Role of leptin in development of liver fibrosis in metabolic syndrome with hepatic steatosis

Thesis

Submitted for partial fulfillment of M.D. Degree
In Medical Biochemistry

Presented by

RADWA TAHA MOHAMED
MBBCh ,M.Sc (Clinical and Chemical Pathology)
M.Sc (Medical Biochemistry)

Supervised by

Prof. Dr. / MOHAMED ALY ABD EL HAFEZ
Professor of Medical Biochemistry
Faculty of Medicine
Cairo University

Prof. Dr. / ESMAT AHMED SHEBA
Professor of Internal Medicine
Faculty of Medicine
Cairo University

Prof. Dr. / SAMAR MARZOUK
Ass. Professor of Medical Biochemistry
Faculty of Medicine
Cairo University

Prof. Dr. / SALWA FAYEZ
Ass. Professor of Medical Biochemistry
Faculty of Medicine
Cairo University

Faculty of Medicine
Cairo University
(2011)

Acknowledgment

First and foremost, thanks to ***ALLAH****, who is most beneficent and most merciful*

I wish to express my sincere thanks and gratitude to Professor Doctor ***Mohamed Abd El-hafiz*** *Professor of Medical Biochemistry department Faculty of Medicine, Cairo University for his support, valuable help,I had the honor to accomplish my work under the supervision of him.*

I also wish to express my deep thanks to Doctor ***Olfat Shaker*** *Professor of Biochemistry Faculty of Medicine, Cairo University for her help in supporting us during molecular biology work.*

Also want to thank Doctor ***Esmat Shiba*** *Professor of internal medicine Faculty of Medicine, Cairo University for his support.*

And for Doctor ***Samar Marzouk,*** *Ass. Professor of Medical Biochemistry, Faculty of Medicine, Cairo University I am really indebted to her for her kind encouragement during this work, and her valuable support and help.*

I like to express my thanks Doctor ***Salwa Fayez,*** *Ass. Professor of Medical Biochemistry, Faculty of Medicine, Cairo University to for her help.*

I like to express my thanks to every one who helped a lot during this work.

Finally,

No words can express my deep thanks and appreciation to my ***family*** *for their continuous support and outstanding encouragement.*

Abstract

The study was carried out on 58 patients suffering from NIDDM (18 lean diabetic, 20 obese diabetic, 20 patients with MetS), and 20 healthy age and sex matched controls all patients were subjected to: thorough clinical evaluation, routine laboratory investigation and abdominal ultrasonography.

Key word: ACTH , AMP, ANOVA, *syndrome,* Biochemistry

Table of Contents

List of Tables

List of Figures

List of abbreviations

β_2-AR	β_2-adrenergic receptor
ACAT-1	Acetyl-Coenzyme A acetyl transferase
ACTH	adrenocorticotropic hormone
AdipoR1	adiponectin receptors
ADSF	adipocyte-derived secretory factor
AHA/NHLBI	American Heart Association/National Heart, Lung, and Blood Institute
AIDS	acquired immunodeficiency syndrome
ALT	alanine aminotransferase
AMP	Adenosine mono phosphate
AMPK	AMP activated protein kinase
ANA	Anti nuclear antibody
ANOVA	analysis of variance
AP-1	activation protein-1
AST	Aspartate transaminase
ATP	Adenosine tri phosphate
AUC	Area under the curve
BAT	brown adipose tissue
BMI	Body Mass Index
BP	Blood pressure
bp	Base pair
CAD	Coronary Artery Disease
CART	cocaine-andamphetamine-regulated transcript
CD	Cluster of differentiation
CE	Cholesterol esters
CETP	cholesterol ester transfer protein
CHO	Carbohydrate
CI	confidence intervals
CNS	Central nervous system
CRH	corticotropin-releasing hormone
CRP	C- reactive protein
CVD	Cardiovascular disease
db/db	Diabetic mice
DNA	Deoxy ribonucleic acid
DNL	De novo lipogenesis
EDTA	Ethylene diamine tetra acetic acid
EGIR	European Group for study of Insulin Resistance
ELISA	Enzyme linked immuno sorbent assay
eNOS	endothelial nitric oxide

EPO	erythropoietin
Erk 1	mitogen-activated protein kinases
ESR	Erythrocyte sedimentation rate
ET-1	endothelin-1
fa/fa	Zucker fatty rats
FBG	Fasting blood glucose
FC	free cholesterol
FFAs	Free fatty acids
FSH	follicle-stimulating hormone
G-CSF	granulocyte colony-stimulating factor
GGT	Gamma glutamyl transaminase
GH	growth hormone
GI	Gastro intestinal
Glc	glucose
GLP-1	glucagons like peptide
GLUT-4	Glucose Transporter-4
GN	Gluconeogenesis
GnRH	gonadotropin-releasing hormone
HBS Ag	Hepatitis B surface antigen
HCV Ab	Hepatitis C antibody
HDL- C	High density lipoprotein- Cholesterol
HFCS	High Fructose Corn Syrup
HPG	hypothalamic pituitary gonadal Axis
HIV	Human immuno deficiency virus
HLA	Human leucocyte antigen
HOMA	Homeostatic model assessment
HGP	Hepatic Glucose Production
hPGH	human placental growth hormone
HSL	Hormone-sensitive lipase
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IFN	interferon
IGF-1	insulin-like growth factor 1
IGF-BP	insulin-like growth factor binding protein
IgG	Immunoglobulin G
IGT	Impaired Glucose Tolerance
IL-6	interleukin 6
iNOS	Inducible nitric oxide synthase
IP-10	interferon-gamma-inducible protein
IR	insulin resistance

IRS-1	Insulin receptor substrate -1
IVGTT	Intravenous glucose tolerance test
JAK	Janus kinase JAK
LAR	leukocyte antigen–related phosphatase
LDL-C	Low Density lipoprotein- Cholesterol
LEPR1	Leptin receptor 1
LFTs	liver function tests
LH	Leutinizing hormone
LHRH	Leutinizing hormone releasing hormone
LIF	leucocyte inhibitory factor
(LpL	lipoprotein lipase
LPS	Lipopoly saccharide
MAP	mitogen-activated protein
MCH	melanin-concentrating hormone
MCP-1	monocyte chemotactic protein-1
MetS	Metabolic Syndrome
MI	Myocardial infarction
mRNA	Messenger Ribonucleic Acid
mTOR	molecular target of rapamycin
MTP	microsomal transfer protein
NAD	Nicotinamide Adenine Dinucleotide
NADPH	Nicotine Amide Dinucleotide Phosphate
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NCEPATP III	National Cholesterol Education Program—Third Adult Treatment Panel
NEFAs	Non-esterified fatty acids;
NIDDM	Non insulin dependent diabetes mellitus
NF κ	Nuclear Factor kappa
NPY	neuropeptide Y
NPY/AgRP	neuropeptideY/Agouti-related peptide
Ob	leptin gene
Ob-R	leptin receptor gene
OGTT	Oral Glucose tolerance testing
PAI-1	plasminogen activator inhibitor type-1
PAMPs	pathogen associated molecular patterns
PBEF	pre-B cell colony-enhancing factor
PCOS	polycystic ovarian syndrome
PCR	Polymerase chain reaction
PGE2	Prostaglandin E2
PIAS	protein inhibitors of activated STATs
PI3K	phosphatidylinositol 3-kinase

PKC	protein kinase C
POMC	Proopiomelanocortin
PPAR γ	Peroxisome proliferator-activated receptor γ
PPS	Post prandial blood sugar
PRRs	pattern recognition receptors
PTP1B	protein tyrosine phosphatase 1B
PTPs	protein tyrosine phosphatases
PUFA	Poly unsaturated fatty acid
PVN	paraventricular nucleus
QUICKI	Quantitative Insulin Sensitivity Check Index
RA	Rheumatoid arthritis
RBP-4	retinol binding protein-4
RF	rheumatoid factor
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen species
RXR	retinoid X receptor
SAPK	stress activated protein kinase
SD	Standard deviation
SGOT	Serum glutamate oxaloacetate transaminase
SGPT	Serum glutamate pyruvate transaminase
SH-2	Src-homology-2
SLIP	serum leptin interacting protein
SLR	soluble leptin receptor
SNPs	Single nucleotide polymorphism
SOCS3	suppressor- of cytokine- signaling-3
SPSS	Statistical Package for Social Science.
STATs	Signal Transducers and Activators of Transcription
T3	triiodothyronine
T4	thyroxin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TAG	Triacyl glycerol
TG	Triglyceraids
Th	T helper cells
TLRs	Toll-like receptors
TNF- α	Tumor Necrosis Factor- α
TPO	thrombopoietin
TRAF-3	tumor necrosis associated factor-3
TRH	thyroid-releasing hormone
TSH	thyroid-stimulating hormone
Tyr	tyrosine
VCAM-1	Vascular Cell Adhesion Molecule-1.

VLDL	Very low density lipoprotein
WAT	white adipose tissue
WHO	World Health Organization

Introduction

The Metabolic Syndrome (MetS) is a cluster of cardiovascular risk factors including obesity, hypertension and dyslipidaemia that increases the risk of the development of type 2 diabetes mellitus and cardiovascular disease. The risk factors of MetS include obesity, aging, sedentary lifestyle, diabetes mellitus, coronary heart disease and lipodystrophy. It is estimated that t a large majority of patients with type 2 DM or impaired glucose tolerance have the metabolic syndrome ***(Ogbera, 2010)***, recent interest has focused on the possible involvement of insulin resistance as a linking factor ***(Alberti, et al., 2009)***

The insulin resistance is associated with a primary cellular defect in insulin action (insulin resistance) and a compensatory increase in insulin secretion. This combination of insulin resistance and hyperinsulinaemia causes a number of metabolic and cardiovascular changes that result in metabolic syndrome ***(Kashyap and Defronzo, 2007)***

The adipose tissue secretes several bioactive mediators that influence inflammation, insulin resistance, diabetes, atherosclerosis and several other pathologic states besides the regulation of body weight. These mediators are mostly proteins and are termed "adipocytokines", The various cell signaling proteins secreted by the mature adipocytes include adiponectin, tumor necrosis factor-α (TNF-α), resistin, retinol binding protein-4 (RBP-4), visfatin, plasminogen activator inhibitor 1 (PAI-1), leptin, omentin, interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) ***(Gandhi, et al.,2010),*** Although inflammatory factors and adipocytokines have different pathophysiological pathways and targeted tissues and organs, the altered systemic balance of inflammatory factors and adipocytokines may result in MetS ***(Yu, et al., 2009)***

Non-alcoholic fatty liver disease (NAFLD) includes a spectrum of liver pathology ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) ***(Charlton, 2004),*** NASH is commonly observed in individuals with metabolic syndrome comprising obesity, type-2 diabetes, hyperlipidemia and hypertension ***(Marchesini et al, 2003).***

Pathogenesis of NASH most likely involves two steps. the initial event is thought to be insulin resistance, leading to the accumulation of lipids in hepatocytes, and the second step involves increased oxidative stress and production of inflammatory cytokines, resulting in the

hepatocellular injury and subsequent progression of hepatic fibrosis ***(Neuschwander-Tetri and Caldwell, 2003).***

Insulin receptor defects responsible for insulin resistance include reduced insulin-stimulated tyrosine kinase activity, reduced activation of tyrosine phosphorylation of the insulin receptor and of IRS-1, and decreased association of p85 protein and phosphatidylinositol-3-kinase activity with IRS-1***(Kashyap and Defronzo, 2007)***

Aim of the work

The present work is designed to estimate the levels of serum IL-6 and leptin in different clinical groups of diabetes mellitus and metabolic syndrome (MetS) to find possible correlations between the serum levels of them & calculate both HOMA (Homeostatic model assessment) and QUICKI (Quantitative insulin sensitivity check index) as indicator of insulin sensitivity and to find the relation between them and both leptin and IL-6.

To find the relation between the frequency of IRS-1 gene mutation and both diabetes mellitus and metabolic syndrome