UNIVERSITI PUTRA MALAYSIA

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

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By

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

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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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Chairman : Goh Yong Meng, PhD

Faculty : Veterinary Medicine

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

TEKELESELASSIE 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 dipengaruh 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 lengkuk 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 menunjukan 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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Finally I want to dignify my parents who have placed my needs ahead of their own.

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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Date: 11 September 2009

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

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ABBREVIATIONS

AA	Arachidonic acid
ACC	*Acetyl coenzyme A carboxylase*
ALA	Alfa linolenic acid
AMPK	Adenosine monophosphate dependent kinase
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AS 160	*Akt* 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	*Glucose 6-phosphatase*
GK	*Glycerol kinase*
GLA	Gamma linolenic acid
GLUT	Glucose transporter
GPO	*Glycerol phosphate oxidase*
GS	*Glycogen synthase*
GSK3	*Glycogen synthase kinase 3*
HDL-C	High densitiy lipoprotein cholesterol
hr	hour
HRP	*Horseradish peroxidase*
HSDA	N-(2-hydroxy-3-sulfopropyl)-3,5,dimethxyaniline
HSL	*Hormone sensitive lipase*
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 *nicotinamide* adenine dinucleotide
ng	Nanogram
NIDDM	Non insulin dependent diabetes mellitus
nM	Nano meter
PBS	Phosphate buffer saline

PDE3B	*Phosphodiesterase 3B*
PDK	*Phosphoinositide dependent kinase*
PEG	Polyethylene glycol
PEPCK	*Phosphoenolpyruvate caboxy kinase*
PGs	Prostaglandins
PI3K	*Phosphoinositide-3 kinase*
PIP2	Phosphatidylinositol di-phosphate
PIP3	Phosphatidylinositol tri-*phosphate*
PKA	Protein kinase A
PKB	Protein kinase B
PKC	Protein kinase C
POD	*Per oxidase*
PUFA	Polyunsaturated fatty acids
RabGTPase	Rab *guanosine triphosphatases*
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
µl	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).