpose of this study is to look at one possible cause of liver disease.

Procedures:

If you agree to take part, you will be asked a few questions, and examined to see if you qualify for this study. Your qualification will depend on your body mass index and medical history. You will be asked to sign the informed consent form. You may require an ultrasound. This is a painless procedure which will not cause you any harm. You will also have some blood drawn which will be about approximately 4 teaspoons and you will also have to provide a urine and breath sample.

Risks:

Drawing blood is normally done as a part of routine medical care and may present with a slight risk of discomfort. It may result in faintness, inflammation of the vein, pain, bruising or bleeding at the puncture site.

Benefits:

Your participation will contribute to a better understanding of this disease that may help identify future therapies.

Your rights:

Your participation is voluntary, and refusal to participate will result in no penalty or affect your access to medical care.

Contact:

If you have any questions, please do not hesitate to contact me on 083 995 3931 or Prof Raal at 011 488 3538 or Prof Song at 011 488 3627.

Confidentiality:

All information obtained in this study, including your hospital records, personal data and research data will be kept strictly confidential. Data that may be reported in scientific journals will not include any information that identifies you as a participant in this study.

Informed consent:

I, ……the undersigned hereby consent to the participation in this study, the nature, conduct, benefits and risks of which have been explained to me and which I understand, on myself/ my spouse/ the patient……
I have also read received, read and understand the above information regarding the clinical study.
Signature of the patient/ spouse/ near relative:

……..

Witness 1……

2……

Date ……

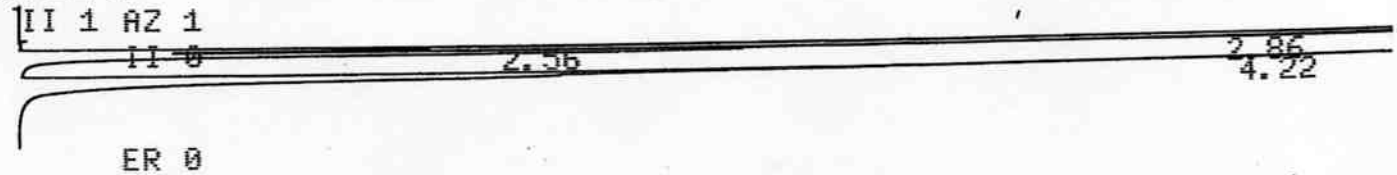
Time……

23.1.04 Pathinath Sharma

Std 0.1% each of CH_3OH, C_2H_5OH, C_3H_7OH - Int. Std.

CHANNEL A INJECT 01/23/04 10:45:44

CHANNEL A INJECT 01/23/04 10:47:03



ER 0

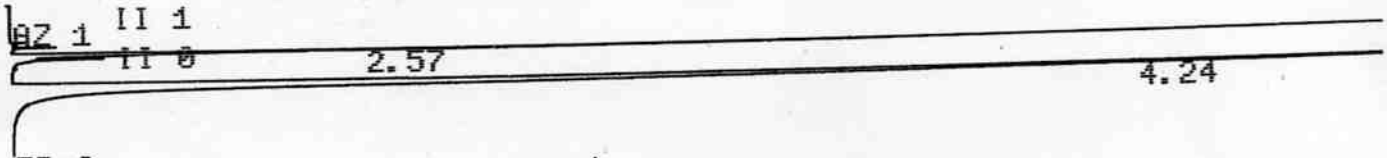
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FILE 1. METHOD 0. RUN 11 INDEX 11

PEAK#	AREA%	RT	AREA BC	
1	19.681	2.56	22158 01	← CH_3OH
2	33.825	2.86	38082 01	← C_2H_5OH
3	46.495	4.22	52347 01	← C_3H_7OH
TOTAL	100.		112587	

#2 CH_3

CHANNEL A INJECT 01/23/04 11:12:12



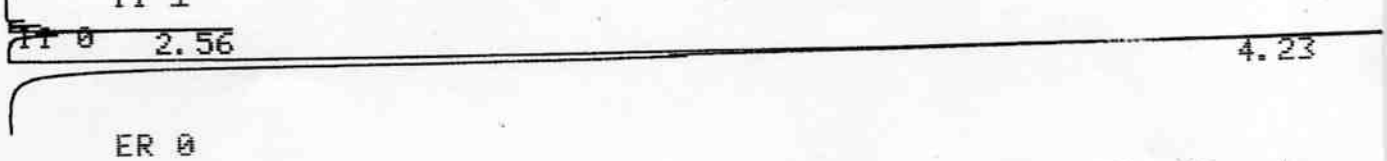
ER 0

ALCOHOL 01/23/04 11:12:12 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 12 INDEX 12

PEAK#	AREA%	RT	AREA BC	
1	13.095	2.57	18062 01	← CH_3OH
2	86.905	4.24	119871 01	← C_3H_7OH
TOTAL	100.		137933	

CHANNEL A INJECT 01/23/04 11:22:58



ER 0

ALCOHOL 01/23/04 11:22:58 CH= "A" PS= 1.

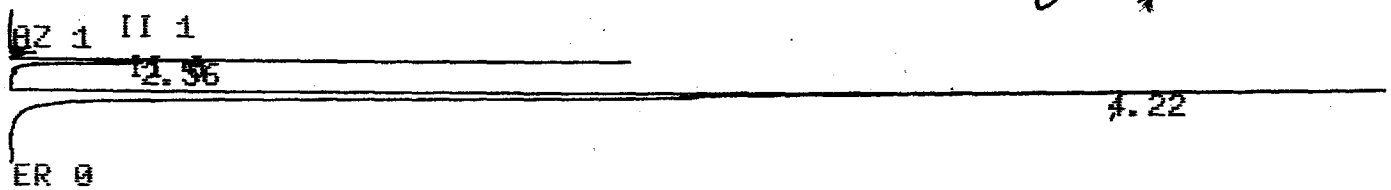
FILE 1. METHOD 0. RUN 13 INDEX 13

PEAK#	AREA%	RT	AREA BC
1	2.476	2.56	1989 01

LE 1. METHOD 0. RUN 19 INDEX 19

AK#	AREA%	RT	AREA	BC
1	100.	4.29	38968	01
TAL	100.		38968	

ANNEL A INJECT 01/23/04 15:27:52

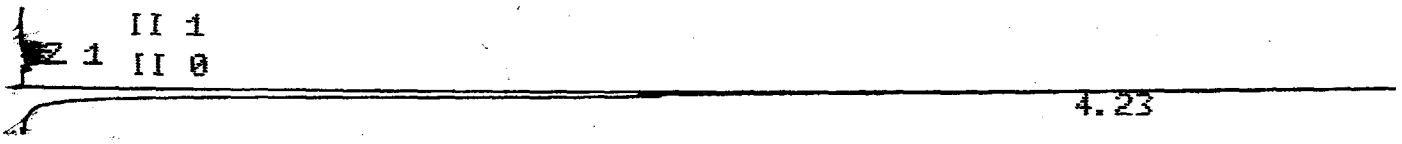


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LE 1. METHOD 0. RUN 20 INDEX 20

AK#	AREA%	RT	AREA	BC
1	10.967	2.56	5597	01
2	89.033	4.22	45440	01
TAL	100.		51037	

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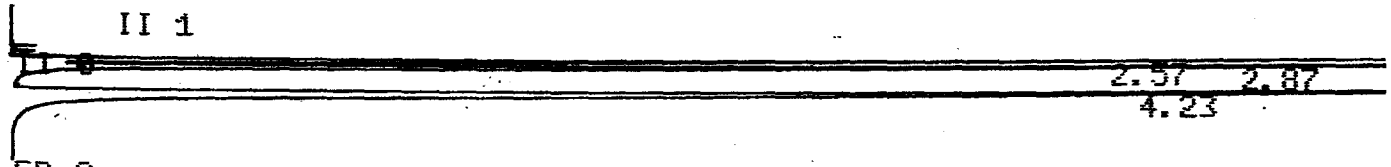


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LE 1. METHOD 0. RUN 21 INDEX 21

AK#	AREA%	RT	AREA	BC
1	100.	4.23	41337	01
TAL	100.		41337	

ANNEL A INJECT 01/23/04 15:57:00



COHOL 01/23/04 15:57:00 CH= "A" PS= 1.

LE 1. METHOD 0. RUN 22 INDEX 22

AK#	AREA%	RT	AREA	BC
1	21.45	2.57	68618	01
2	34.86	2.87	111515	01
3	43.689	4.23	139758	01
TAL	100.		319891	

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Menezes

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M03-09-25

PROJECT The Role of Increases Gastrointestinal Ethanol Production in Obese Patients: Implications for the Pathogenesis of Non-Alcoholic Fatty Liver Disease

INVESTIGATORS Dr CN Menezes

DEPARTMENT School of Clinical Medicine, Johannesburg hospital

DATE CONSIDERED 03-09-26

DECISION OF THE COMMITTEE Approved unconditionally

Unless otherwise specified the ethical clearance is valid for 5 years but may be renewed upon application
This ethical clearance will expire on 1 January 2008.

DATE 03-10-13 CHAIRMAN.....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof FJ Raal
Dept of School of Clinical Medicine, Johannesburg Hospital
Works2\lain0015\HumEth97.wdb\M 03-09-25

==========

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress form. I/we agree to inform the Committee once the study is completed.

DATE 15/10/03 SIGNATURE .....

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 9: NORMAL BIOCHEMICAL VALUES [50, 76]

BIOCHEMISTRY	NORMAL VALUES
Total Cholesterol (mmol/L)	≤5
Triglycerides (mmol/L)	≤1.5
HDL-C (mmol/L)	≥1.2
LDL-C (mmol/L)	≤3.0
Alanine Aminotransferase (U/L)	5-40
Aspartate Aminotransferase (U/L)	5-40
hs-CRP (mg/L)	< 0.5

CHAPTER 1

LITERATURE REVIEW

1.1 NON ALCOHOLIC FATTY LIVER DISEASE

1.1.1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is an increasingly recognized as one of the commonest causes of chronic liver disease worldwide that may progress to end stage liver disease. The pathological picture resembles that of alcohol induced liver disease even though alcohol is not abused.[1, 2] Risk factors for NAFLD include obesity, type 2 diabetes, hyperlipidaemia, total parenteral nutrition, jejuno-ileal bypass surgery and the use of certain drugs.[3] A variety of other terms have been used to describe this entity such as fatty liver disease, diabetic hepatitis, alcohol like liver disease, and hepatic steatosis, a more serious subset of NAFLD.[3, 4]

1.1.2 Definition

NAFLD comprises a spectrum of liver disease, ranging from simple steatosis to steatohepatitis, advanced fibrosis and the potential to

progress to cirrhosis with its inherent complications of hepatic failure and hepatocellular carcinoma.[3, 4] Non-alcoholic steatohepatitis or NASH represents a stage within the spectrum of NAFLD and is defined pathologically by the presence of steatosis together with necro-inflammatory activity.[5]

1.1.3 Epidemiology

NAFLD affects 10% to 24% of the general population in various countries.[4] This prevalence increases to 57.5%[6] to 74%[7, 8] in obese persons. It is a common explanation for abnormal liver function tests in blood donors and a cause of asymptomatic elevation of liver enzymes in up to 90% of cases once other causes of liver diseases are excluded.[9] With the increasing prevalence of obesity, it is estimated that at least about a quarter of the American adult population currently has NAFLD. Approximately 20% to 30% of patients with NAFLD will develop fibrosing steatohepatitis that may progress to cirrhosis, end-stage liver disease, and hepatocellular carcinoma.[10] The prevalence of NAFLD in the different population groups of South Africa is unknown.

1.1.4 Characteristics

a) Clinical features

Patients with NAFLD are generally asymptomatic.[3, 4] It is often diagnosed after abnormalities are noted during “routine “laboratory testing.[11] Patients may report fatigue or malaise and a sensation of fullness or discomfort on the right side of the upper abdomen.[3, 4] Hepatomegaly may be the only physical sign.[3, 4] Acanthosis nigricans may be found in children with NAFLD.[12, 13] Other signs may include signs of chronic liver disease such gynaecomastia, spider naevi, testicular atrophy and palmar erythema.

b) Laboratory Abnormalities

Elevated levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) or both are a common laboratory finding in patients with NAFLD. The ratio of AST to ALT in subjects with NAFLD is usually less than 1, but this ratio increases as fibrosis advances, leading to a loss of its diagnostic accuracy in patients with cirrhotic NAFLD.[14]

Serum alkaline phosphatase, gamma glutamyltransferase or both are often above the normal range in many patients.[15, 16, 17] Other laboratory abnormalities include hypoalbuminaemia, a prolonged prothrombin time, and hyperbilirubinaemia, but this is usually only found in end stage liver disease.[4]

c) Imaging Studies

Fatty infiltration of the liver produces a diffuse increase in echogenicity as compared with that of the kidneys on ultrasonography.[4] Regardless of the cause, cirrhosis has a similar appearance on ultrasonography.[4] Ultrasonography has a sensitivity of 89%, and a specificity of 93% in detecting steatosis. It has a sensitivity of 77% and a specificity of 89% in detecting fibrosis.[18] Fatty infiltration of the liver produces a low density hepatic parenchyma on computed tomographic scanning. Steatosis is generally diffuse however, occasionally it may be focal, and hence may be misinterpreted as showing malignant liver masses.[19] In such cases, magnetic resonance imaging can help distinguish the two.[20]

d) Histology

The histologic features of NAFLD are indistinguishable from those of alcohol-induced liver disease.[4] This includes steatosis (fatty liver), which is predominantly macrovesicular and generally diffusely distributed throughout the liver lobule. Microvesicular steatosis has been reported occasionally. It can progress to a hepatitis (parenchymal inflammation with or without accompanying focal necrosis) where there is lobular infiltration with both acute and chronic inflammatory cells and Mallory body formation, and it may result in variable degrees of perivenular and sinusoidal fibrosis progressing to cirrhosis.[1, 3, 16, 21-24]

1.1.5 Pathogenesis of NAFLD

The pathogenesis of NAFLD has remained poorly understood since the earliest description of the disease. A growing body of literature have documented the close relationship of NAFLD with the metabolic syndrome, which is characterized by abdominal obesity, insulin resistance with or without frank hyperglycaemia, dyslipidaemia, and hypertension.[10]

However, much of the current thinking remains hypothetical, since the mechanism or mechanisms remain uncertain.[4] A current concept in the pathogenesis of NAFLD involves a “two-hit” hypothesis in which an initial metabolic disturbance, namely insulin resistance causes steatosis, and a second pathogenic stimulus causes oxidative stress, reactive oxygen species generation, lipid peroxidation, and resultant steatohepatitis.[10] Another theory put forward is that NAFLD is almost certainly a polygenic disease affected by environmental factors and stressors, with disease pathogenesis related to “multiple hits.” Although the metabolic syndrome and insulin resistance are important risk factors for the development of NAFLD, our current knowledge of the genetic, biochemical, metabolic, inflammatory, endocrine, and environmental mechanisms responsible for the pathogenesis of NAFLD are poorly understood.[10]

It is also not fully understood why simple steatosis develops in some patients, whereas steatohepatitis and progressive disease develop in others.[4]

Differences in Body Mass Index (BMI) or antioxidant systems in the context of genetic predisposition may be an explanation. Retention of

lipids within hepatocytes, mostly in the form of triglycerides is a prerequisite for the development of NAFLD.[4] The primary metabolic abnormality leading to this, is not well understood. It is thought that it may be as a result of alterations in the pathways of uptake, synthesis, degradation or secretion in hepatic lipid metabolism resulting in insulin resistance which is a reproducible factor in the development of NAFLD.[25]

The molecular pathogenesis of insulin resistance is multifactorial, and several molecular targets involved in the inhibition of insulin action have been identified (Figure 1). These include Rad (Ras associated with diabetes, an enzyme, which is a member of the Rad-guanosine triphosphatase superfamily), [26] which interferes with several essential cell functions, such as growth, differentiation, vesicular transport and signal transduction; PC-1, a membrane glycoprotein that has a role in insulin resistance, [27] reduces insulin-stimulated tyrosine kinase activity; leptin, [28] which induces dephosphorylation of insulin-receptor substrate-1; fatty acids, [29] which inhibit insulin-stimulated peripheral glucose uptake; and tumour necrosis factor α, [30] which down regulates insulin-

induced phosphorylation of insulin receptor substrate-1 and reduces the expression of the insulin-dependent glucose-transport molecule Glut 4. Insulin resistance leads to fat accumulation in hepatocytes by two mechanisms: lipolysis and hyperinsulinemia. Significant amounts of dicarboxylic acids, which are potentially cytotoxic, can be formed by microsomal ω-oxidation. This pathway of fatty acid metabolism is closely related to mitochondrial β-oxidation and peroxisomal β-oxidation. Deficiency of the enzymes of peroxisomal β-oxidation has been recognised as an important cause of microvesicular steatosis and steatohepatitis.[31] Deficiency of acyl-coenzyme A oxidase disrupts the oxidation of very-long-chain fatty acids and dicarboxylic acids, leading to extensive microvesicular steatosis and steatohepatitis. Loss of this enzyme also causes sustained hyperactivation of peroxisome-proliferator-activated receptor-α (PPAR-α), leading to transcriptional up-regulation of PPAR-α regulated genes.[31] PPAR-α has been implicated in promoting hepatic synthesis of uncoupling protein-2, which is expressed in the liver of patients with NAFLD.[32]

Increased intrahepatic levels of fatty acids provide a source of oxidative

stress, which may in large part be responsible for the progression from steatosis to steatohepatitis to cirrhosis. Mitochondria are the main cellular source of reactive oxygen species, which may trigger steatohepatitis and fibrosis by three main mechanisms, a) lipid peroxidation, b) cytokine induction and c) induction of Fas ligand. Patients with steatohepatitis have ultrastructural mitochondrial lesions, [33] that is absent in patients with simple steatosis and in healthy subjects.[34] They also slowly resynthesize ATP in vivo after a fructose challenge, which usually causes acute hepatic ATP depletion.[35] This impaired ATP recovery may reflect the mitochondrial injury found in patients with steatohepatitis.[33, 34]

Although, symptoms of liver disease rarely develop in patients with the metabolic syndrome, the steatotic may be vulnerable to further injury when challenged by additional insults. This has lead to the presumption that progression from simple steatosis to steatohepatitis and advanced fibrosis results from two distinct events.[36]Firstly, insulin resistance leads to fat accumulation within hepatocytes and secondly, mitochondrial reactive oxygen species cause lipid peroxidation, cytokine oxidation and

the induction of Fas ligand (Figure 2). Lipid peroxidation results in end-products, malondialdehyde and 4-hydroxynonenal, which have proinflammatory and profibrogenic properties, may account for the typical histological features observed in NASH.[37]The correlation between the degree of lipid peroxidation and the amount of fat in the liver also provides an explanation for the association between the severity of steatosis and the risk of NASH.[38]

In summary, the two metabolic abnormalities most strongly associated with NAFLD are insulin resistance and an increased supply of fatty acids to the liver. These advances in the understanding of the pathogenesis of NAFLD have lead to the rational design of therapy for this disorder.

Figure 1: The First Hit for NAFLD: [Adapted from Ref 4]

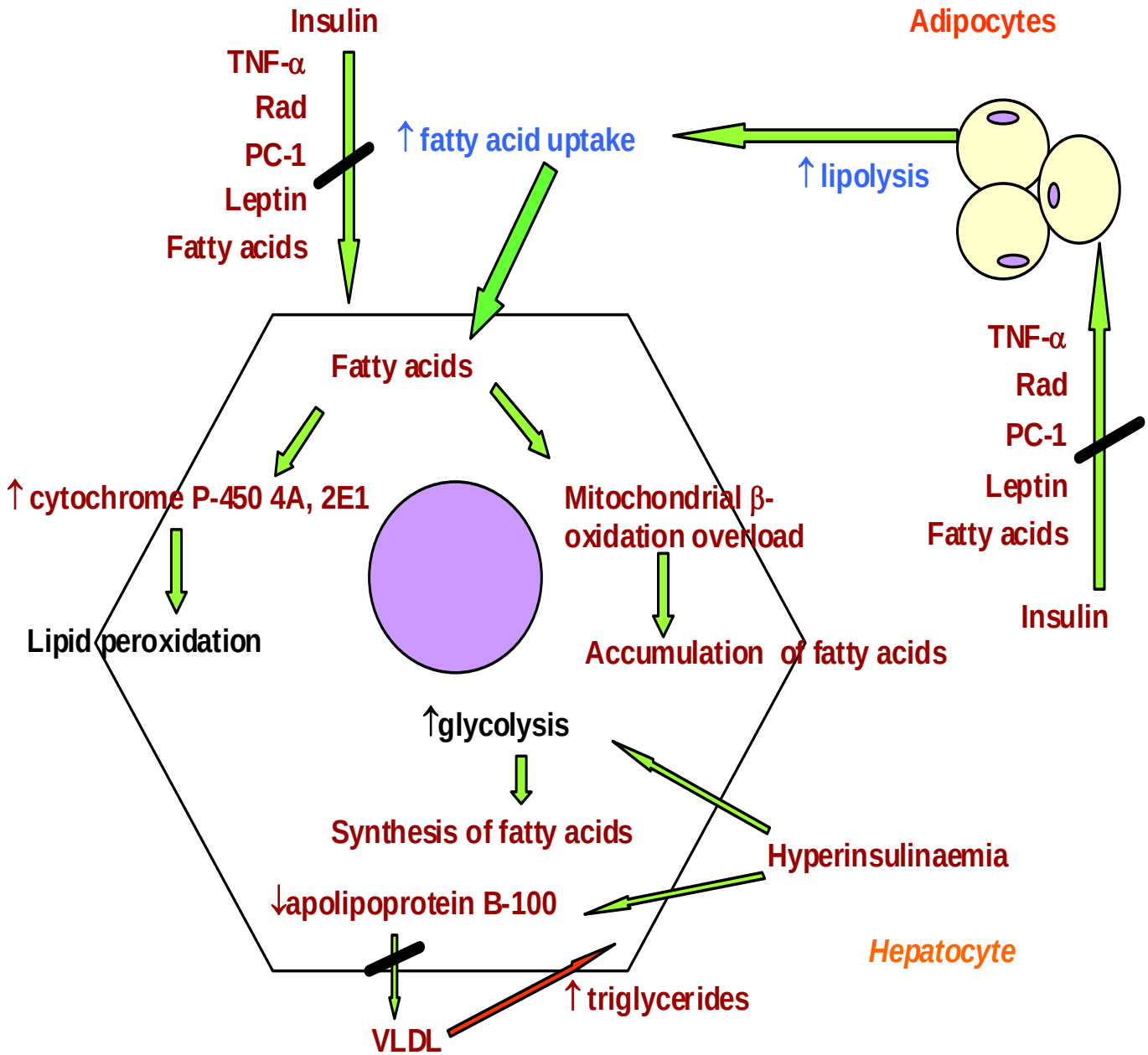
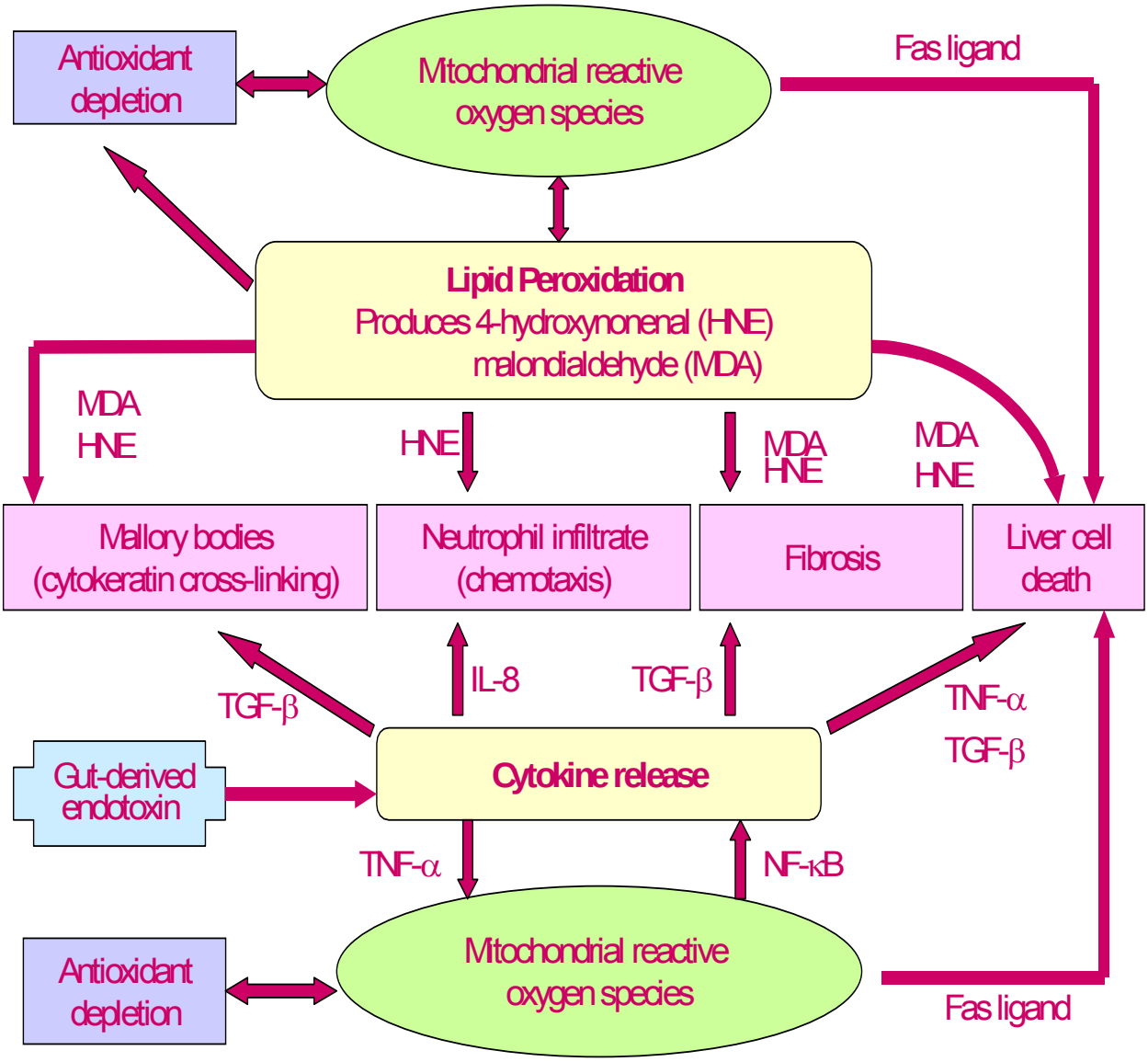


Figure 2: The Second Hit for NAFLD: [Adapted from Ref 4]



1.1.6 Diagnosis of NAFLD

NAFLD is usually suspected in persons with asymptomatic elevation of aminotranferase levels, radiologic findings of fatty liver, or unexplained persistent hepatomegaly.[4] Histology of a liver biopsy specimen is generally the gold standard. Whilst the typical features on liver biopsy are well described, given the anticipated high numbers of patients having NAFLD in the context of a high prevalence of both obesity and diabetes, it could be argued that the number of patients likely to warrant a biopsy would be enormous.[40]

However, a liver biopsy may be considered when other hepatic pathologies need to be excluded and it may also be helpful in ascertaining the extent of inflammation so as to separate simple steatosis from NASH, which may have important prognostic implications. A biopsy is also expensive and invasive, and it is associated with the risk of complications. It has therefore been argued that although there is promise of new treatments, the only effective therapy at present for the obese patient is weight loss, and why subject these patients to a liver biopsy? [40] There is also the opportunity for sampling errors. Histologic lesions of NASH are unevenly distributed throughout the liver parenchyma, therefore, sampling error of liver biopsy

can result in substantial misdiagnosis and staging inaccuracies.[41]Therefore, each case has to be assessed individually. Recently, measurements of serum concentration of adipocytokines have been used as an assessment for separating patients with bland steatosis from NASH, suggesting that liver biopsies may become unnecessary in the majority of patients with suspected NAFLD. Recent studies have found that serum levels of leptin and resistin were significantly higher in patients with NASH as compared to those with simple steatosis, whereas serum levels of adiponectin were significantly lower. This suggests that these adipocytokines may play a role in the pathogenesis of NAFLD.[42-44] Leptin is a mediator of hepatic inflammation and fibrosis in NAFLD, and resistin and adiponectin are important modulators of insulin resistance, a central factor in the pathogenesis of NAFLD.[42]

1.1.7 Role of Obesity and Alcohol

Obesity increases the risk of both fatty liver and cirrhosis in patients who consume alcohol, the mechanism of which remains obscure. The pathological picture of NASH resembles that of alcohol induced liver injury, but it occurs in patients who do not abuse alcohol as stated earlier.[45]

Obesity increases hepatic exposure to alcohol; this is because ingested alcohol accumulates in the excessive adipose tissue of obese individuals due to its lipid solubility, prolonging the alcohol exposure from social drinking.[45] Long-term alcohol intake can also alter enteric flora, resulting in the overgrowth of gram negative bacteria. Increased permeability produces endotoxaemia, which activates Kupffer cells to up-regulate lipopolysaccharide receptors and mediators such as macrophage inflammatory protein 2, prostaglandin E2, tumour necrosis factor-α, and α-hydroxyethyl. This can result in steatohepatitis. Subsequently, fibrogenesis results from the activation of hepatic stellate cells, which are responsible for the overproduction of the extracellular matrix that produces the fibrous scar.[47]

It is important to note that even small increases in gut derived ethanol might increase portal blood alcohol levels enough to induce hepatic steatosis, given evidence that alcohol produces obligatory redox changes that promote the accumulation of triglycerides in hepatocytes.[45]

Evidence has also suggested that obesity is associated with alterations in gut motility, because of decrease in sensitivity to neuropeptides such as cholecystokinin and bombesin.[48] This may favour bacterial overgrowth.

Therefore, there is a possibility that obese individuals, who do not consume alcohol, are capable of producing and metabolising alcohol endogenously, and this possibility may play a role in the pathogenesis of obesity related fatty liver disease and merits further consideration.[45]

A recent study showed that breath collected from genetically obese mice, ob/ob mice showed a higher alcohol content than their lean littermates. Hence, even in the absence of alcohol ingestion, alcohol could be detected in exhaled breath, reflecting an increase production of alcohol by intestinal bacteria and hence probably contributing to the pathogenesis of obesity related fatty liver disease.[45]

Another study in human subjects found that the breath alcohol levels in obese individuals where higher as compared to lean controls.[46]

Of note, because of efficient hepatic metabolism of alcohol, this eliminates most of the toxin before it reaches the systemic circulation, resulting in blood alcohol determinations which underestimate the actual production of alcohol in the gut.[45] Hence it is important to look at urine and breath levels of alcohol, apart from just blood levels.

1.1.8 Alcohol Metabolism and the Liver

Normal liver function is essential for life. Alcohol-induced liver damage disrupts the body's metabolism, eventually impairing the function of other organs. Most of the alcohol that a person imbibes is eventually broken down by the liver. Alcohol is readily absorbed from the gut.[49]

The metabolism of ethanol occurs in the cytosol via hepatic alcohol dehydrogenase (ADH) and certain microsomal enzymes. Alcohol cannot be stored and must be metabolised. which produces acetaldehyde, this is then rapidly biotransformed by aldehyde dehydrogenase (ALDH) in the cytosol and mitochondria to acetoacetate.[49]

These by-products are toxic to the liver and other organs. Methanol is metabolized primarily in the liver through similar sequential oxidative steps to formaldehyde, formic acid and carbon dioxide. The initial step involves oxidation to formaldehyde by hepatic ALDH. Formaldehyde is then oxidized by formaldehyde dehydrogenase to formic acid/or formate depending on the pH. In the final step, formic acid is detoxified to carbon dioxide by folate-dependent reactions.[49]

If methanol is co-ingested with a significant amount of alcohol, the

methanol conversion is temporarily blocked since ethanol has nine times greater affinity for alcohol dehydrogenase than does methanol. This allows elimination of methanol from the blood via the urine and exhaled air before conversion to formaldehyde.[49]

The by-products of alcohol are more toxic than alcohol itself. These metabolites accumulate within cells and interfere with normal cellular function, sometimes without ever reaching levels that are detectable in body fluids or tissues, and affecting virtually every system in the body, especially the liver.[49]

1.2 METABOLIC SYNDROME

1.2.1 Introduction

The metabolic syndrome is a metabolic disorder that is becoming more common with the increased prevalence of obesity.[50] The disorder is defined in various ways, and a new definition was recently proposed by the International Diabetes Federation (IDF). [51] In my study, the latest National Cholesterol Education Program's Adult Treatment Panel III (NCEP: ATP III) criteria [53] were used.

The NCEP: ATP III identified the metabolic syndrome as a multiplex risk factor for cardiovascular disease (CVD). However, according to the new stricter IDF definition, for a person to be defined as having the metabolic syndrome they must have central obesity plus any two other factors as listed in figure 4.

The concept of the metabolic syndrome has existed for at least 80 years. In 1988, Reaven resurrected the syndrome and noted that several risk factors, in particular dyslipidaemia, hypertension, hyperglycaemia, commonly cluster together in what he called Syndrome X.[54]

Even though CVD is a primary clinical outcome of the metabolic syndrome, most people with this syndrome have insulin resistance, which increases the risk for type 2 diabetes.[52] Other associations of the metabolic syndrome include hyperuricaemia, gout, gallbladder disease, polycystic ovary syndrome, hypertension, sleep disturbances, asthma, some forms of cancer as well as NAFLD.[52]

1.2.2 Components of the Metabolic Syndrome

Six components of the metabolic syndrome that relate to CVD, have been Identified by the ATP III: [52,53]

a) Abdominal obesity as measured by waist circumference
b) Atherogenic dyslipidaemia, manifested by raised concentrations of serum triglycerides and low concentrations of HDL cholesterol.
c) Hypertension, which is strongly associated with obesity and insulin resistance.
d) Glucose intolerance, that may later evolve into frank diabetes mellitus, constituting a major independent risk factor for CVD.
e) A proinflammatory state, manifests clinically by a raised hs C-reactive protein (hs CRP)
f) A prothrombotic state, recognized by an elevation in the levels of plasminogen activator inhibitor-1 (PAI-1) and fibrinogen.

1.2.3 Pathogenesis of the Metabolic Syndrome

There are several proposed aetiological factors. These include the following:

a) Obesity and Abdominal Body Fat Distribution

Obesity is a chronic condition characterised by an excess of body fat. It is most often defined by the Body Mass Index (BMI), a mathematical formula

that is highly correlated with body fat. BMI is the weight in kilograms divided by the height in metres squared (kg/m²). The World Health Organization recognises the following classification system for health consequences of obesity using the BMI. A BMI of more than or equal to 25kg/m² is defined as overweight, which is further classified as pre-obese with index between 25.0 and 29.9 kg/m², and a BMI above 30 kg/m² is categorised as obese.[55] Those with a BMI of more than and equal 40.0 kg/m² are morbidly obese.[56] Obesity has reached epidemic proportions in America, effecting 25% to 30% of the population.[10] The epidemic however, is no longer confined to the USA. In South Africa, the prevalence of obesity is about 19%.[57] These figures parallel the rise in insulin-resistant states such as the metabolic syndrome and type 2 diabetes.[10] Obesity contributes to hypertension, high serum cholesterol, low HDL cholesterol and hyperglycaemia and is associated with a higher CVD risk. Abdominal obesity is strongly associated with the other metabolic risk factors. Excess adipose tissue releases several by-products which aggravate the risk. These include nonesterified fatty acids (NEFA), which overload muscle with lipids that are later diverted to the liver enhancing insulin resistance and promoting fatty liver. Other products include, high interleukin 6, resistin, TNF α and CRP

levels signifying a proinflammatory state and an elevated PAI-1 contributing to a prothrombotic state. Low adiponectin levels on the other hand correlate inversely with the degree of obesity.

b) Insulin Resistance:

It is thought that insulin resistance plays a greater role than obesity in the pathogenesis of the metabolic syndrome, where it directly causes other metabolic risk factors.[54, 58] However, trying to assign a unique role for insulin resistance is complicated by the fact that it is linked to obesity making the identification of the cause and effects difficult.

Insulin resistance rises with an increasing BMI, yet a broad range of insulin sensitivities exist at any given BMI.[59] Insulin resistance may exist even with BMIs of less than 25 kg/m² such as in the South Asians. In fact, it may contribute to a high prevalence of type 2 diabetes and premature CVD in this population group. They can be said to have primary insulin resistance. However, even with primary insulin resistance, weight gain may worsen insulin resistance and the metabolic syndrome. Thus, the two appear to be closely linked and are not easily dissociated.[52]

c) Other Independent and Contributing Factors that mediate specific components of the Metabolic syndrome:

Each risk factor of the metabolic syndrome is subject to its own regulation by both genetic and acquired factors and, as a result, there is a variation in their expression. For example, lipoprotein metabolism is modulated by genetic variation, hence the expression of dyslipidaemias in response to obesity and/or insulin resistance varies considerably. Glucose levels depend on insulin secretory capacity as well as insulin sensitivity. Endocrine factors, advancing age and proinflammatory states have also been implicated.[52, 60]

1.2.4 Diagnosis of the Metabolic Syndrome

Using the ATP III criteria for the diagnosis of the metabolic syndrome, the clinical signs of the metabolic syndrome can be easily detected (Figure 3). It is critical to ensure proper diagnosis, dissemination of information to patients regarding their risks and appropriate therapies, and initiation of appropriate therapies.

The evaluation of patients who present features suggestive of metabolic syndrome should include the following:

a) Measurement of vital signs including blood pressure, and body weight;

b) Measurement of waist circumference;

c) Measurement of fasting blood sugar; and

d) A fasting lipid profile (total cholesterol, HDL-C, TG, and a calculated LDL-C)

According to the ATP III definition, a subject has the metabolic syndrome if 3 or more of the criteria listed in Figure 3 are present. These are similar to the Guidelines for Type 2 Diabetes at Primary Care in 2003 published by the Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA). [61]

Figure 3. The ATP III Diagnostic Criteria for the Metabolic Syndrome [53]

1. Abdominal obesity * (waist circumference > 102 cm [40 in] in men, > 88 cm [35 in] in women)
2. Hypertriglyceridaemia (>/=1.7mmol/L [>/= 150 mg/dL]) or drug treatment for elevated TG).
3. Low HDL-C (<1.03mmol/L [< 40 mg/dL] in men, <1.3mmol/L [< 50 mg/dL] in women or drug treatment for reduced HDL-C).
4. High blood pressure (>/= 130/85 mm Hg or drug treatment for hypertension).
5. High fasting glucose (>/=5.6mmol/L [>/= 100 mg/dL] or drug treatment for elevated glucose)

*These cut offs are lower for Europids, >/= 94 cm in men and >/= 80 cm in women, and even lower for South Asians >/= 90 cm in men and >/= 80 cms

in women. For South Africa (Sub-Saharan Africans), the Europid cut offs should probably be used, and for Asians, the South Asian cut offs. [51]

Figure 4. The IDF Diagnostic Criteria for the Metabolic Syndrome [51]

According to the new IDF definition, for a person to be defined as having the metabolic syndrome they must have:

Central obesity (waist circumference >/= 94 cm in men, >/= 80 cm in women)*

Plus any two of the following four factors:

1. Hypertriglyceridaemia (>/=1.7 mmol/L [>/= 150 mg/dL]), or specific treatment for this lipid abnormality
2. Low HDL-C (<1.03mmol/L [< 40 mg/dL] in men, <1.3 mmol/L [< 50 mg/dL] in women), or specific treatment for this lipid abnormality
3. High blood pressure (systolic BP >/= 130 or diastolic BP >/= 85 mm Hg), or treatment of previously diagnosed hypertension
4. High fasting glucose (>/=5.6 mmol/L [>/= 100 mg/dL]), or previously diagnosed type 2 diabetes

* Using the Europid cutoffs

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CHAPTER 2

THE ROLE OF INCREASED GASTROINTESTINAL ALCOHOL PRODUCTION IN PATIENTS WITH THE METABOLIC SYNDROME

2.1 AIMS OF THE STUDY

It has been shown that small increases in gut derived alcohol might increase portal blood alcohol levels which are enough to induce hepatic steatosis. Animal studies have demonstrated the presence of increased gut alcohol production by intestinal bacteria which has probably lead to the pathogenesis of NAFLD.

The main aim of this study was to evaluate patients with the metabolic syndrome who had clinical features of non-alcoholic fatty liver disease (NAFLD) for the presence of increased intestinal production of alcohol. The study was done in an attempt to increase our level of understanding of the aetiology and pathogenesis of NALFD, and thereby possibly identify new approaches to the management of the disease.

2.2 SUBJECTS UNDER STUDY

The study group included 20 patients attending both state and private

Endocrine and Liver clinics at the Johannesburg Hospital. The control group included 20 healthy hospital staff members or their relatives. Informed consent was obtained from every subject before enrollment into the study. Ethics Committee clearance was obtained from the University of the Witwatersrand (M03-09-25, see Appendices).

Inclusion criteria included:

1. **NCEP:ATP III criteria for the diagnosis of the metabolic syndrome.**
2. No history of alcohol use.
3. Deranged liver function tests.
4. The presence of fatty liver disease on abdominal ultrasound.

The inclusion criteria for the control group were:

1. **Absence of the metabolic syndrome as defined by the NCEP: ATP III criteria.**
2. No history of alcohol use/ abuse.
3. Normal liver function tests.
4. No history of medical illness.

Fasting blood, breath, and urine samples were obtained from the study group and the control group to assess the levels of alcohol in blood, breath and urine.

2.3 DATA COLLECTION

2.3.1 Data

Data were collected from patients' records. This included details of risk factors for NAFLD such as type 2 diabetes, hypertension and dyslipidaemia. Diabetes mellitus was defined as a fasting blood glucose >/= to 5.6 mmol/L or a previous diagnosis of diabetes. Hypertension was defined as a blood pressure of >/= to 130/85 or on antihypertensive therapy. Dyslipidaemia was defined as a fasting total-cholesterol of > 5 mmol/l, a triglyceride of > 1.5 mmol/l, a HDL-cholesterol of < 1.2 mmol/l and/or a LDL-cholesterol of > 3 mmol/l, or was assumed to be present if the subject was on lipid modifying therapy (Appendix 9).

Details of current and previous drug as well as supplement medication was also taken.

They were all physically examined, and anthropometric measurements

included weight, height and waist circumference. BMI was calculated using weight in kilograms divided by height in metres squared. All subjects were also examined for complications of their medical problems. Blood tests included the total cholesterol, HDL-C, TG, calculated LDL-C, HbA1C levels, fasting glucose levels, AST as well as ALT levels.

Ultrasound scanning of the liver was also performed to assess the degree of steatosis. Healthy control subjects were age, sex and race matched. Blood samples were obtained for liver enzymes, a fasting lipogram and a fasting glucose. Both the study and control subjects were screened to exclude other causes of chronic liver disease. This included screening for Hepatitis B and C infection.

2.4 SPECIMEN ANALYSIS

2.4.1 Alcohol Analysis

Blood, urine and breath samples were collected and analysed for alcohol levels. This was performed using Gas Chromatography (GC), which has been accepted as a worldwide standard for forensically estimating blood alcohol concentrations.

The columns used for alcohol GC analyses were

mainly supplied by Sigma-Aldrich being one of them. Their recommendations (Sigma-Aldrich catalogue, 2004/2005, page 318) were adapted to suit the experimental procedures and sensitivity required. Sources of error were greatly reduced by limiting the number of steps in the protocol for the sample procedure. For this reason the direct injection method was used.[62]

Most samples were analysed on the same day. If not possible, the samples were stored at –20ºC and thawed slowly at ambient room temperature and mixed well before analysis. In order to verify the stability of these preserved samples, the samples that were analysed on the day of collection were aliquoted and frozen. After 30 days, these samples were thawed to room temperature and the alcohol concentration estimated by GC. This was a standard practice in the estimation of volatile substances such as alcohol.

Samples were measured using a Hewlett Packard 5890A GC, Spectra Physics 4820 Integrator, Supelco Carbowax Capillary column (30 metre x 0.32 mm ID), Hamilton 5 µL syringe, Amican Ultra- MC centrifugal filter.

Laboratory pure ethanol, methanol and n-Propanol standards were used for calibration.The carbowax column was inserted into the GC as per the

manufacturer’s instructions.

Nitrogen was used as the carrier gas and set to a flow rate of approximately 1.0mL/min. The oven was controlled at 45ºC. Stock standard solutions of methanol, ethanol and n-propanol were made to 2% in distilled water.

A 190µL aliquot was placed in a Millipore filter with a cut-off of 10kD and 10µL of the internal standard of 2% n-propanol was added. This was then centrifuged in a microfuge at 12rpm (xG) for 30 minutes.

Four microlitres of the filtrate was then injected into the GC and the integrator activated at the same time. Both methanol and ethanol were quantified by preparing a range of concentrations between 0.01%-0. 1% and including n-propanol at a fixed concentration of 0.1% for each standard.

The calculation of the ratios of the integrated area of each alcohol to the area of the 0.1% n-propanol gave a figure that could be plotted on a graph, the vertical y-axis being the ratio and the horizontal x-axis, the alcohol concentration. The graph thus depicted yielded a straight line indicating that the ratio of alcohol to n-propanol was linear, confirming a directly propotional relationship. Incorporation of the 0.1% n-propanol in

each unknown sample permitted the direct calculation of the alcohol being sought.

The carbowax column separated the alcohols presented according to their chain length, it therefore followed that methanol was the first to be eluted, this was followed by ethanol and then the n-propanol standard. Once the ratio to n-propanol to either the methanol or the ethanol standards was established, one was able to directly calculate the concentration of the alcohol eluted. Since methanol had a molecular mass of only 32, it became fairly difficult to establish its presence using mass spectrometry. However, by fitting a less polar capillary column (DB24) in the GC, the elution profile of the different alcohols did vary with time. This was definitive in identifying the alcohols eluted when compared with the known alcohol standards. Analytes that appeared to be methanol were identified and confirmed using this technique.

Of note, urine and breath samples were injected directly onto the GC column without preparation, however serum samples have a more complex matrix and the proteins present could form deposits on the capillary column in the GC thus affecting the efficiency of the column. It was therefore deemed necessary to remove most of the protein prior to

analysis by employing Millipore cut-off filters that excluded any molecules greater than 10kD. Alcohols have a molecular masses much lower than the cut-off and would therefore pass freely through the filter. This was verified by incorporating the propanol as the internal standard into the serum samples to be tested. The propanol from these serum samples gave results comparable to the aqueous propanol standard.

2.4.2 Analysis of Blood

Blood samples were collected from the study and control subjects for liver enzymes, a fasting lipogram and a fasting glucose. Hepatitis B and C serologies were also done.

Blood was assessed for adiponectin, leptin, NEFA, and hsCRP levels. Blood specimens were centrifuged and their supernatants (serum) were dispensed into 5ml labelled plastic tubes and stored at -70°C for batch analysis at a later stage.

Serum NEFA analysis was performed manually using the Half-micro test (Roche), an enzymatic colorimetric assay. Leptin analysis was performed using the Quantikine® Human Leptin Immmunoassay (R&D systems) while

Adiponectin analysis was done using the Quantikine® Adiponectin Immunoassay (R&D systems). These assays employed the quantitative sandwich enzyme immunoassay technique.

Immunoturbidimetric analysis for the in vitro quantitative determination of hs-CRP in serum was carried out using the Tina-quant (Roche) C-reactive (latex) high sensitive assay.

All tests were performed following the manufacturer's instructions and the concentrations were calculated as per instructions. To avoid inter-assay variability, all assays were performed as single batch assays. All assays had an inter-assay variability of less than 5%.

2.5 STATISTICAL ANALYSIS

Data was analysed using the GB- Stat v4 program (Dynamic Microsystems, Inc. Silver Springs, MD 20904). Quantitative or continuous data were either expressed as the mean ± SD, with data that was normally distributed, or as median with a range for data that was non-parametric. Categorical variables which were expressed as percentages were evaluated using the Fisher's 2-tailed exact test. Continuous variables were analysed with the unpaired Student's t test. All reported P values were considered as significant at a level of $P< 0.05$.

CHAPTER 3

RESULTS

3.1 Table1: Anthropometric Data

The study group and control group were comparable in terms of age, gender and ethnicity. All the study subjects were already on treatment for their medical conditions.

Although 45% of the control group were found to have dyslipidaemia this was mild in all cases (TC < 7.5 mmol/l), and none were on lipid modifying treatment.

There was a significant difference between the two groups in the waist circumference and body mass index ($p < 0.0001$).

	Study Group (n=20)	Control Group (n=20)	P value
Gender (M/F)	8/12	8/12	NS
Age (years)	48±15.3	48±16.2	NS
WC (cm)	111±14.9	82.±8.8	<0.0001
BMI (kg/m²)	35.1±8.2	23.3±2.1	<0.0001
Hypertension	12/20 (60%)	None	<0.01
Dyslipidaemia	16/20 (80%)	9/20 (45%)	<0.01
Diabetes/IFG	14/20 (70%)	None	<0.01
Ethnicity:			
Black	2/20 (10%)	2/20 (10%)	NS
White	12/20 (60%)	12/20 (60%)	NS
Indian	4/20 (20%)	4/20 (20%)	NS
Chinese	1/20 (5%)	1/20 (5%)	NS
Coloured	1/20 (5%)	1/20 (5%)	NS

Values are mean ± SD. NS – Not Significant

3.2 Table 2: Fasting Lipid Profiles

HDL levels were significantly lower and NEFA concentrations significantly higher in the study group versus the control group. Total cholesterol and triglyceride levels were also higher in the study group but this was not significant.

	Study Group (n=20)	Control Group (n=20)	P value
Total Cholesterol (mmol/L)	5.71±3.25	5.27±1.10	NS
Triglycerides (mmol/L)	7.16±18.50	1.11±0.58	NS
HDL C (mmol/L)	1.23±0.36	1.58±0.42	<0.01
NEFA (µmol/L)	863.5±0.46	432.9±0.16	<0.001

Values are mean ± SD. NS – Not Significant

3.3 Table 3: Serum Aminotransferases

The differences between the study and control groups were significant with the study groups' ALT levels being 57.4±44.79 U/L versus 17.4 ±4.60 U/L in the control group (95% CI 18.02 - 61.42, P < 0.01) and the study groups'AST levels being 52.5 ±36.21 U/L versus 23.4 ±4.86 U/L in the

control group (95% CI 11.99 – 46.20, P < 0.01).

	Study Group (n=19)	Control Group (n=20)	P value
ALT (U/L)	57.4±44.79	17.7±4.60	<0.001
AST (U/L)	52.5±36.21	23.4±4.86	<0.01
AST:ALT ratio	1.01	1.37	<0.01

Values are mean ± SD

3.4 Table 4: Ultrasound Results

Seventy five percent had features of fatty liver disease on sonar.

Five of the study subjects had liver biopsies confirming the diagnosis of NAFLD. These were done prior to inclusion into the study.

	Study Group (n=20)	Control Group (n=20)
Yes	15(75%)	ND
No	5(25%)	ND

ND – Not Done

3.5 Table 5: Alcohol Measurements

Of the twenty patients with NAFLD screened for any alcohol present, 60% tested positive. Fifty five percent tested positive for methanol, while 35% were positive for ethanol. Of note, no alcohol was detected in the control subjects.

Alcohol	**Study Group (n=20)**	**Control Group (n=20)**	**P value**
Ethanol Present	7 (35%)	0 (0%)	=0.0084*
Methanol present	11(55%)	0 (0%)	=0.0000*
Ethanol and Methanol present	6 (30%)	0 (0%)	=0.0202*
Any alcohol present	12 (60%)	0 (0%)	=0.0000*

*2 tailed Fisher exact test.

3.6 Table 6: Study Subjects with detectable Alcohol concentration levels

The methanol concentration was highest in the blood and significantly higher when compared to that in the urine and breath.

Case No.	**Ethanol (mg %)**			**Methanol (mg %)**		
	Blood	**Urine**	**Breath**	**Blood**	**Urine**	**Breath**
3	9	ND	ND	2	1.6	2.4
4	2	ND	ND	1.8	ND	ND
6	ND	ND	ND	50	ND	ND
7	8	44.6	ND	29	32.3	ND
8	8	ND	ND	2	ND	8
9	10	ND	ND	ND	ND	ND
10	10	ND	ND	21	ND	8
11	ND	ND	ND	7.1	ND	ND
12	ND	ND	ND	3	ND	ND
13	3	ND	ND	9	38	1
14	ND	ND	ND	7	ND	ND
15	ND	ND	ND	46	9.7	5.3
Mean ± SD	7.14± 3.28	3.71± 12.87	0	16.17± 17.95	6.8± 13.58	2.05± 3.19

ND - Not Detected.

3.7 Table 7: Adipocytokines

There was a significant difference in the adiponectin and leptin concentrations between the two groups, the adiponectin levels being significantly lower and the leptin levels being significantly higher in the study group than in the controls.

	Study Group (n=20)	**Control Group (n=20)**	**P value**
Adiponectin (ng/L)	6875 (1090-29600)	15475 (4750-95200)	<0.01
Leptin (ng/L)	13.56 (0-76.1)	3.05 (0-35.3)	<0.05

Values are the median with the minimum-maximum range.

3.8 Table 8: Inflammatory Markers

The hs-CRP levels were significantly higher in the study group as compared to the control.

	Study Group (n=20)	**Control Group (n=20)**	**P value**
hs-CRP (mg/L)	6.46 (0.36-141.8)	0.86 (0.17-16)	<0.01

Values are the median with minimum-maximum range.

CHAPTER 4

DISCUSSION

NAFLD is considered to represent the hepatic expression of the Metabolic Syndrome. NAFLD can lead to NASH and subsequent chronic liver disease and cirrhosis. With the increasing prevalence of obesity, coupled with diabetes, dyslipidaemia and hypertension, a very large segment of the population is at risk for impending liver disease in the coming decades. It still remains an active area of research and a better understanding of the pathophysiology of NAFLD would help develop future therapies.

The study examined the possibility that endogenous production of alcohol may be responsible as a part of the 'second hit' theory for the pathogenesis of NAFLD. The fact that alcohol was detected in blood, urine and breath is important evidence for its pathogenesis in the development of NAFLD. This was in keeping with previous animal and human studies. [45,46]

Contrary to previous studies, methanol was also detected. [45, 46] A significantly higher percentage of patients had detectable levels of methanol than ethanol, 55% compared to 35%. No alcohol was detected in the controls, in keeping with the reported non consumption of

alcohol by our patients during their interviews. Of note, no ethanol was detected in the breath of the study subjects, which is not consistent with previous studies. [45, 46] This may be explained by the fact that the intestinally derived ethanol indeed underwent hepatic metabolism, and could not reach the lungs. However, methanol was detected in all body fluids measured. The methanol concentration was highest in blood and significantly higher when compared to that in urine and breath. It is a natural constituent of body fluids. [64] It is available in the diet and sourced principally from vegetables, fresh fruits and fruit juices. Other sources include diet drinks, in which the artificial sweetener aspartame is widely used, and when hydrolysed is partly converted to methanol. The amount of methanol ingested from fruit juices or diet beverages is debatable, but litres would have to be ingested before even being considered to be toxic.[64]

Intestinal bacterial overgrowth has been implicated to explain the increased production of endogenous ethanol. Alterations in gut motility associated with obesity, and complications associated with diabetes, such as visceral autonomic neuropathy, may be responsible for an increase in the intestinal bacterial overgrowth.

This study suggests that importance of endogenous alcohol production may be important in the pathogenesis of NAFLD in patients with the metabolic syndrome. The origin of the alcohol is uncertain, but the importance of intestinal bacterial flora and the role of foods, may be important in the pathology of this disease. If so, it may be that therapeutic alternatives such as the use of antibiotics and probiotics as new treatment strategies may have a role as been alluded to by recent studies.[64]

Rapid weight loss and long lasting fasting periods should be avoided, since they lead to an increase in the flow of NEFA to the liver. A gradual weight reduction is suggested as it is associated with improvement of hepatic lesions, including fibrosis.[65-69]

Control of diabetes, dyslipidaemia and hypertension with diet and drugs, with simultaneous treatment of obesity as suggested above, is of paramount importance.

Oral antibiotics such as metronidazole (flagyl) may be efficacious in reverting steatosis, and in some cases, inflammation and fibrosis. [70, 71] Doses may have to be reduced in severe liver disease. However, side effects such as gastrointestinal intolerance and an unpleasant taste, may affect long term compliance.

Prolong use may also cause a reversible peripheral neuropathy and it is contraindicated in the first trimester during pregnancy.

Probiotics may be a better alternative. They have shown promising results in recent studies. [72-74] They may interfere with the development of NAFLD at various levels: 1) decrease proinflammatory cytokines, 2) alter the inflammatory effects of pathogenic strains of intestinal bacteria, through changes in cytokine signaling, 3) improve epithelial barrier function and thereby avoid excessive exposure of the liver to cytokines and bacterial alcohol. Probiotics have shown to improve liver enzymes in treated patients.

Several cytoprotective agents and antioxidants have shown promising results. These include ursodeoxycholic acid, vitamin E, lecitin, B carotene, selenium, S-adenosyl-methionine, metadoxine and silimarin.[63]

In conclusion, this study therefore suggests that endogenous alcohol production may indeed be responsible for the ‘second hit’ theory in the pathogenesis of NAFLD.

CHAPTER 5

LIMITATIONS

The sample size may have been to small, and further larger studies are required to confirm these results. Eight control subjects have a high total cholesterol. However, none of them had severe hyperlipidaemia (defined as a TC > 7.5 mmol/L or TG > 5). This may have limited the overall result of the study.

Even though the waist circumference and BMI are considered to be reliable anthropometric indices of abdominal visceral adipose tissue accumulation, MRI or CT scans would precisely identify the proportion of abdominal adipose tissue, but these are expensive and surrogate measures of visceral fat had to be used instead, these being the waist circumference and the BMI.

No liver biopsies were performed. It has been argued that although a fatty liver may be readily detected on ultrasonography, a liver biopsy is necessary for the diagnosis of NASH, especially since imaging techniques do not detect hepatic fibrosis or inflammation easily in the

presence of fat. Since the only effective therapy at the moment is weight loss, it can be argued that these patients should not be subjected to liver biopsy and its associated risks. Only five patients had confirmed biopsies done prior to recruitment in to this study. No permission is granted generally by the Ethics Committee for invasive procedures. The use of other radiological studies such as the MRI or CT scans are expensive, and maybe nonspecific. We therefore made use of alternative surrogate markers such as hepatic enzymes, abdominal ultrasounds and adipocytokines as markers of steatosis.

In terms of alcohol levels detected, it would be a better to analyse portal blood for alcohol rather then peripheral blood. This is to avoid the first pass effect of metabolism of the liver, where most the body's toxins are metabolised, but this would be invasive and not practical. There are other factors also playing a role in the levels of alcohol detected such as the amount of adipose tissue present as this is a storage site. However, analysis of portal blood may be difficult and would require sophisticated techniques, but they should be considered.

Four patients had higher than normal levels of methanol, however.

This could have been due to porto-systemic shunting, explaining that some alcohol may have escaped directly into the systemic circulation escaping the portal blood, but this was not in keeping with their clinical picture as all the patients were clinically well.

REFERENCES

1. Lugwig J, Viggiano T, McGill D and Oh B. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980; **55**: 434-8.
2. Schaffner F and Thaler H. Nonalcoholic fatty liver disease. Prog Liver Dis 1986; **8**: 283-98.
3. Diehl AM. Non-alcoholic steatohepatitis. Seminars in Liver Disease. 1999; **19**:221-229.
4. Angulo P. Non-alcoholic fatty liver disease. NEJM 2002; **346**:1221-1231.
5. Alba LM et al. Non alcoholic fatty liver disease. Alimentary Pharmacology and Therapeutics 2003; **17**:977.
6. Nomura H, Kashiwagi S, Hayashi J, Kajiyama W, Tani S and Goto M. Prevalence of fatty liver in a general population of Okinawa, Japan. Jpn J Med 1988; **27**: 142-9.
7. Bellentani S, Saccoccio G, Masutti F et al. Prevalence of, and risk factors for, hepatic steatosis in northern Italy. Ann Intern Med 2000; **132**:112-7.

8. Luyckx F, Desaive C, Thiry A et al. Liver abnormalities in severely obese subjects: effect of drastic weight loss after gastroplasty. Int J Obes Related Metab Disord 1998; **22**: 222-6.
9. Daniel S, Ben-Menachem T, Vasudevan G, Ma CK and Blumenkehl M. Prospective evaluation of unexplained chronic liver transminase abnormalities in asymptomatic and symptomatic patients. Am J Gastroenterology 1999; **94**: 3010-4.
10. Green RM. NASH-hepatic metabolism and not simply the metabolic syndrome. Hepatology 2003; **38**: 14-17.
11. Sheth S, Gordon F and Chopar S. Nonalcoholic steatohepatitis. Ann Intern Med 1997; **126**: 137-145.
12. Rashid M and Roberts E. Nonalcoholic steatohepatitis in children. Journal of Pediatrics Gastroenterology Nutrition 2000; **30**: 48-53.
13. Manton N et al. Non-alcoholic steatohepatitis in children and adolescents. Med J Aust 2000; **173**: 476-9.
14. Angulo P, Keach J, Batts K and Lindor K. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999; **30**: 1356-62.

15. Itoh S, Yougel T and Kawagoe K. Comparison between non-alcoholic steatohepatitis and alcoholic hepatitis. Am J Gastroenterology 1987; **82**: 650-4.
16. Diehl A, Goodman Z and Ishak K. Alcohol-like liver disease in nonalcoholics: a clinical and histologic comparison with alcohol-induced liver disease. Gastroenterology 1989; **95**: 1056-62.
17. Pinto H, Baptista A, Camilo M, Valente A, Saragoca A, de Moura MC. Non-alcoholic steatohepatitis: clinicopathological comparison with alcoholic hepatitis in ambulatory and hospitalized patients. Dig Dis Sci 1996; **41**: 172-9.
18. Joseph A, Saverymuttu S, al-Sam S, Cook M and Maxwell J. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. Clin Radio 1991; **43**:26-31.
19. Debaere C, Rigauts H and Laukens P. Transient focal fatty liver infiltration mimicking liver metastasis. J Belge Radiol 1998; **81**: 174-5.
20. Mitchell D. Focal manifestations of diffuse liver disease at MR imaging. Radiology 1992; **185**:1-11.

21. Itoh S, Igarashi M, Tsukada T et al. Non-alcoholic fatty liver with alcoholic hyaline after long term glucocorticoid therapy. Acta Hepatogastoenterol 1977; **24**:415-419.
22. Lee R. Non-alcoholic steatohepatitis: Tightening the morphological screws on a hepatic rambler. Hepatology 1995; **21**:1742-1744.
23. Falchuk K, Fiske S, Hagit R et al. Pericentral hepatic fibrosis and intracellular hyalin in diabetes mellitus. Am J Gastroenterol 1980; **78**: 535-540.
24. Silverman J, O'Brien K, Long S et al. Liver pathology in morbidly obese patients with and without diabetes. Am J Gastroenterol 1990; **85**: 1345-1355.
25. Marchesini G, Brizi M, Morselli-Labate A et al. Association of non-alcoholic fatty liver disease with insulin resistance. Am J Med 1999; **107**:450-5.
26. Reynet C and Kahn C. Rad: A member of the Ras family overexpressed in muscle of type II diabetic humans. Science 1993; **262**:1441-4.
27. Maddux B, Sbraccia P, Kumakura S et al.Membrane glycoprotein

PC-1 and insulin resistance in non-insulin dependent diabetes mellitus. Nature 1995; **373**; 448-51.

28. Cohen B, Novick D and Rubinstein M. Modulation of insulin activities by leptin. Science 1996; **274**: 1185-8.
29. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 1997; **46**: 3-10. (Erratum, Diabetes 1997; 46:536)
30. Hotamisligil G, Peraldi S, Budvari A, Ellis R, White M and Spiegelman B. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF alpha and obesity-induced insulin resistance. Science 1996; **271**: 665-8.
31. Fan C, Pan J, Usuda N, Yeldandi A, Rao M and Reddy J. Steatohepatitis, spontaneous peroxisome proliferation and liver tumours in mice lacking peroxisomal fatty acyl-CoA oxidase: implications for peroxisome proliferator-activated receptor alpha natural ligand metabolism. J Biol Chem 1998; **273**:15639-45.
32. Chavin K, Yang S, Lin H et al. Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. J Bio Chem 1999; **274**: 5692-700.

33. Caldwell S, Swerdlow R, Khan E et al. Mitochondrial abnormalities in non-alcoholic steatohepatitis. J Hepatol 1999; **31**: 430-4.

34. Sanyal A, Campbell-Sargent C, Mirshani F et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001; **120**: 1183-92.

35. Cortez-Pinto H, Chatham J, Chacko V, Arnold C, Rashid A and Diehl A. Alterations in liver ATP homeostasis in human non-alcoholic steatohepatitis: a pilot study. JAMA 1999; **282**:1659-64.

36. Day C and James O. Steatohepatitis: a tale of two “hits”? Gastroenterology 1998; **114**: 842-5.

37. James O and Day C. Commentary: Non-alcoholic steatohepatitis: another disease of affluence. The Lancet. 1999; **353**: 1634-1636.

38. Wanless I and Lentz J. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology 1990; **12**: 1106-10.

39. McNair A. Non-alcoholic steatohepatitis (NASH): why biopsy? Gut

2002; **51**(6): 898-899.

40. Ratziu V, Charlotte F, Heurtier A et al. Sampling variability of liver biopsy in Non-alcoholic fatty liver disease. Gastroenterology 2005; **128**(7): 1898-906.

43. Gawrieh S, Rosdo B, Lindor M and Charlton M. Adipokine levels are predictive of histology in patients with non-alcoholic fatty liver disease. Hepatology. 2004; **40**: 237A.

44. Uygun A, Kadayifci A, Yesilova Z, Erdil A, Yaman H et al. Serum leptin levels in patients with non-alcoholic steatohepatitis. Am J Gastroenterology 2000; **95**(12): 3584-9.

45. Vuppalanchi R, Marri S, Kolwankar D, Considine R and Chalasani N. Is adiponectin involved in the pathogenesis of non-alcoholic steatohepatitis? A preliminary human study. J Clin Gastroenterology 2005; **39**(3): 237-42.

46. Cope K, Terence R and Diehl AM. Increased gastrointestinal ethanol production in obese mice: Implications for fatty liver disease pathogenesis. Gastroenterology 2000; **119**:1340-1347.

47. Nair S, Cope K, Terence R and Diehl AM. Obesity and female

gender increase breath ethanol concentration: Potential implications for the pathogenesis of non alcoholic steatohepatitis. Am J Gastroenterol 2001; **96**: 1200-1204.

48. Friedman S. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 2000; **275**: 2247-2250.

49. Bansil R, Stanley P and LaMont J. Mucin biophysics. Annu Rev Physiol 1995; **57**: 635-657.

50. The Merck Manual of Diagnosis and Therapy. Liver disease due to alcohol. 16th Edition; Chap 67: 890-93.

51. Eckel R, Grundy S and Zimmet P. The metabolic syndrome. The Lancet. 2005; **365**: 1415-1428.

52. The metabolic syndrome- a new world-wide definition. The Lancet 2005; **366**: 1059-62.

53. Grundy S et al. Definition of the metabolic syndrome. Circulation. 2004; **109**: 433-438.

54. Grundy et al. Diagnosis and Management of the Metabolic Syndrome. Circulation 2005; **112**: e285-e290.

55. Reaven G. Banting lecture 1988: Role of insulin resistance in

human disease. Diabetes. 1988; **37**: 1595-1607.

56. Arterburn D and Noel P H. Extracts from “Clinical Evidence”: obesity. BMJ 2000; **322**: 1406-1409.

57. Despres JP, Lemieux I and Prud’homme. Treatment of obesity: Need to focus on high-risk abdominally obese patients. BMJ 2001; **322**: 716-720.

58. Data from South Africa’s first National Demographic and Health Survey, 1998: Guidelines for the prevention and management of overweight and obesity in South Africa. SA Society for the Study of Obesity; Appendix A, Table A1.

59. Ferrannini E, Haffner S, Mitchell B et al. Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. Diabetologia. 1991; **34**: 416-422.

60. Abbasi F, Brown B, Lamendola C et al. Relationship between obesity, insulin resistance and coronary heart disease risk. J Am Coll Cardiol. 2002; **40**: 937-943.

61. Ford E, Giles W and Dietz W. Prevalence of the metabolic syndrome among US adults: Findings from the third National Health and Nutrition Examination Survey. JAMA. 2002; **287**: 356-

359.

62. Guidelines for Type 2 diabetes at primary care in 2003. Society for Endocrinology, Metabolism and Diabetes of South Africa.
63. Tangerman A. The high sensitive gas chromatographic analysis of ethanol in whole blood, serum, urine and faecal supernatant by the direct injection method. Clinical Chemistry. 1997; **43**(6): 1003-1009.
64. Medina J, Fernandez-Salazar L, Garcia-Buey L and Moreno-Otero R. Approach to the pathogenesis and treatment of non-alcoholic steatohepatitis. Diabetes Care. 2004; **27**: 2057-2066.
65. Environmental Health Criteria 196: Methanol pp. 1-9 (1997) by The International Programme on Chemical Safety (IPCS) under the joint sponsorship of the UN Environment Programme, the ILO and the WHO.
66. Kugelsmas M et al. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. Hepatology 2003; **38**: 413-419.
67. Ueno T et al. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. J Hepatol 1997; **27**: 103-107.

68. Palmer M and Schaffner F: Effect of weight reduction on hepatic abnormalities in overweight patients. Gastroenterology 1990; **99**: 1408-1413.

69. Drenick E et al. Effect on hepatic morphology of treatment of obesity by fasting, reducing diets and small-bowel bypass. N Engl J Med 1970; **282**: 829-834.

70. Neuschwander-Tetri B and Bacon E. Nonalcoholic steatohepatitis. Med Clin North Am 1996; **80**: 1147-1166.

71. Sheth S et al. Non-alcoholic steatohepatitis. Ann Intern Med 1997; **126**; 137-145.

72. Lugwig J et al. Review: Non-alcoholic steatohepatitis. J Gastroenterol Hepatol 1997; **12**: 398-403.

73. Li Z et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve non-alcoholic fatty liver disease. Hepatology 2003; **37**: 343-350.

74. Solga S and Diehl. Non-alcoholic fatty liver disease: lumen-liver interactions and possible role for probiotics. J Hepatol 2003; **38**: 681-687.

75. Loguercio C et al. Gut-liver axis: a new point of attack to treat

chronic liver damage? Am J Gastroenterol 2002; **97**: 2144-2146.

76. Diagnosis, management and prevention of the common dyslipidaemias in South Africa. Clinical Guideline 2000. S Afr Med J 2000; **90**: 164-178.