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Effects of Dietary Fatty Acids on Insulin Resistance, Tissue Lipid Profile and Adipose Tissue Cellularity in Sprague-Dawley Rat

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**Effects of Dietary Fatty Acids on Insulin Resistance, Tissue Lipid Profile
and Adipose Tissue Cellularity in Sprague-Dawley Rat**

By

Tekeleselassie Ayalew Woldemariam

**A thesis submitted to School of Graduate Studies, Universiti Putra Malaysia
in fulfilment of the requirement for the Degree of Master of Science**

July 2009



DEDICATION

This thesis is dedicated to my family



Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

Effects of Dietary Fatty Acids on Insulin Resistance, Tissue Lipid Profile and Adipose Tissue Cellularity in Sprague-Dawley Rat

By

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Insulin resistance describes a dysfunctional state of glucose metabolism which often occurs in advance of any metabolic diseases in human population. Dietary fatty acids are closely linked to insulin resistance as they are known to modulate fatty acid and glucose metabolism in mammals. In this study, fatty acids from butter, soybean and menhaden oil were separately incorporated into rat chow diet to assess the differential effect of dietary fatty acids on the various indicators and risk factors of insulin resistance. These include glucose clearance functions, plasma insulin, body composition, tissue and plasma fatty acid profiles, blood lipids, adipose cellularity and leptin level. A total of 40 male Sprague-Dawley rats of 9 weeks age, randomly allocated to four treatment groups of ten animals each, were employed in this study. The treatment groups consisted of rats fed with chow diet (CD), rats fed chow diet fortified with 10% w/w butter (BCD), rats fed chow diet added with 6.67 % w/w menhaden oil and 3.33% w/w soybean oil (MCD), and rats fed chow diet added with 3.33 % w/w menhaden oil and 6.67 % w/w soybean oil (SCD). The rats were subjected to their respective treatment



diets for 22 weeks and body weight was measured weekly. Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were carried out on day 0, and then later in the 12th and 20th weeks of dietary intervention to assess changes as a result of insulin resistance. Serial plasma insulin levels were also quantified on day 0 and in the 20th week. Upon termination of the trial at the end of the 22nd week, post mortem body composition and inguinal fat cellularity were performed on the rats. Plasma leptin and blood lipids in all treatment groups were measured. Determination of fatty acid profile of selected tissues (plasma, red blood cell membrane, liver and skeletal muscle) were also carried out. Generally, tissue and plasma fatty acid profiles were reflective of the dietary fatty acid composition. Results showed that glucose clearance in all treatment groups was not compromised as a result of dietary intervention. However, the BCD group consistently showed higher blood glucose spike 15 minutes after initial glucose loading, and higher blood glucose readings even after insulin challenge during IPITT compared to the other groups. The glucose clearance capacities of MCD and SCD fed animals remained similar to that of their initial baseline values even after 20 weeks of treatment. Unlike glucose concentration, plasma insulin level was significantly ($P < 0.05$) higher in a majority of time points in the BCD rats compared to the MCD and SCD rats in the 20th week. The corresponding total amount of plasma insulin by time as indicated by the area under the plasma insulin curve, (AUC) for the BCD rats was 456.7 ± 27.7 ng/L min. This was significantly higher ($P < 0.05$) than those of the CD (335.5 ± 38.5 ng/L min), MCD (273.7 ± 37.6 ng/L min) and SCD (265.9 ± 21.7 ng/L



min) rats. Area under the curve (AUC) values also showed that all treatment groups, (CD, MCD and SCD) had much higher ($P<0.05$) plasma insulin values after 20 weeks of treatment, compared to their baseline concentration of 200.3 ± 21.6 ng/L min. Apart from being hyperinsulinaemic, the insulin sensitivity index of BCD rats was found to be significantly ($P<0.05$) compromised unlike those of the MCD and SCD rats. Risk factors associated with insulin resistance such as excessive body fat accumulation and adipocyte cellularity were altered by dietary fatty acids. Inguinal fat cellularity results showed large and hypertrophied adipocytes in the BCD rats, while adipocytes in the MCD and SCD rats became hyperplastic but significantly smaller ($P<0.05$) than those of BCD rats. Plasma leptin was elevated significantly ($P<0.05$) in the BCD rat (3.22 ± 0.32 ng/mL) compared to MCD (2.37 ± 3.2 ng/mL), SCD (2.29 ± 0.35 ng/mL) and CD (2.16 ± 0.11 ng/mL) groups. Blood lipid picture was found to be healthier in the MCD and SCD supplemented groups. These two groups had significantly ($P<0.05$) lower total cholesterol and triacylglycerol (TAG) contents than the BCD-fed rats. This was accompanied by significantly reduced high density lipoprotein cholesterol (HDL-C) in the MCD (0.15 ± 0.05 mmol/L) and SCD (0.19 ± 0.05 mmol/L) rats, compared to a value of 0.34 ± 0.07 mmol/L observed for the BCD rats. Therefore, it was concluded that 10% dietary fat supplementation from menhaden and soybean oil could delay the onset of hyperinsulinaemia, and possibly insulin resistance in the rat model. Furthermore, PUFA was also shown to have an effect on the risk factors and other indicators for insulin resistance such as adipocyte cellularity, blood lipids and leptin.

KEYWORDS: fatty acids, insulin resistance, lipid profile, adipose, rat.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai sebahagian keperluan untuk ijazah Master Sains

KESAN ASID LEMAK KE ATAS KERINTANGAN INSULIN, PROFIL LIPID DAN KEBERSELAN TISU ADIPOS PADA TIKUS SPRAGUE-DAWLEY

oleh

TEKELESELISSIE AYALEW WOLDEMARIAM

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Kerintangan insulin merupakan satu keadaan disfungsi akibat kegagalan metabolisme glukos. Ia sering berlaku sebelum kemunculan penyakit metabolik. Asid lemak mempunyai perkaitan yang rapat dengan kerintangan insulin. Hal ini memandangkan metabolisme glukos dan asid lemak pada haiwan mamalia dipengaruhi oleh komposisi asid lemak. Penyelidikan ini mengkaji kesan penambahan asid lemak daripada mentega, minyak kacang soya dan minyak Menhaden dalam rangsum tikus ke atas penunjuk dan faktor risiko kerintangan insulin. perkara Ini termasuklah kadar pembersihan glukos, insulin plasma, komposisi badan, profil asid lemak tisu & plasma, lipid darah, keberselan adipos dan leptin. Sejumlah 40 ekor tikus yang dibahagikan secara rawak kepada empat kumpulan (n=10) telah digunakan dalam penyelidikan ini. Kumpulan rawatan terdiri daripada tikus yang diberi makan makanan tikus sahaja (CD), makanan tikus ditambah 10% (b/b) mentega (BCD), makanan tikus ditambah 6.67 % (b/b) minyak Menhaden serta 3.33 % (b/b) minyak kacang soya (MCD), dan makanan



tikus ditambah 3.33 % (b/b) minyak Menhaden serta 6.67 % (b/b) minyak kacang soya (SCD). Tempoh rawatan berlangsung selama 22 minggu dan berat badan diukur seminggu sekali. Ujian toleransi glukosa intraperitoneal (IPGTT) dan ujian toleransi insulin intraperitoneal (IPITT) telah dilakukan pada hari 0, dan pada minggu ke-12 dan ke-20. Ukuran insulin plasma secara bersiri telah dibuat pada hari 0 dan minggu ke-20. Penentuan komposisi badan dan keberselan lemak inguinal telah dibuat dalam minggu ke-20 semasa post mortem. Leptin dan lipid darah telah dicerap untuk semua subjek. Ini juga termasuk lipoprotein kolesterol plasma, tahap trigliserida dan profil asid lemak untuk plasma, membran eritrosit, hati dan otot rangka. Secara amnya, profil asid lemak plasma dan tisu mencerminkan komposisi asid lemak makanan. Keputusan juga menunjukkan bahawa fungsi pembersihan glukosa pada semua kumpulan rawatan tidak terjejas di akhir ujikaji. Walabagaimanapun, kumpulan BCD menunjukkan secara konsisten, tahap glukosa darah yang lebih tinggi 15 minit selepas suntikan glukosa, dan kandungan glukosa darah yang lebih tinggi walaupun selepas insulin disuntik berbanding kumpulan rawatan yang lain. Keupayaan pembersihan glukosa pada haiwan MCD dan SCD pada akhir ujikaji adalah lebih kurang sama dengan ukuran dasar yang diambil pada awal ujikaji. Bagaimanapun, tahap insulin plasma telah meningkat dengan mendadak ($P < 0.05$) pada kumpulan BCD berbanding dengan semua kumpulan lain pada akhir tempoh ujikaji. Jumlah insulin plasma dengan masa yang diwakili oleh luas permukaan di bawah lengkung insulin plasma (AUC) adalah 456.7 ± 27.7 ng/L min untuk kumpulan BCD, jauh lebih tinggi ($P < 0.05$) berbanding dengan kumpulan

CD (335.5 ± 38.5 ng/L min ng/L min), MCD (273.7 ± 37.6 ng/L min) dan SCD (265.9 ± 21.7 ng/L min). Nilai AUC juga menunjukkan bahawa semua kumpulan (CD, MCD dan SCD) merekodkan peningkatan insulin plasma yang ketara ($P < 0.05$) selepas 20 minggu. Selain menunjukkan tahap insulin yang tinggi, indeks kepekaan insulin untuk tikus BCD juga didapati terjejas secara signifikan ($P < 0.05$) berbanding dengan tikus MCD dan SCD. Faktor risiko yang menjurus kepada kerintangan insulin seperti pengumpulan lemak badan, dan peningkatan tahap keberselan adipos juga berubah mengikut jenis asid lemak yang ditambah ke dalam makanan tikus. Lemak inguinal memperlihatkan hipertrofi adiposit pada tikus BCD, sementara adiposit pada tikus MCD dan SCD menunjukkan saiz yang kecil dengan hiperplasia yang nyata ($P < 0.05$). Leptin plasma juga adalah lebih tinggi ($P < 0.05$) pada tikus BCD (3.22 ± 0.32 ng/mL), berbanding MCD (2.37 ± 3.2 ng/mL), SCD (2.29 ± 0.35 ng/mL) dan CD (2.16 ± 0.11 ng/mL). Profil lipid darah adalah lebih sihat pada kumpulan yang diberi makan rangsum MCD dan SCD. Kedua-dua kumpulan menunjukkan tahap kolesterol dan trigliserida yang lebih rendah ($P < 0.05$) berbanding dengan tikus BCD. Kandungan HDL-C juga lebih rendah ($P < 0.05$) di kalangan ahli kumpulan MCD dan SCD. Kesimpulannya, penambahan 10 % (b/b) asid lemak politaktepu (PUFA) pada diet mampu melambatkan hiperinsulinemia dan barangkali kerintangan insulin pada tikus. Oleh itu, PUFA ternyata mempunyai kesan yang signifikan ke atas factor-factor risiko dan penunjuk kerintangan insulin lain seperti komposisi lemak badan, lipid darah dan leptin.

KATA KUNCI : asid lemak, kerintangan insulin, profil lipid, adipos, tikus



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I certify that a Thesis Examination Committee has met on 13 July 2009 to conduct the final examination of Tekeleselassie Ayalew on his thesis entitled "Effects of Dietary Fatty Acids on Insulin Resistance, Tissue Lipid Profile and Adipose Tissue Cellularity in Sprague-Dawley Rat" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not currently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

Tekeleselassie Ayalew

Date: 27 April 2009



TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vii
ACKNOWLEDGEMENT	x
APPROVAL	xi
DECLARATION	xiii
LIST OF TABLES	xvii
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xix
CHAPTER	
I GENERAL INTRODUCTION	1
II LITERATURE REVIEW	6
Introduction to lipids and fatty acids	6
Role of dietary fatty acids in mammals	9
Glucose homeostasis	14
Glucose transport	16
Glycogen synthesis	19
Glycogenolysis	20
Gluconeogenesis	21
Insulin resistance	22
Determinants of insulin resistance	24
Dietary carbohydrates	25
Dietary fats	26
Types of dietary fat and obesity	27
Obesity and insulin resistance	29
Adipocytokines and insulin resistance	32
III EFFECTS OF DIETARY FATTY ACIDS SUPPLEMENTATION ON FATTY ACID COMPOSITION OF PLASMA AND BODY TISSUES IN THE RAT MODEL	37
Introduction and objective	37
Materials and methods	39
Experimental design	39
Experimental animals and housing	39
Experimental diet preparation	40
Sampling	41
Fatty acid profile determination	41
Data analysis	46
Results	47



Fatty acid profile of treatment oils	47
Fatty acid profile of treatment diets	47
Fatty acid profile of plasma	51
Fatty acid profile of RBC membrane	53
Fatty acid profile of muscle tissue	55
Fatty acid profile liver tissue	57
Discussion	59
Conclusions	62
IV INFLUENCE OF DIETARY FATS ON INSULIN SENSITIVITY AND BLOOD LIPID IN SPRAGUE- DAWLEY RATS	63
Introduction and objective	63
Materials and methods	65
Rat and dietary intervention	65
Measurement of insulin sensitivity	65
Plasma cholesterol and triacylglycerol profile assay	68
Data analysis	71
Results	72
Intraperitoneal glucose tolerance test	72
Intraperitoneal insulin tolerance test	76
Plasma insulin and insulin sensitivity index	80
Plasma cholesterol and triacylglycerol	83
Discussion	85
Conclusions	90
V INFLUENCE OF DIETARY FATTY ACIDS ON BODY COMPOSITION, INGUINAL ADIPOCYTE CELLULARITY AND PLASMA LEPTIN CONCENTRATION	91
Introduction and objective	91
Materials and methods	93
Animal and diet	93
Body composition determination	93
Adipocyte cellularity	94
Plasma leptin measurement	95
Data analysis	96
Results	97
Body weight, body composition and fat accretion	97
Adipocyte cellularity	98
Plasma leptin concentration	99
Discussion	101
Conclusions	106
VI GENERAL DISCUSSION	107
VII GENERAL CONCLUSION	114



REFERENCES	116
APPENDICES	146
BIODATA OF STUDENT	149
LIST OF PUBLICATIONS	150



LIST OF TABLES

Table	Page
1. Trivial (common), IUPAC, and shorthand (carboxyl and n/ ω reference) nomenclature of common fatty acids	8
2. Composition of standard rat chow diet	40
3. Fatty acid profile of treatment oils	48
4. Fatty acid composition of treatment diets	49
5. Plasma fatty acid profile of treatment groups	52
6. Fatty acid composition of RBC membrane of rats	54
7. Muscle tissue fatty acid profile of treatment groups	56
8. Liver tissue fatty acid profile of treatment groups	58
9. Plasma triacylglycerol and lipoprotein cholesterol profile of rats	84
10. Body composition, fat accretion and inguinal fat cellularity of rats	99



LIST OF FIGURES

Figure		Page
1.	Schematic diagram showing the metabolism of essential fatty acids (LA and ALA) and formation of eicosanoids	11
2.	Schematic diagram showing the action of insulin on carbohydrate, protein and lipid metabolism	15
3.	Schematic diagram showing the insulin signaling pathway mediating the metabolic roles of insulin	18
4.	Schematic presentation of the link between obesity and insulin resistance	31
5.	Baseline and 12 th week IPGTT curve of rats	73
6.	Glucose AUC of base line and 12 th week IPGTT	73
7.	Baseline and 20 th IPGTT curve of rats	75
8.	Glucose AUC of base line and 20 th week IPGTT	75
9.	Baseline and 12 th IPITT curve of rats	77
10.	Glucose AUC of base line and 12 th week IPITT	77
11.	Baseline and 20 th IPITT curve of rats	79
12.	Glucose AUC of base line and 20 th week IPITT	79
13.	Insulin concentration curve of base line and 20 th week IPGTT of rats	82
14.	Insulin AUC of base line and 20 th week IPGTT	82
15.	Insulin- glucose AUC index at day zero and after 20 weeks	83
16.	Weekly average body weight of rats	97
16.	Plasma leptin concentration of rat in the four treatment groups	100
17.	Flow diagram showing the overall experimental approach	146



ABBREVIATIONS

AA	Arachidonic acid
ACC	<i>Acetyl coenzyme A carboxylase</i>
ALA	Alfa linolenic acid
AMPK	Adenosine monophosphate dependent kinase
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AS 160	<i>Akt</i> substrate of 160
ATP	Adenosine triphosphate
AUC	Area under the curve
BCD	Butter plus chow diet
cAMP	Cyclic adenosine monophosphate
CD	Chow diet
DAG	Diacylglycerol
DAP	Dihydroxyacetone phosphate
DGLA	Dihomogamma linolenic acid
DHA	Docosahexanoic acid
DPA	Docosa pentanoic acid
EDTA	Ethylene diamine tetra-acetic acid
EFA	Essentail fatty acid
EGP	Endogenous glucose production
EIA	Enzyme immuno assay
ELISA	Enzyme-linked immunosorbent assay



EPA	Ecosapentanoic acid
FAME	Fatty acid methyl ester
FOXO1	Forkhead transcription factor 1
G-1-P	Glucose-1- phosphate
G-6-P	Glucose -6- phosphate
G6Pase	<i>Glucose 6-phosphatase</i>
GK	<i>Glycerol kinase</i>
GLA	Gamma linolenic acid
GLUT	Glucose transporter
GPO	<i>Glycerol phosphate oxidase</i>
GS	<i>Glycogen synthase</i>
GSK3	<i>Glycogen synthase kinase 3</i>
HDL-C	High density lipoprotein cholesterol
hr	hour
HRP	<i>Horseradish peroxidase</i>
HSDA	N-(2-hydroxy-3-sulfopropyl)-3,5-dimethylaniline
HSL	<i>Hormone sensitive lipase</i>
IL	Interleukin
IPGTT	Intraperitoneal glucose tolerance test
IPITT	Intraperitoneal insulin tolerance test
IR	Insulin receptor
IRS	Insulin receptor substrate
IUPAC	International union of pure and applied chemistry



Kcal	Kilocalorie
kg	Kilogram
KJ	Kilo joule
LA	Linoleic acid
LCACoA	Long chain acetyl coenzyme A
LDL	Low density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol
LTs	Leukoterines
MAP	Mitogen activated pathway
MCD	Chow diet added with one part of soybean oil and two parts of menhaden oil
mg	Miligram
min	minutes
mL	Mililitre
mmol	Milimole
mTOR	Mammalian target of rapamycin
MUFA	Monounsaturated fatty acid
N	Normal
NADH	Reduced <i>nicotinamide</i> adenine dinucleotide
ng	Nanogram
NIDDM	Non insulin dependent diabetes mellitus
nM	Nano meter
PBS	Phosphate buffer saline

PDE3B	<i>Phosphodiesterase 3B</i>
PDK	<i>Phosphoinositide dependent kinase</i>
PEG	Polyethylene glycol
PEPCK	<i>Phosphoenolpyruvate caboxy kinase</i>
PGs	Prostaglandins
PI3K	<i>Phosphoinositide-3 kinase</i>
PIP2	Phosphatidylinositol di-phosphate
PIP3	Phosphatidylinositol tri- <i>phosphate</i>
PKA	Protein kinase A
PKB	Protein kinase B
PKC	Protein kinase C
POD	<i>Per oxidase</i>
PUFA	Polyunsaturated fatty acids
RabGTPase	Rab <i>guanosine triphosphatases</i>
RBC	Red blood cell
SCD	Chow diet added with 2 parts of soybean oil and one part of menhaden oil
SEM	Standard error of the mean
SFA	Saturated fatty acids
SGLT	Sodium coupled glucose transporter
SOCS3	Suppressor of cytokine signaling-3
TAG	Triacylglycerol
TXs	Thrombxanes



TNF- α	Tumor necrosis factor- α
UDP	Uridine diphosphate
UTP	Urindine triphosphate
VLDL	Very low density lipoprotein
WHO	World Health Organization
μ l	Microlitre



CHAPTER I

GENERAL INTRODUCTION

The dietary habits and lifestyle of human societies have undergone significant shifts as a result of industrialization and urbanization (WHO, 2003; Hawkes, 2006). Better economic status also resulted in significantly improved nutritional status, and these brought about the emergence of lifestyle diseases, such as diabetes mellitus. The nutrition transition includes both quantitative and qualitative changes in the diet (Popkin, 2004). The adverse dietary changes include shifts in the structure of the diet towards a higher energy density diet with a greater role for fat and added sugars in foods, greater saturated fat intake, reduced intakes of complex carbohydrates and dietary fiber, and reduced fruit and vegetable intakes (Drewnowski and Popkin, 1997; Popkin and Gordon-Larsen, 2004). However, studies on the evolutionary aspects of diet indicate that major changes have taken place in the type and amount of essential fatty acids (EFA) (Cordain *et al.*, 2005; Simopoulos, 2006). The advent of oil seed processing industry increased vegetable oils production. Besides, increased meat consumption from grain fed animals attributed to the high n-6 to n: 3 fatty acid ratios (Cordain *et al.*, 2005).

Diet, while critical to prevention, also plays a key role as a risk factor for chronic diseases (WHO, 2003).

